BIODEGRADABLE POLYMERS IN CLINICAL USE AND CLINICAL DEVELOPMENT

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PREFACE

The use of biodegradable polymers has been increasing in recent years, specifically toward various biomedical applications as these materials not only serve the desired purpose but also get eliminated from the body due to their biodegradable nature. Ideally, a material should possess specific physical, chemical, biological, functional, biomechanical, and degradation properties that fit a particular biomedical application. Biodegradable polymers have been used as surgical aids, extended-release drug carriers, and as scaffolds for tissue engineering, to name a few applications. Traditional biodegradable medical devices such as orthopaedic pins and nails, fixation plates, suture nets, filaments, and hemostatic sponges, have been used for decades with few modifications. However, recent advances in drug delivery, tissue engineering, gene therapy, and medical devices have resulted in an increased need for biopolymers with tailored properties. It should be noted that introducing new polymeric devices or drug delivery systems requires extensive clinical development, which may take several years and involve a significant financial investment. Thus, the trend has been to rely on varying compositions and modifications of clinically used biodegradable polymers. Considering the importance of such an area, this new reference text, *Biodegradable Polymers in Clinical Use and Clinical Development*, focuses on biodegradable polymers and their importance in biomedical research and presents their clinical status and development scenario along with their patent landscape. This book has been divided into eight parts based on different types of biodegradable polymers and their applications. Every attempt has been made to exclude the polymers that have not yet reached clinical application.

Part I provides a brief overview of different classes of biodegradable polymers elaborated upon in the book with an emphasis on drug delivery, detailing their key features and degradation patterns. Polymer selection criteria for specific biomedical applications have also been discussed. Additionally, an overview of different products based on biodegradable polymers is provided.

There are two major classes of biodegradable polymers, i.e. natural and synthetic, where natural polymers can be further classified based on their source of generation. Natural polymers are discussed in Parts II through IV. Part II discusses two main protein-based polymers of natural origin, namely, collagen

and gelatin. The chapters focus on detailing their structure, occurrence, types, properties, manufacturing processes, degradation, and clinical applications.

Part III focuses on polysaccharide-based polymers of natural origin. A chapter on chitosan and its derivatives highlights its role as biosensor, permeation enhancer, delivery vehicle, tissue-engineering scaffold, and wound-healing material. Another chapter focuses on various sources, clinical applications, preclinical experiments, and possible future options for alginates. Furthermore, dextran, pentosan sulphate, and arabinogalactans are detailed in terms of their occurrence, structure, biochemistry, chemical nature, pharmacokinetics, side effects and toxicity, and medicinal applications.

Polyhydroxyalkanoates are discussed in Part IV, focusing on their production, characterization, and current applications in the medical field. Results from *in vitro* and *in vivo* efficacy studies are highlighted along with insights on toxicity and biocompatibility. Also, a patent landscape is provided.

Furthermore, Part V details important synthetic biodegradable polymers used in clinical settings. A chapter is dedicated specifically to the use of lactide and glycolide polymers and their copolymers in drug delivery applications, detailing their synthesis, processing, and properties *vis-a-vis* toxicity and safety considerations. Another chapter discusses the use of polyanhydrides in localized delivery with special attention to their degradability behavior, toxicological profile, uses in different disease conditions, and recent advances in the medical field. Also, synthesis, physicochemical characteristics, and biomedical applications of poly(ε-caprolactone-co-glycolide) copolymers and their applications as monocryl sutures, suture coatings, dermal tissue repair agents, and buttressing materials are detailed. This part also includes chapters on polycyanoacrylates and PEGylation technology, PEGylated drugs, chemistry, safety and toxicity of PEGylation, and applications of PEG at the clinical level and in the market.

Part VI deals with biomedical applications of calcium phosphate-based ceramics where synthesis, characterization, and properties of calcium phosphate materials are discussed. Moreover, the biocompatibility and toxicity profiles are reviewed along with a summary on some of the clinical results and commercially available calcium phosphate products.

Emerging clinical uses of biodegradable polymers are dealt with in Part VII, where a chapter focuses on polymers described for nucleic acid delivery, showing promising *in vivo* activity. A description of the structural design, physicochemical properties, and preclinical evaluation of different polymeric carriers and progress in clinical development is also provided. A separate chapter has been added focusing on applications of biodegradable polymers in tissue engineering, with a major focus on clinical applications thereof. The chapter compiles biodegradable polymer-based products currently under clinical trials or in the market for tissue engineering applications and also discusses the advances in discovery of need-specific polymeric biomaterials and biomaterial design.

Additionally, clinical use and applications of various injectable polymers of both natural and synthetic origin are discussed and offer insight into the underlying chemistry and design of *in situ* forming materials. The relevant design considerations affecting material properties and functionality are described along with highlights of possible future trends in the field.

Part VIII discusses the aspects of biodegradable polymers relating to *intellectual property rights*. It was felt that inclusion of patent database search would be useful to researchers in giving an overview of the technological developments and innovations that have taken place on a global level. This section may serve as a useful guide and reference chapter for research as it gives an overview of the innovations and technological challenges, and it also supplies a global map of the players involved, that is, industry and academia working on the applications of biodegradable polymers in drug delivery and tissue engineering.

Given the history of research and development in the applications and use of biodegradable polymers, it seems certain that new modifications in existing polymers and a wide variety of new polymers used in drug delivery and other biomedical applications will emerge in the coming years. The readers of this text are expected to have a broad base of backgrounds ranging from the basic sciences to more applied disciplines. Keeping this in mind, each section is planned such that it provides an overview of the specific subject and also goes into a detailed discussion with extensive references. With these details, the book will be valuable to both novices and experts. We trust that this in-depth coverage shall assist recent inductees to the subject of biodegradable polymers for various biomedical applications.

Lastly, and most importantly, the editors are thankful to all the internationally recognized authors who have played a vital role in making this book a reality through their contributions.

PART I

GENERAL

CHAPTER 1

BIODEGRADABLE POLYMERS IN DRUG DELIVERY

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1.1 INTRODUCTION

Polymers have become an indispensible part of daily life. Biodegradable polymers are of special interest because they do not accumulate in nor harm the environment and thus can be considered "green" [1–3]. The use of biodegradable polymers has increased in the vast array of application, but it is staggering in its use in biomedical applications. A whole new genera of "polymer therapeutics" has developed because of the wide applicability of these

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polymers, which includes but is not limited to their function in pharmacological uses, mechanical support, mechanical barrier, artificial tissue/organs, preparation of prodrugs, and as carriers for cells, drugs, and the like [4–15] with and without targeting. These functions can also be executed by nonbiodegradable devices, but such devices would permanently remain in biological tissues if not removed surgically. Because of the inherent difficulty in retrieving small-scale devices from tissues, it is advantageous to use biodegradable polymers that would naturally degrade and disappear in tissues over a desired period of time [16, 17].

In addition, the most important criteria for polymers that should be considered is their toxicological profiles. The polymer and any of its degradation products should not invoke any unacceptable toxicity and immune response. General criteria for selecting a polymer for use as a degradable biomaterial are to match the mechanical properties and the degradation rate to the needs of the application, shelf life/stability, processability, cost, and the like [1, 2, 18].

Biodegradable polymers can be either natural or synthetic. In general, synthetic polymers offer greater advantages over natural ones as they can be tailored to give a wider range of possibilities with a variety of properties. Some of the natural polymers have functional groups suitable for applications such as tissue engineering and are less prone to produce toxic effects. However, the presence of such functional groups and contaminants present in the material of natural origin may produce undesirable immunoligcal effects [19–21]. On the other hand, synthetic polymers are available with a wide range of chemical linkages that can greatly affect their degradation and other derived properties. To obtain an intermediate property, two or more polymers can be blended or chemically linked (copolymerized) [1, 22]. This latter approach has basically attracted a lot of attention because of the possibility of generating polymers with desired properties without limitations such as phase separation.

To date, due to the versatility of polymeric materials, specifically biodegradable ones, they are rapidly replacing other biomaterial classes, such as metals, alloys, and ceramics for use in biomedical applications. In 2003, the sales of polymeric biomaterials exceeded $7 billion, accounting for almost 88% of the total biomaterial market for that year [8]. The global market for biodegradable polymers increased from 409 million pounds in 2006 to an estimated 541 million pounds by the end of 2007. It should reach an estimated 1203 million pounds by 2012, a compound annual growth rate (CAGR) of 17.3% [23, 24].

This chapter provides a brief overview of different classes of polymers with their key features. Different degradation patterns affect the release of entrapped molecules as well as other derived properties, and these patterns are also discussed in this chapter. There are various successful products in clinical practice, and the number of such products is ever increasing and at a faster rate from the past few decades. Several products are discussed in this chapter with their major properties; however, the focus is on drug delivery systems available on the market. A general decision tree, based upon these properties, for the

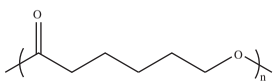
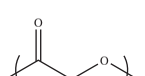
selection of material is also given, which could be of help in selecting polymers for a particular end use. Finally, the future of the polymeric biomaterial is briefly discussed.

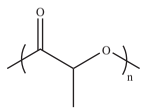
1.2 TYPES OF BIODEGRADABLE POLYMERS

Biodegradable polymers can be divided in two major classes of natural and synthetic origin. However, the properties of polymers in these classes vary widely and give a selection of individual polymers for individual requirements. Most of the polymers utilized for biomedical application are listed in Table 1.1 with their chemical structure, properties, major applications, and marketed product. The properties given are those for the particular type of polymer; however, customarily copolymers are employed to achieve a hybrid of individual properties. Because of constant advancements the list is ever increasing and many new copolymers as well as polymers are entering the panorama of biodegradable polymers for biomedical application.

Most of properties required for use as a biomaterial are fulfilled by many natural polymers, for example, polysaccharides and protein derivatives and synthetic polymers (e.g., polyesters, polyanhydride, and polyorthoesters). However, when biomedical applications are considered, the requirement diversifies in terms of mechanical strength required, degradation time, surface properties, physicochemical parameters, degree of crosslinking, presence of functional group for modification and tagging, and so forth. Some of the applications such as bone grafting and bone repair, in addition to biocompatibility, require purely mechanical function. Hence polymers that can withstand load and have long degradation time are suitable for these applications [25–27]. Some applications such as surgical dressings, sutures, and the like require varying strength and degradation time, which usually depends on the type of tissue and the type of injury. In tissue engineering application growth factors are considered vital for rapid healing of the tissue and generating more biofuncational tissue. Hence, the strategy is to mimic matrix and provide the necessary information or signaling for cell attachment, proliferation, and differentiation to meet the requirement of dynamic reciprocity for tissue engineering [28–34]. Natural polymers in this regard are conceived better than noninformational synthetic polymers [20, 35]. However, the flip side of natural polymers is that even though they are available in ample quantity, they suffer from some limitations such as immunogenecity, difficulty in processing, a potential risk of transmitting origin related/associated pathogens, and batch-to-batch variability [36, 37]. Synthetic polymers on the other hand can be produced in a reproducible manner with better quality control. This particular fact is more important when these are used as carriers for bioactives where reproducible delivery is required from the carrier, as the change in the in vivo release of the bioactive carrier can change the course of the treatment. Choice among the synthetic polymers is again based on the individual application; however, when

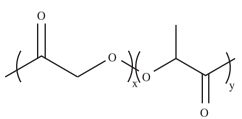
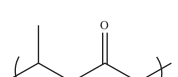
TABLE 1.1 Biomedical Applications of Polymers

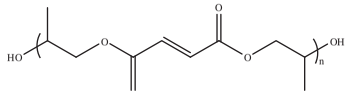
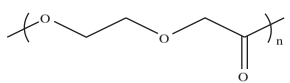
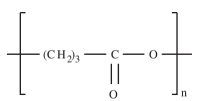
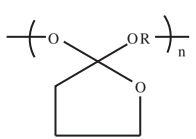
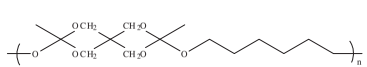
Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
Synthetic Polyesters					
Poly(ε-caprolactone)		Most widely used polymer class for drug delivery and tissue engineering Synthesized usually by ring-opening polymerization (ROP), polycondensation Bulk degrading polymer Semicrystalline (varying with mol. wt.) polymer having a low glass transition temperature (−60°C) and low melting point (55−60°C) Due to its crystallinity and hydrophobicity, degradation of poly(caprolactone) (PCL) is very slow, rendering it suitable for long-term delivery over a period of more than a year It has the ability to form compatible blends with other polymers, which provides opportunities to manipulate the drug release rate from matrix Undergoes bulk hydrolytic degradation of hydrolytically labile aliphatic ester linkages and autocatalyzed by carboxylic end groups; however, the rate of degradation is rather slow (2−3 years) Its slow degradation, high permeability to many drugs and nontoxicity leads to study for a long-term drug/vaccine delivery vehicle	Sutures, tissue engineering, drug delivery	Capronor Monacryl SynBiosys	63−71
Poly(glycolic acid)		The first biodegradable synthetic polymer investigated for biomedical applications It is a highly crystalline polymer (45−55% crystallinity) and, therefore, exhibits a high tensile modulus with very low solubility in organic solvents Glass transition temperature ranges from 35 to 40°C Melting point 200°C	Sutures, tissue engineering, drug delivery	Dexon Biofix	72−77

		Due to its excellent fiber-forming ability, it is used for bioresorbable sutures, e.g., Dexon Polyglycolic acid is a bulk-degrading polymer, degrades by the nonspecific scission of the ester backbone Polyglycolide shows excellent mechanical properties due to its high crystallinity The high rate of degradation, acidic degradation products, and low solubility, however, limit the biomedical applications for polyglycolic acid Therefore, several copolymers containing glycolide units are being developed to overcome the inherent disadvantages of polyglycolide, e.g., poly(lactide-*co*-glycolide)			
Poly(lactic acid) PLA 		Chiral and exists in two optically active forms, L- and D-Lactide; L-lactide is naturally occurring monomer Polymerization of racemic (D,L)-lactide and mesolactide, however, results in the formation of amorphous polymers, whereas poly(L-lactic acid) (PLLA) is semicrystalline (37% crystalinity) PLLA is a more hydrophobic and slow-degrading polymer compared to polyglycolic acid and has good tensile strength, thus, used for sutures High-molecular-weight PLLA can take between 2 and 5 years for total resorption in vivo More hydrophobic compared to poly(glycolic acid) (PGA) but less than PCL Can be produced in crystalline form using racemic pure form or amorphous using racemic monomer	Bone fixtures, sutures, drug delivery, tissue engineering	Phantom Soft Thread Soft Tissue Fixation Screw, Phantom Suture Anchor, Full Thread Bio Interference Screw, BioScrew, Bio-Anchor, Meniscal Stingers, Clearfix, Meniscal Dart, Sculptra, Atridox, Trenantone, Genexol	78–84

(*Continued*)

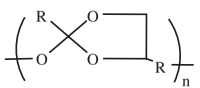
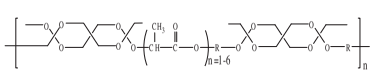
TABLE 1.1 (Continued)

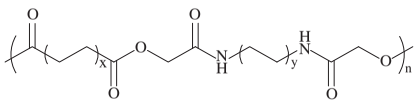
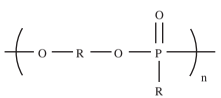
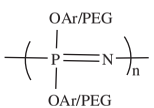
Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
		Several copolymers of L-lactides with glycolides or DL-lactides are currently under investigation for the development of polymers with better property modulation for controlled drug delivery poly(D,L-lactic acid) (PDLLA) is amorphous polymer due to the random distribution of L- and D-lactide units Glass transition temperature of 55–60°C Due to its amorphous nature, the polymer shows much lower strength compared to poly(L-lactic acid)			
Poly(lactide-*co*-gycolide)		One of the most widely used polymers for biomedical applications especially in drug delivery Depending on the ratio of lactide and gylcolide, properties of the copolymer can vary Undergo bulk erosion through hydrolysis of the ester bonds and the rate of degradation depends on a variety of parameters including the lactic acid/glycolic acid (LA/GA) ratio, molecular weight, and the shape and structure of the matrix	Tissue fixation, sutures, tissue engineering, drug delivery	PuraSorb, Vicryl, Panacryl, Dermagraft, Cytoplast Resorb Risperdal Consta, Arestin, Lupron Depot, Zoladex, Trelstar, Enatone, Procin, Eligard, Sandostatin LAR, Nutropin Depot Decapeptyl, Pamrelin, Vivitrol, Proffact depot, Plenaxis	1, 6, 18, 56, 59, 60, 74
Poly(β-hydroxybutyrate)		Stereoregular polymers, synthesized by numerous bacteria Slowly degrading Too brittle for many applications Blends are used mainly, such as 3-hydroxyvalerate, 3-hydroxyhexanoate, and 4-hydroxybutyrate	Tissue engineering, drug delivery	—	67, 85–89

Poly (propylene fumarate)		High-strength polymeric biomaterial Undergo bulk erosion via hydrolysis of the ester bonds Unsaturated fumarate groups can be used for crosslinking Composites with various ceramics have also been used to enhance mechanical strength	Orthopaedic applications, bone tissue engineering	—	90–99
Poly(p-dioxane) Poly(1,5-dioxipian-2-one)		Very low glass transition temperature ranging from −10 to 0°C. Undergoes degradation by nonspecific scission of the ester back bone Slow to moderately degrading polymer, is known to lose its strength within 1–2 months and its mass within 6–12 months by hydrolytic degradation	Monofilament suture, orthopedic application	PDS Pins	100–107
Trimethyl carbonate polymers		An elastomeric aliphatic polyester with excellent flexibility and poor mechanical strength Undergoes surface degradation and degradation at faster rate in vivo because of enzymatic degradation	Soft tissue regeneration, low mol. wt. polymers for drug delivery	Maxon, Acufex BioSyn (ter polymer)	108–113
Poly(ortho esters)					114–133
Poly(ortho esters) I		Hydrolyzed under aqueous environment thus producing γ-butyrolactone that is rapidly converted to γ-hydroxybutyric acid This hydrolytic product acid has an autocatalytic effect on the further degradation of the polymer It should be stabilized with a base such as Na_2CO_3 to prevent an uncontrolled, autocatalytic hydrolysis reaction Such disadvantages of POE I have limited applications in biomedical applications	Drug delivery	Alzamer	118, 124
Poly(ortho esters) II		Synthesized to overcome autocatalytic effect of POE I	Drug delivery		117, 121, 133

(*Continued*)

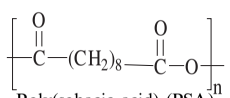
TABLE 1.1 (Continued)

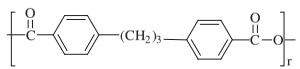
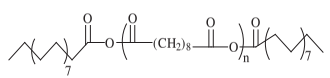
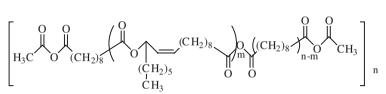
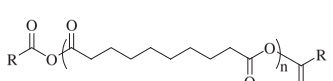
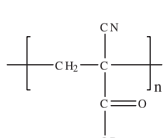
Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
		The initial products of the polymer hydrolysis are neutral, and thus it is not necessary to use a basic excipient However, the polymers belonging to this family are extremely hydrophobic, which limits the access of water to the hydrolytically labile ortho ester linkage Hence, in order to achieve the increase in the surface erosion rate, it is necessary to incorporate acidic excipients into the polymer matrix These acidic excipients often accelerate the autocatalytic reaction which is limitation of POE II to design surface eroding devices			
Poly(ortho esters) III		Semisolid material at room temperature (polymer chains are highly flexible) that enables to prepare the injectable delivery system Drug delivery system made by simple mixing with the therapeutic agents without the need of organic solvents or elevated temperatures In addition, no autocatalysis occurs during degradation, since the initial hydrolysis generates one or more isomeric monoesters The ortho ester bonds of the polymer are only sensitive to the acidic products	Drug delivery		117, 121, 133
Poly(ortho esters) IV		A modified POE II that can be used without acidic excipients To surmount the disadvantages of POE II, such as extreme hydrophobicity and stability, a short segment based on lactic	Drug delivery		117, 125, 130

		acid or glycolic acid is incorporated into the backbone of POE IV Such segments are included as latent acids, which catalyze hydrolysis of the ortho ester linkages in the polymer backbone The erosion rate of the polymer can be precisely controlled by adjusting the content of such segments			
Poly(ester amide)		Due to the hydrogen bonding ability of the amide bonds and biodegradability imparted by the ester bonds, these copolymers have good mechanical and thermal properties The degradation of poly(ester amides) has been shown to take place by the hydrolytic cleavage of the ester bonds, leaving the amide segments more or less intact	Bioresorbable suture materials	Cameo	134–140
Polyphospho ester		Polyphosphoesters degrade under physiological conditions due to the hydrolytic and enzymatic cleavage of the phosphate bonds in the backbone Higher synthetic flexibility Pentavalency of the phosphorous atoms allows for chemical linkages to be made and enabling the development of novel polymer prodrugs Copolymerized with poly(urethanes) and poly(lactide) to improve the flexiblity of these biomaterials	Drug delivery, scaffold for tissue engineering	Paclimer	141–147
Poly(phosphazenes)		Biocompatibility, unique functionality, matrix permeability and the ability to control the rate and mode of degradation Among the different classes of degradable polyphosphazenes investigated,	Non-load-bearing tissue engineering, drug delivery		148–153

(*Continued*)

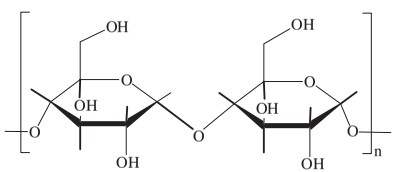
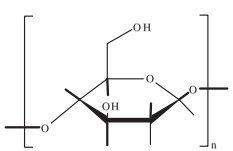
TABLE 1.1 (Continued)

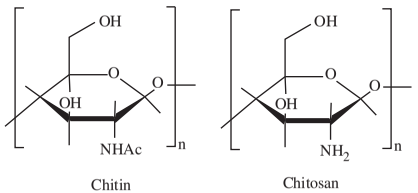
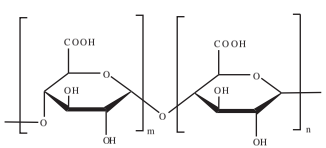
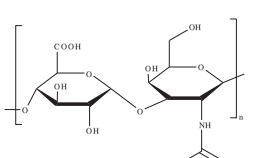
Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
		poly[(amino acid ester) phosphazenes] have been met with the most success in terms of potential biomedical applications All of the amino acid ester substituted polyphosphazene were found to be degradable with the rate of degradation varying, ranging from a few hours to years, with the type of amino acid esters used Undergo both surface and bulk erosion, depending on the lability of the bond and hydrophobicity of polymer attached to side group of backbone			
Pseudo poly(amino acid)		Amino acids linked by nonamide bonds such as esters, imino carbonates, and carbonates Most extensively investigated system is the tyrosine-derived poly(amino acids) Mechanically competent, biodegradable polymer system for load-bearing biomedical applications Amorphous, hydrophobic, and undergo slow hydrolytic degradation	Drug delivery, tissue engineering		154–158
Polyanhydrides		Usually synthesized by melt-polycondensation Surface-eroding polymers Drug release usually follow zero-order kinetics			51, 54, 159
Alipahtic polyanhydrides	 Poly(sebacic acid) (PSA)	Polyanhydirdes with both statured and unsaturated diacid moners are studied	Usually copolymerized with other class		159

		Aliphatic polyanhydrides are brittle, crystalline, and melt at temperatures below 100°C Soluble in chlorinated hydrocarbons Degrade and are eliminated from the body within weeks Usually these, especially sebacic acid, are copolymerized with other classes of polyanhydrides	of polyanhydrides and used in drug delivery		
Aromatic polyanhydrides		Crystalline, insoluble, slow degradation, melting point 200°C Various aromatic polyanhydrides have been studied, however 1,3-bis-carboxy phenoxy propane (CPP)-based polyanhydrides are the most investigated class	Drug delivery	Gliadel	160–162
Fatty acid polyanhydrides Stearic acid-terminated [P(StA-SA)] Ricinoleic acid-based [P(RA-SA)] Euricic-acid-dimer-based [P(EAD-SA)]	 P(StA-SA)  P(RA-SA)  n=25-50 P(EAD-SA)	Hydrophobic, soluble, non- or semicrystalline, low melting point (20–90°C) Low mechanical strength, degrades into natural body components Surface eroding and restricts water penetration into the polymer matrix Very high content of fatty acid leads to increase in bulk degrading properties RA (ricinoleic acid)-based polyanhydrides and polyesters are liquid-injectable polymers and form gel in contact with aqueous media	Drug delivery	Septacin (EADSA)	163–170
Polycaynoacrylates		One of the fastest degrading polymers having degradation times ranging from a few hours to a few days Degradation rate depends on the length of the alkyl side groups	Synthetic surgical glue, skin adhesive and an embolic material, one of the first biodegradable polymers	Dermabond	171–174

(*Continued*)

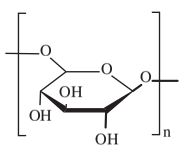
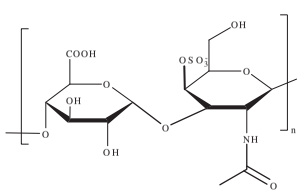
TABLE 1.1 (Continued)

Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
		Lower alkyl derivatives, leads to toxic degradation products such as cyanoacetic acid and formaldehyde Higher alkyl derivatives, such as octyl and isobutyl cyanoacrylates, are used Easy preparation, high utility size ranges, absence of solvent residues, ability to form stealth nanoparticles and the ability of Poly(cyanoacrylate) (PCA) to absorb or encapsulate a wide range of drug or protein molecules	used for nanoparticles for drug delivery		
Natural Polymers **Polysaccharides**					
Starch		It is a physical combination of branched (amylopectin) and linear polymers (amylose) with *d*-glucopyranoside repeating units Amylose is crystalline with molecular weight (MW) of 500 kDa and soluble in boiling water Amylopectin is insoluble in boiling water Stability under stress is not high Hyrodxy functionality can be used for chemical modifications Acetylated starch is more hydrophobic and have better structural fiber or film-forming polymer as compared to native starch Usually blended with nonbiodegradable polymers to aid their film-forming properties	Film formation, filler in different forms, in solid dosage form used as a binder, disintegrant etc.		175–182
Cellulose		It is a very highly crystalline, high-molecular-weight polymer, which is infusible and insoluble in water and organic solvents Ether and ester derivatives are the useful derivatives of cellulose	Film-forming agent		183–189

Chitin and chitosan	 Chitin Chitosan	Chitin is a macromolecule found in the shells of crabs, lobsters, shrimps, and insects Chitin is insoluble in its native form but chitosan, the partly deacetylated form, is water soluble The degree of deacetylation of typical commercial chitosan is usually between 70 and 95%, and the molecular weight between 10 and 1000 kDa Biologically renewable, biodegradable, biocompatible, nonantigenic, nontoxic, and biofunctional Ability to absorb heavy metal ions Modified chitosans have been prepared for use in cosmetics and in wound treatment	Artificial skin and absorbable sutures, in cosmetics as moisturizer, water-soluble prodrug, particulate drug delivery		190–205
Alginic acid		Block copolymer composed of two uronic acid units The composition of alginate (the ratio of the two uronic acids and their sequential arrangements) varies with the source High acid content of alginates allow spontaneous and mild gelling in the presence of divalent cations They are high-molecular-weight polymers having molecular weights up to 500 kDa High reactivity of acid allows appropriate modification for various applications Unable to undergo enzymatic degradation γ Irradiation and other mild chemical crosslinking is done to make them degradable Poor cell adhesion on alginate gels	Scaffolding material for bone regeneration wound dressings and as drug and cell delivery vehicles, chemically immobilizing the drug to the polymer backbone		19, 20, 35, 206–208
Hyaluronic acid (HA)		Largest glycosaminoglycan having molecular weights up to several millions Water-soluble and forms highly viscous solutions with unique viscoelastic properties	Wound dressing, vitreous humor and synovial fluid substitute, tissue engineering and drug delivery	Hyaff, Ossigel, Amvisc, Amvisc, plus Synvisc, Orthovis	209–217

(*Continued*)

TABLE 1.1 (Continued)

Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
		Forms three-dimensional structures in solution with extensive intramolecular hydrogen bonding Undergoes degradation within the body by free radicals Ability to promote angiogenesis Due to the high functionality and charge density of HA, it can be crosslinked by a variety of physical and chemical methods			
Dextrans		Available in a wide range of molecular weights Contain a high density of hydroxyl groups that make the polymers highly hydrophilic and capable of being further functionalized chemically In vivo degraded through the action of dextranases Also been used as hydrophilic part of amphiphilic block-*co*-polymers	As a macromolecular prodrug and plasma expander		35, 218–222
Chondroitin sulfate		Major component of aggrecan, the most abundant glycosaminoglycan found in the proteoglycans of articular cartilage Involved in intracellular signaling, cell recognition, and the connection of extracellular matrix components to cell-surface glycoproteins Several physical and chemical crosslinking techniques have been developed to form hydrogels for biomedical applications	Wound dressing, scaffolding material for cartilage tissue engineering		223–230

Carrageenans (Poly galactans)		Extracted from red marine algae Form reasonably stiff and thermoreversible thixotropic gels in the presence of gel-promoting salts at room temperature Three types of carrageenan on the basis of their patterns of sulfate esterification: κ (kappa), ι (iota), and λ (lambda)	Food products, drug delivery	Genia-Beads CG	231–234
Arabinogalactan		A branched polysaccharide consisting of a (1-3)-ß-D-galactan backbone having (1-6)-ß-galactan side chains, which are terminally modified by arabinose Highly water soluble and posses a high degree of biocompatibility	Drug delivery, prodrugs		235–238
Polypeptide					
Albumin	66.5 kDa protein, without glycosylation with molecular dimensions of 8 nm × 3.8 nm	Albumin is a natural carrier of hydrophobic molecules (such as vitamins, hormones, and other plasma constituents), with favorable, noncovalent binding characteristics (hydrophobic interactions) Human albumin functions as a surface-active polymer providing charge and steric stabilization to the nanoparticles to prevent aggregation Tumors are able to trap plasma proteins and utilize degradation products for proliferation, e.g., Abraxane (albumin) nanoparticles of paclitaxel where interaction between paclitaxel and albumin is weak and both substances freely dissociate after reconstitution and drug released at cell surface	Carrier vehicle for intravenous drug/gene delivery, coating materials for cardiovascular devices, surgical adhesives	Abraxane	239–245
Collagen	Inert, rigid protein with fundamental structural unit is a tropocollagen, a molecular rod about 2600 Å in length and 15 Å in diameter with a molecular weight of 300 kDa. Twenty-five different types of collagen	In physiological solutions, spontaneously self-assemble into higher-order structures Porous matrices, films, gels, or monofilaments that can be fabricated	Hemostats, implants, device coatings, and stabilizers for biologics.	Apligraf, Revitix, Vctoi, Forta-Derm, Infuse,	246–255

(*Continued*)

TABLE 1.1 (Continued)

Type of Polymer	Chemical Structure	Key Features	Application	Marketed Product	Reference
	based on the sequence of amino acid, commercial product contain ~95% type I collagen and ~5% type III collagen	Drugs may be incorporated into or added after the construction of the final forms	Collagen sponges, collagen membranes, collagen stents, and vascular graft coatings	Collagraft, Healos, Biomend	
Gelatin	Denatured collagen and is typically isolated from bovine or porcine skin or bone by acid or base extraction and consists of a distribution of polypeptide fragments of different sizes	Often exhibit lot-to-lot variability Physicochemical properties of gelatin vary depending on method of extraction, amount of thermal denaturation employed and electrolyte content of the resulting material	Hemostats, implants, device coatings, and stabilizers for biologics	Gelfoam, Surgifoam, CultiSpher-G, H.P. Acthar gel	250, 252, 256–258
Fibrin	Fibrin is a protein matrix	Fibrin naturally contains sites for cell binding and, therefore, has been investigated as a substrate for cell adhesion, spreading, migration, and proliferation Rapid degradation can represent a problem for use as a shape-specific scaffold in tissue engineering	Biological adhesives, tissue engineering	Tisseel	35, 259–263
Ceramic					
Hydroxyapatite	$[Ca_{10}(PO_4)_6\text{-}(OH)_2$	Main inorganic compounds of bones and teeth Biodegradable, but the process is very slow Promote osseointegration Used as a component in many composites for bone tissue engineering Because of its fragility, low mechanical strength, easy rupture, and weak fatigue resistance in the humoral surrounding it is being combined with other materials	Widely used in the orthopedic and dental fields as a paste, granules, or porous blocks; bioceramic coatings, bone tissue engineering, long-term drug delivery	Hapex	264–270

it comes to drug delivery, time of release is one of the most important characteristic, which not only determines the type of polymer but also the shape and size of the carrier device [38–40].

Usually, polymers having higher hydrophobicity sustain the release of bioactive for longer times; however, it is not always the requirement since sometimes instant release is required upon triggered by the external stimuli, which may be present at the target site [41–49]. In this type of case, polymers with functional groups are suitable so that targeting moieties can be attached. On the other hand, if the polymer is the only governing factor for the release of bioactives, then predictable release is of considerable importance. If the system releases the active moiety in a zero-order pattern at a predetermined rate, then it is considered ideal in terms of release kinetics from the system [11, 50–52]. Surface-eroding polymers are considered to follow zero-order release kinetics and release rate differs depending on the type of polymer/monomer. However, surface-eroding polymers have very labile links, thus often not a suitable candidate for nanoscopic carriers, and thus polyesters are preferred and used most widely for this purpose [51, 53–55]. Polyesters can sustain the release for a longer time, and moreover they have very well established safety and disposition profiles from a clinical point of view, and this can be well appreciated in a number of products approved by the Food and Drug Administration (FDA) [56–62]. Of the many polyesters poly(lactic-co-glycolic acid) (PLGA) is more widely utilized because of the ratio and molecular weight of the blocks and/or of the whole polymer, which can be varied flexibly to give a wide variety of properties for diverse biomedical applications.

1.3 BIODEGRADATION PATTERNS

Similar to the production and properties of biodegradable polymers equally important is their degradation, and it is of utmost significance when it comes to biomedical applications. The polymer should degrade and/or be disposed off completely in a predictable manner from the body, unless it is to perform some permanent function. The degradation products generated should not cause any adverse effect at the site of use or on any other body organs/functions. Usually, the polymer matrix begins to degrade by hydrolytic and/or enzymatic attack. Each reaction results in the scission of a molecule, slowly reducing the weight of the matrix until the entire material has been digested.

Degradation of the polymer occurs through the process of chain cleavage [271] while erosion is the sum of all processes that lead to the loss of mass from a polyanhydride matrix [272, 273]. Erosion of the polymer matrices depends on processes such as the rate of degradation, swelling, porosity, and ease of diffusion of oligomers and monomers from the matrices [271]. Considering the diffusion of water into the polymer matrix the degradation process can be divided into bulk and surface-eroding polymer (Fig. 1.1). In a bulk erosion process polymer mass is lost uniformly throughout the matrix, and the erosion

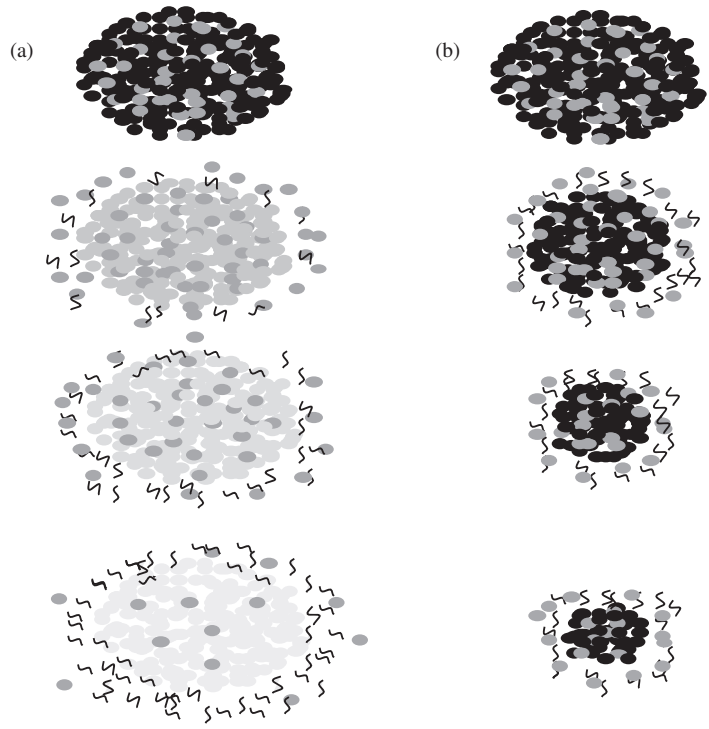


FIGURE 1.1 Erosion patterns in polymeric matrices. (a) Bulk erosion and (b) surface erosion. In bulk erosion, degradation of the matrix occurs throughout the matrix simultaneously with solubilization of oligomers in the surrounding media and drug release occurs by diffusion, which is a concentration-dependent phenomenon. On the other hand, surface erosion occurs from the front of the device, which continuously moves to the core of the device. As these types of polymers are made up of very hydrophobic monomers they do not allow aqueous media to penetrate the core; moreover, the oligomers usually deposit on the device itself and further hinder the release of entrapped bioactives. As a particular volume of the device is exposed to the environment, release occurs in zero-order fashion. These surface-eroding polymers have very labile hydrolytic bonds, thus usually not suitable for sustaining the release in nanoscopic carriers.

rate is dependent on the volume of the polymer rather than its thickness. Consequently, the lifetime of polymer disks of different thicknesses is the same. In contrast, surface-eroding systems display material loss from the outside to the inside of the matrix, so that the erosion rate is dependent on the surface area of the polymer rather than its volume. The lifetime of surface-eroding polymers is dependent on the thickness of the polymer disk, and so thicker samples have a longer lifetime. In the case of controlled drug delivery applications, a surface-eroding device is the better option for drug release [274–276].

In general biodegradation of the polymers is affected by following factors:

- Chemical nature: Type of linkage between the monomers
- Water solubility and permeability of polymer: Hydrophlic/hydrophobic
- Method of chain scission: Hydrolytic/enzymatic
- Mechanism of hydrolysis: Noncatalytic/pH catalytic/autocatalytic
- Water solubility and diffusion of degradation products: Hydrophlic/hydrophobic—slow/fast
- Molecular weight and polydispersity: Low/medium/high
- Molecular level assembly: Crystalline/amorphous
- Glass transition temperature: High/low
- Morphology of device: Size/shape/dimensions/surface-to-volume ratio
- Porosity before and during degradation: Low/medium/high
- Additives: Drug/modifiers/stabilizers
- Method of sterilization: Moist heat/irradiation/other
- Site of application: Local (site)/systemic

All these factors affect the overall degredation rate and lifetime of the polymer. However, the basic governing factor is the chemical nature of the backbone and the hydrophilicity/hydrophobicity of the polymer [277–279]. Polymers that are hydrophilic or not so hydrophobic to diffusion of surrounding aqueous media into the matrix degrade all over the matrix and are called bulk-degrading polymers. Polymers that have very hydrophophobic bone as well as hydrolytically nonlabile bonds are very slow degrading polymers, whereas polymers with hydrophobic monomers but labile linkage, which do not allow water to penetrate, erode from the surface only are called surface-degrading polymers. However, these monomers are so hydrophobic that they do not diffuse away from matrix and keep on depositing on the matrix, thus overall the mass of the device does not reduce significantly. Moreover, they hinder and sustain the diffusion of entrapped bioactive molecules. Natural polymers are usually hydrophilic and undergo bulk degradation [19–21, 35]. Enzymatic degradation is a major contributor in their degradation process, whereas few synthetic polymers undergo enzymatic degradation such as polyesters. Polymers that are substrate for enzymatic degradation are considered better for biomedical applications as they are excreted readily form the body. Hydrophilicity is a major determinant in the time of degradation: the more hydrophilic the polymer, the faster is the degradation when other factors are kept constant. The solubility of the degradation product is another important parameter that governs their removal from the body. Lower molecular weight of the oligomers and their higher aqueous solubility lead to faster degradation and excretion from the body. Some of the oligomers, as in the case of polyanhydrides, are very hydrophobic and deposit on the polymer matrix itself and, in turn, making the degradation slow. Some degradation products have an autocatalytic effect on

the chain scission [280–284]. The catalytic effect is usually because of a change in pH by degradation products in microenvironments, which can cause either acid or base catalysis. Free radical generation during degradation also catalyzes the chain scission in some polymers. Solubility of the degradation product in changed pH can also change the course of degradation as their diffusion away from the matrix is increased. Increase in the molecular weight is usually proportional, though not directly, to the degradation time [285–290]. It is just not because the number of bonds to be broken are more; it also increases the hydrophobicity of the device and makes hydrolysis proportionally slower.

Increase in molecular weight distribution on the other hand usually increases degradation rate as there are more free groups for the chain scission reaction, and if the free groups are hydrophilic, then they ingress water more readily into the device, which thus increases degradation. Only a few polymers occur in completely crystalline form, and they have long-range order in their molecular arrangement, thus making penetration of the water difficult. Because of higher lattice energy, they degrade slowly as compared to their amorphous counterparts. Semicrystalline polymers have an intermediate degradation period, depending on the degree of crystallinity [291–297]. Among amorphous polymers, the glass transition temperature (T_g) is the factor that dictates the degradation behavior. Polymers with higher T_g, in the same class, usually have a higher molecular weight, thus naturally will require a longer time to degrade. On the other hand, high T_g also means that the polymer is stable in that particular molecular configuration, which may be because of forces such as hydrogen bonding and hydrophobic interaction, in turn making polymer degradation slower. Crystallinity and T_g also determine the polymer fragmentation and crumpling of the polymer device, thus affecting degradation. As the size of the device increases, the time required for complete degradation of the device also increases, but the effect is more pronounced in the case of a surface-degrading polymer as compared to a bulk-degrading polymer where degradation takes place throughout the matrix.

Shapes or factors that make surface-to-volume ratios high also have varied effects on polymers with different degradation patterns, as an increase in surface area exposes more surface to the hydrolytic media and thus enhances the degradation process more in the case of heterogonous degradation [53, 298–301]. Porosity is one of the factors that increase the surface-to-volume ratio, thus affecting the degradation similarly. For formulating particular devices, additives are included in the polymer, and it also undergoes some processes to make the device suitable for application. Additives that include a drug can make the polymer more hydrophobic or more hydrophilic and can change the degradation profile accordingly. In practice, two or more polymers are chemically linked (copolymer) or blended to achieve particular degradation or some other derived properties. Processing such as application of heat, pressure, and sterilization can modify the polymer's physical and more importantly chemical properties to further affect the degradation [302–306].

Generally, all these stresses lead to a decrease in molecular weight, but, occasionally, it can also give rise to a phenomenon such as crosslinking, and may thereby hamper the degradation rate. Finally, location or the site of application in the body of the polymer also affects its degradation by secondary effects such as blood flow, movement of the device, load on the device, hardness of the tissue, and the like. If the device is in an environment that causes faster removal of degradation product, it can cause an increased degradation rate in the case of surface-degrading polymers. On the other hand, a balance effect can be in the case of bulk-degrading polymers as autocatalysis will be reduced at one hand but better sink conditions for degredants on the other hand, which reduces matrix mass will be faster. Conditions that subject the polymer to pH conditions and enzymes favorable to the degradation, then naturally polymer disappearance from the body will be faster, and such a case, for example, could be the presentation of large nanoparticles made up of polyesters in a liver microtonal condition.

1.4 OVERVIEW OF DIFFERENT PRODUCTS BASED ON BIODEGRADABLE POLYMERS

Over the past four decades controlled-release polymer technology has impacted virtually every branch of medicine, including oncology, ophthalmology, pulmonary, pain medicine, endocrinology, cardiology, orthopedics, immunology, neurology, and dentistry, with several examples of these systems in clinical practice today (Table 1.2). Several controlled-release formulations based on biodegradable polymers have been approved and marketed where the polymer matrix can be formulated as microspheres, nanospheres, injectable gel, or implant.

1.5 POLYMER SELECTION FOR BIOMEDICAL APPLICATION

Polymers, both synthetic and those derived from a natural origin, are a promising class of biomaterials that can be engineered to meet specific end-use requirements if proper selection is made based on their biomedical application. Polymers can be selected according to key device characteristics, such as mechanical resistance, degradability, permeability, solubility, and transparency, in which all can influence manufacturing characteristics and performance of device. Moreover, it requires a thorough understanding of the surface and bulk properties of the polymer that can give the desired chemical, interfacial, mechanical, and biological functions. The choice of polymer in addition to its physicochemical properties is dependent on the need for the extensive biomedical characterization and specific preclinical tests to prove its safety. Hence, its selection must be carefully tailored in order to provide the combination of chemical, interfacial, mechanical, and biological functions necessary for the manufacturing of biomaterials.

TABLE 1.2 Marketed Controlled-Release Formulations Based on Biodegradable Polymers

Product	Drug	Polymer	Delivery System Description	Application	Company	Reference
Atridox	Doxycycline hyclate	PLA	A subgingival controlled-release gel composed of a 2-syringe mixing system. Syringe A contains 450 mg of the ATRIGEL Delivery System, which is a bioabsorbable, flowable polymeric formulation composed of 36.7% poly(*d*,*l*-lactide) (PLA) dissolved in 63.3% *N*-ethyl-2-pyrrolidone (NMP). Syringe B contains 50 mg doxycycline hyclate which is equivalent to 42.5 mg doxycycline making 10% of doxycycline hyclate. Upon contact with the crevicular fluid, the liquid product solidifies and then allows for controlled release of drug for a period of 7 days. Duration: 4 months	Periodontal disease	Collagenex pharmaceuticals	307–309
Zoladex	Goserelin acetate	PLGA	A depot formulation of goserelin acetate dispersed in a cylindrical rod of biodegradable and biocompatible *d*,*l*-lactide–glycolide (PLGA) copolymers. Goserelin acetate (equivalent to 3.6 or 10.8 mg of goserelin) is dispersed in a matrix of PLGA acids copolymer to a total weight of 18 and 36.0 mg per depot, for subcutaneous injection with continuous release over 1- or 3-month periods, respectively. The encapsulated drug is released by a combination of diffusion and erosion-controlled mechanisms. However, because the delivery device is a monolithic, heterogeneous hydrolysis is thought to be the predominant erosion process. Duration: 1 and 3 months	Prostate cancer, endometrioses	AstraZeneca/Promed/ Pharma-stern	310–313

Lupron Depot	Leuprolide acetate; when used, body stops producing testosterone hormones in males and estrogen hormones in females	PLGA	A microsphere formulation based on the biodegradable polymer of PLA and PLGA. Available in prefilled dual chamber of which the front chamber contains leuprolide acetate (22.5 mg), polylactic acid (198.6 mg), and D-mannitol (38.9 mg). The second chamber of diluent contains carboxymethylcellulose sodium (7.5 mg), D-mannitol (75.0 mg), polysorbate 80 (1.5 mg), water for injection, USP, and glacial acetic acid, USP, to control pH and intended as intramuscular injection once in every 3 months. Other products include Enantone, Porcin as microsphere formulations manufactured by Takeda and Abbott, respectively. Duration: 3 months	Prostate cancer	Abbott and Takeda laboratories Joint venture (TAP)	314–316
Trenantone	Leuprolin acetate	PLA	Microspheres		Takeda	317, 318
Eligard	Leuprolide acetate	PLGA	Eligard is a sterile polymeric matrix formulation of leuprolide acetate for subcutaneous injection. It is designed to deliver leuprolide acetate at a controlled rate over a 1-, 3-, 4-, or 6-month therapeutic period containing 7.5, 22.5, 30, and 45 mg of Leuprolide equivalent, respectively. The polymeric system is the ATRIGEL Delivery System consisting of a biodegradable copolymer (PLGA in the ratio of 50 : 50, 75 : 25, 75 : 25, 85 : 15, repectively) formulation dissolved in a biocompatible solvent, N-methyl-2-pyrrolidone (NMP). The polymer delivered is 82.5, 158.2, 211.5, and 265 mg, respectively. It is administered	Prostate cancer, breast cancer, endometroses	Tolmar, Inc., for QLT, USA, Inc. and distributed by Sanofiaventis U.S. http://www.eligard.com	319–321

(*Continued*)

TABLE 1.2 (Continued)

Product	Drug	Polymer	Delivery System Description	Application	Company	Reference
			subcutaneously, where it forms a solid drug delivery depot.			
Sandostatin LAR	Octreotide acetate	PLGA-glucose	A long-acting dosage form consisting of microspheres of the biodegradable PLGA-glucose co-polymer containing octreotide. It maintains all of the clinical and pharmacological characteristics of the immediate-release dosage form octreotide acetate (Sandostatin LAR) injection with the added feature of slow release of octreotide from the site of injection, reducing the need for frequent administration. Duration: 1 month	Growth hormone suppression	Novartis Pharmaceuticals Corporation	322–325
Nutropin Depot	Somatropin (hGH)	PLGA	The Nutropin Depot formulation consists of micronized particles of recombinant human growth hormone (rhGH) embedded in biocompatible, biodegradable PLGA microspheres. Nutropin Depot is packaged in vials as a sterile, white to off-white, preservative-free, free-flowing powder. Before administration, the powder is suspended in diluent for Nutropin Depot (a sterile aqueous solution). Available strengths: Nutropin Depot is supplied as single-use vials with 13.5, 18, or 22.5 mg sterile, preservative-free somatropin powder per vial. • Each 13.5-mg 3-mL single-use vial of Nutropin Depot contains 13.5 mg somatropin, 1.2 mg zinc acetate, 0.8 mg zinc carbonate, and 68.9 mg PLGA.	Growth hormone deficiency	Genentech, Inc.	326, 327

			• Each 18-mg 3-mL single-use vial of Nutropin Depot contains 18 mg somatropin, 1.6 mg zinc acetate, 1.1 mg zinc carbonate, and 91.8 mg PLGA. • Each 22.5-mg 3-mL single-use vial of Nutropin Depot contains 22.5 mg somatropin, 2.0 mg zinc acetate, 1.4 mg zinc carbonate, and 114.8 mg PLGA.			
Gliadel	Carmustin	Polyanhydride (Polifeprosan)	Gliadel Wafer (polifeprosan 20 with carmustine implant) is a sterile, off-white to pale yellow wafer approximately 1.45 cm in diameter and 1 mm thick designed to deliver carmustine directly into the surgical cavity created when a branin tumor is resected. Each wafer contains 192.3 mg of a biodegradable polyanhydride copolymer and 7.7 mg of carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea, or BCNU]. Carmustine is a nitrosourea oncolytic agent. The copolymer, polifeprosan 20, consists of poly[bis(p-carboxyphenoxy) propane : sebacic acid] in a 20 : 80 molar ratio and is used to control the local delivery of carmustine. Carmustine is homogeneously distributed in the copolymer matrix. Immediately after a neurosurgeon operates to remove the high-grade malignant glioma, up to eight wafers are implanted along the walls and floor of the cavity that the tumor once occupied. Each wafer contains a precise amount of carmustine that dissolves slowly, delivering carmustine to the surrounding cells. Duration: 1 month	Brain cancer along with surgery and irradiation therapy	Guilford Pharmaceuticals Inc.	54, 328–332

(*Continued*)

TABLE 1.2 (Continued)

Product	Drug	Polymer	Delivery System Description	Application	Company	Reference
Septacin	Gentamicin sulfate	Polyanhydride [Erucic acid dimer (EAD) and sebacic acid in a 1 : 1 weight ratio].	Septacin is a sustained-release formulation consisting of gentamicin sulfate dispersed in a biodegradable polyanhydride matrix. The polyanhydride matrix is a copolymer of erucic acid dimer (EAD) and sebacic acid in a 1 : 1 weight ratio. The drug loading is 20% (w/w) gentamicin sulfate. Sebacic acid is the more hydrophilic monomer while EAD is the more hydrophobic component. Drug release can be tailored by varying the ratio of the two monomers. After incorporation of gentamicin sulfate in the copolymer by melt mixing, the drug polymer blend is injected and molded into a bead (12 mm × 3 mm) form that is suitable for use. Septacin is designed to be implanted at the surgical site when a hip or knee prosthesis is replaced as a result of infection. The beads slowly release gentamicin as the polymer degrades. This provides a relatively high local concentration of drug while minimizing systemic exposure.	Osteomyelitis, a deep bone infection that can occur after hip or knee replacement surgery		54, 333
Risperdal Consta	Risperidone	PLGA	The extended-release microsphere formulation is a white to off-white, free-flowing powder that is available in dosage strengths of 12.5, 25, 37.5, or 50 mg risperidone per vial. Risperidone is microencapsulated in 75 : 25 PLGA at a	Antipsychotic agent	Janssen-Cilag pharmaceuticals (J and J)	334–336

			concentration of 381 mg risperidone per gram of microspheres. Duration: 2 weeks			
Arestin	Minocycline hydrochloride	PLGA	It consists of minocycline hydrochloride encapsulated in a bioresorbable polymer of PLGA. It is supplied in a unit dose dispenser as subgingival administration into periodontal pockets that delivers 1 mg of minocycline. Exposure to moisture in the periodontal pocket triggers hydrolysis of the polymer and release the active ingredient. It is a sustained-release product.	Adult periodontitis	OraPharma Inc.	337, 338
Capronor	Levonoregesrel	Polycaprolactone (PCL)	Capronor is a biodegradable polymer reservoir system for the sustained subdermal delivery of contraceptive steroids. It is a hollow capsules of poly(ε-caprolactone) containing steroid in ethyl oleate (suspension vehicle). Capsule ends are heat-sealed reservoir-type devices. It is an implantable biodegradable contraceptive. It is implanted under skin. It dissolves in the body and does not require removal. Degradation of the poly (ε-caprolactone) matrix occurs through bulk hydrolysis of ester linkages, which is autocatalyzed by the carboxylic acid end groups of the polymer, eventually forming carbon dioxide and water. Capronor II consists of 2 rods of poly(ε-caprolactone) each containing 18 mg of levonorgestrel. Capronor III is a single capsule of copolymer (ε-caprolactone and trimethylenecarbonate) filled with 32 mg of levonorgestrel. For both systems, the implant remains intact during the first year of use, thus could be removed if needed. Over the second year, it	Contraceptive		339–341

(*Continued*)

TABLE 1.2 (Continued)

Product	Drug	Polymer	Delivery System Description	Application	Company	Reference
			biodegrades to carbon dioxide and water, which are absorbed by the body. Duration: Degradable polymer over 18–24 months			
H.P. Acthar Gel	Adrenocorticotropic hormone	Gelatin	Highly purified sterile preparation of the adrenocorticotropic hormone in 16% gelatin to provide a prolonged release after intramuscular or subcutaneous injection. Also contains 0.5% phenol, not more than 0.1% cysteine (added), sodium hydroxide, and/or acetic acid to adjust pH and water for injection, quantity sufficient (q.s.) Acthar Gel is 40–80 units given intramuscularly or subcutaneously every 24–72 h. In the treatment of acute exacerbations of multiple sclerosis daily intramuscular doses of 80–120 units for 2–3 weeks may be administered.	Endocrine disorders, nervous system disease, rheumatic disorder, collagen disease, etc.	Questcor Pharmaceuticals, Inc. **U.S. Pat. No. 2,992,165**	342, 343
Genexol-PM	Paclitaxel	PEG-PLA	Genexol-PM is a polymeric micelle formulated paclitaxel, free of Cremophor EL. Objectives with Genexol-PM are to reduce Cremophor-EL-related toxicities and to increase therapeutic efficacy. Polymeric micelles are composed of hundreds of amphiphilic diblock copolymers. Block copolymers include poly-(ethylene glycol) used as nonimmunogenic carriers and biodegradable core-forming poly(D,L-lactic acid) to solubilize hydrophobic drug [mPEG-PLA]. One vial of Genexol-PM contains 30 mg of paclitaxel and	Cancer (breast, lung, prostate, and pancreatic)	Samyang	344–347

			150 mg of mPEG-PLA as a solubilizer. Genexol-PM has been marketed in Korea in 2007 and started to compete with Abraxane in the cremophore-free paclitaxel market.			
Abraxane	Paclitaxel	Albumin	Abraxane (injectable suspension of paclitaxel protein-bound particles) is an albumin-bound form of paclitaxel with a mean particle size of approximately 130 nm. Abraxane is supplied as a white to yellow, sterile, lyophilized powder for reconstitution with 20 mL of 0.9% sodium chloride injection, USP, prior to intravenous infusion. Each single-use vial contains 100 mg of paclitaxel and approximately 900 mg of human albumin. Each milliliter of reconstituted suspension contains 5 mg paclitaxel. Abraxane is free of solvents. The suspension consists only of albumin-bound paclitaxel nanoparticles, eliminating the need for standard steroid or antihistamine premedication to prevent solvent-based hypersensitivity reactions. As a result of lowered toxicity, higher doses of paclitaxel may be administered, for example, up to 300 mg/m^2.	Cancer (breast, lung, prostate, and pancreatic)	Bristol-Myers Squibb (BMS)	241, 348–352
Nanoxel	Paclitaxel	VP, NIPAM, functionalized PEG	Cremophor-free paclitaxel formulation in which the drug is delivered as nanomicelles using a polymeric carrier made of a pH-sensitive copolymer of N-isopropyl acrylamide (NIPAM) and vinylpyrrolidone (VP) monomers.	Cancer (breast, lung, prostate, and pancreatic)	Dabur Pharma	353, 354

(*Continued*)

TABLE 1.2 (Continued)

Product	Drug	Polymer	Delivery System Description	Application	Company	Reference
Decapeptyl, Diphereline, and Pamorelin LA	Triptorelin acetate that acts by lowering the level of testosterone in the body	PLGA	Microsphere formulation and available in 1, 3, and 6 month periods for advanced prostate cancer	Advanced and metastatic prostate cancer	Ferring, IPSEN Pharma	355–359
Vivitrol	Naltrexone	PLGA	Vivitrol (naltrexone for extended-release injectable suspension) is supplied as a microsphere formulation of naltrexone for suspension, to be administered by intramuscular injection. Naltrexone is an opioid antagonist with little, if any, opioid agonist activity. Vivitrol microspheres consist of a sterile, off-white to light-tan powder that is available in a dosage strength of 380 mg naltrexone per vial. Naltrexone is incorporated in 75 : 25 PLGA at a concentration of 337 mg of naltrexone per gram of microspheres. The diluent is a clear, colorless solution. The composition of the diluent includes carboxymethylcellulose sodium salt, polysorbate 20, sodium chloride, and water for injection. The microspheres must be suspended in the diluent prior to injection. The recommended dose of Vivitrol is 380 mg delivered intramuscularly every 4 weeks or once a month. It is supplied as Vivitrol (naltrexone for extended-release	Alcohol dependence	Cephalon	360–362

			injectable suspension) in single-use cartons. Duration: 1 month			
Profact Depot	Buserelin	PLGA	Implant		Sanofi-Aventis Pharma	363–367
Plenaxis	Abarelix	PLGA	Plenaxis is a synthetic decapeptide with potent antagonistic activity against naturally occurring gonadotropin releasing-hormone (GnRH). It is initially manufactured as an acetate water complex and converted to a carboxymethylcellulose (CMC) water complex in manufacturing the drug product. The single-dose vial contains 113 mg of anhydrous free base abarelix peptide supplied in an abarelix CMC complex. This complex also contains 19.1–31 mg of CMC. After the vial is reconstituted with 2.2 mL of 0.9% sodium chloride injection, 2 mL is administered to deliver a dose of 100 mg of abarelix as the abarelix CMC complex at a pH of 5±1.	Prostate cancer	Praecis	368–370

In general, there are varieties of polymer attributes to be considered when selecting a biodegradable polymer for biomedical application [371–373]:

Regulatory and Toxicology Status. One of the most critical considerations is the regulatory requirement for a particular application. If an application requires a rapid development and commercialization, then the polymer selection will most likely be made from among those that have already received regulatory approval, for instance, polyesters.

Polymer (Monomer or Copolymer) Composition. Whether to use homopolymers consisting of a single monomeric repeat unit or copolymers containing multiple monomer species has to be considered before a decision is made of which polymer to be used. If copolymers are to be used, then the relative ratio of the different monomers may be manipulated to change polymer physicochemical properties including bulk hydrophilicity, morphology, structure, and the extent of drug–polymer interactions (e.g., drug solubility in the polymer). Ultimately, these properties will all influence the performance of the drug delivery system, for instance, via changes to the relative rates of mass transport and the degradation rate of both the polymer and the device.

Thermal Properties. The thermal attributes of the polymer, as described by the glass transition temperature (T_g) and the melting temperature (T_m), can also affect the mass transport rates through the polymer as well as the polymer processing characteristics and the stability of the device at the end. Below the glass transition temperature, the polymer will exist in an amorphous, glassy state. When exposed to temperatures, above T_g, the polymer will experience an increase in free volume that permits greater local segmental chain mobility along the polymer backbone. Consequently, the mass transport through the polymer is faster at temperatures above T_g. Often, the polymer processing, such as extrusion or high shear mixing, is performed above T_g. On the other hand, the greatest stability during the storage of a polymer device may be obtained at temperatures below T_g, where solute diffusion is much slower and more subtle changes in polymer properties are reduced.

Ionization. The presence of charged groups on a polymer can also influence the physicochemical properties of the polymer, the device, and the drug release pattern from the device. The number and density of ionized groups along the polymer backbone, on the side-chain groups, or at the terminal end groups of the polymer chains can all vary the extent of polymer–polymer and polymer–drug interactions. As drug delivery systems, the polymer properties can affect the performance of the drug delivery system as ionizable groups can affect drug solubility in the polymer and, correspondingly, the release rate from the polymer.

Molecular Weight and Molecular Weight Distribution. Molecular weight of a monodisperse or polydisperse polymer is expressed in terms of its relative

molar mass, which is related to the degree of polymerization and relative molecular mass of the repeat unit. The properties that have enabled polymers to be used in a diversity of biomedical applications derive almost entirely from their long-chain macromolecular nature. We are concerned about molecular weight and its distribution in polymer selection because many physicochemical properties of polymers are influenced by the length of the polymer chain, including viscosity, the glass transition temperature, mechanical strength, and the like and, consequently, the use of polymers in various biomedical applications will be affected.

Molecular Architecture. An important microstructural feature determining polymer properties is the polymer architecture. Molecular architecture of polymers can be described as linear polymers, branched polymers, crosslinked network polymers, and the like. The simplest polymer architecture is a linear chain: a single backbone without branches. A related unbranching architecture is a ring polymer. A branched polymer is composed of a main chain with one or more short or long substituent side chains or branches. Special types of branched polymers include star polymers, comb polymers, brush polymers, ladders, and dendrimers among others. Branching of polymer chains affects the ability of chains to slide past one another by altering intermolecular forces, in turn affecting bulk physical properties of polymers. Long-chain branches may increase polymer strength, toughness, and the glass transition temperature due to an increase in the number of entanglements per chain. Different representative polymer architectures are shown in Figure 1.2.

Polymer Morphology. Polymer morphology generally refers to the arrangement of chains in space and the microscopic ordering of many polymer chains and described as amorphous, semicrystalline, and crystalline structures that can affect the manufacturing characteristics and performance. There are some polymers that are completely amorphous, although the morphology of most polymers is semicrystalline. That is, they form mixtures of small crystals and

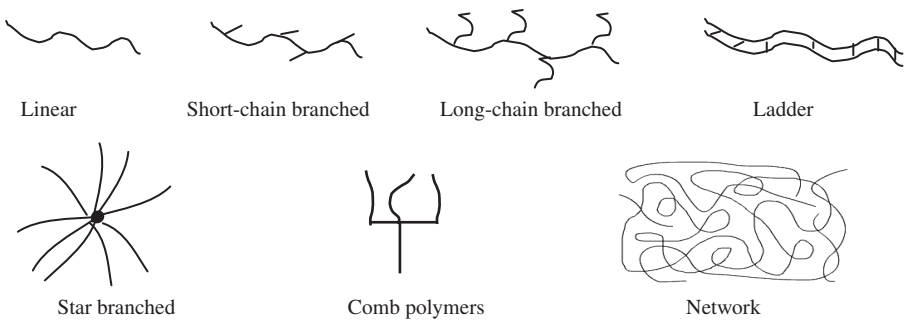
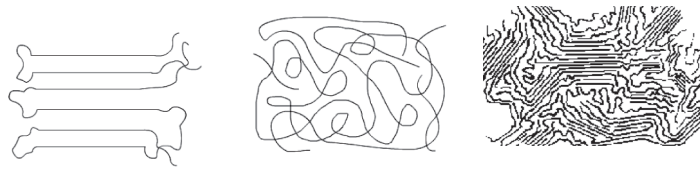


FIGURE 1.2 Types of molecular architectures of polymers.



(a) Crystalline (b) Amorphous (c) Semicrystalline

FIGURE 1.3 Types of molecular arrangement of polymeric chains in crystal lattice.

are amorphous in combination with the tangled and disordered surrounding crystalline material and melt over a range of temperatures instead of at a single melting point. In most polymers the combination of crystalline and amorphous structures forms a material with advantageous properties of strength and stiffness so that manufacturing characteristics and performance can be tailored as desired. Different polymer morphologies are shown in Figure 1.3.

In general, when the polymer selection is made, it has to fulfill the following end-use requirements:

- The mechanical properties must match the application. For instance, in tissue engineering application, it should remain sufficiently strong until the surrounding tissue has healed.
- The degradation time must match the time required for biomedical application.
- It does not invoke a toxic response upon in vivo degradation.
- It is metabolized in the body after fulfilling its purpose into nontoxic constituents that can be easily eliminated.
- It is easily processable in the final product form with an acceptable shelf life and easily sterilized.

1.6 FUTURE PROSPECTS

Biodegradable materials are highly desired for most biomedical applications in vivo, such as transient implants, drug delivery carriers, and tissue engineering scaffolds. Biodegradable polymers remain the most versatile and promising class of biomaterials that can be engineered to meet specific end-use requirements in biomedical application. Given the importance of biodegradable polymers in the various biomedical applications, the currently available polymers need to be further improved by altering their surface and bulk properties in order to provide the desired functions necessary for manufacturing of new and improved biomaterials, for instance, the generation of stimuli-responsive polymeric biodegradable materials. Stimuli-responsive biomaterials resembling natural living tissues that undergo changes in physicochemical properties in response to a variety of physical, chemical, and biological stimuli are attracting increasing

interest because of their potential application in biomedical fields. Hence, biomedical systems that are both biodegradable and stimuli responsive have therefore been studied intensively and significant progress in this field has been achieved. Although biodegradable stimuli-responsive materials are highly attractive for biomedical applications, most such materials are currently at a developmental research stage. Additionally, single stimulus-responsive property limits the practical applications of these materials. To achieve more favorable applications for these materials, further efforts are still necessary, especially for developing multi-stimuli-responsive functions of materials and improving the stimuli-responsive properties of such materials in a biological environment. Bearing in mind the great prospect of these biodegradable stimuli-responsive materials, there is great hope in the future for the development of stimuli-responsive polymers or systems that could be reliably employed in biomedical applications for further clinical practices.

REFERENCES

1. Gunatillake, P., R. Mayadunne, and R. Adhikari. Recent developments in biodegradable synthetic polymers. *Biotechnol. Annu. Rev.* 2006;**12**:301–347.
2. Pachence, J. M., M. P. Bohrer, J. Kohn, L. Robert, and V. Joseph. *Biodegradable polymers*. In *Principles of Tissue Engineering*, 3rd ed. Academic: Burlington, 2007, pp. 323–339.
3. Scott, G. "Green" polymers. Polym. Degrad. Stab. 2000;**68**(1):1–7.
4. Tuzlakoglu, K. and R. L. Reis. Biodegradable polymeric fiber structures in tissue engineering. *Tissue Eng. Part B Rev.* 2009;**15**(1):17–27.
5. Chiellini, F., A. M. Piras, C. Errico, and E. Chiellini. Micro/nanostructured polymeric systems for biomedical and pharmaceutical applications. *Nanomed*, 2008;**3**(3):367–393.
6. Heller, J. Biodegradable polymers in controlled drug delivery. *Crit. Rev. Ther. Drug Carrier Syst.* 1984;**1**(1):39–90.
7. Ratcliffe, A. Tissue engineering of vascular grafts. *Matrix Biol.* 2000;**19**(4):353–357.
8. Ju, X. J., R. Xie, L. Yang, and L. Y. Chu. Biodegradable "intelligent" materials in response to physical stimuli for biomedical applications. *Expert Opin. Ther. Pat.* 2009;**19**(4):493–507.
9. Gunja, N. J. and K. A. Athanasiou. Biodegradable materials in arthroscopy. *Sports Med. Arthrosc.* 2006;**14**(3):112–119.
10. Chitkara, D., A. Shikanov, N. Kumar, and A. J. Domb. Biodegradable injectable in situ depot-forming drug delivery systems. *Macromol. Biosci.* 2006;**6**(12):977–990.
11. Commandeur, S., H. M. van Beusekom, and W. J. van der Giessen. Polymers, drug release, and drug-eluting stents. *J. Interv. Cardiol.* 2006;**19**(6):500–506.
12. Friedman, J. A., A. J. Windebank, M. J. Moore, R. J. Spinner, B. L. Currier, and M. J. Yaszemski. Biodegradable polymer grafts for surgical repair of the injured spinal cord. *Neurosurgery* 2002;**51**(3):742–751; discussion 751–752.

13. Piskin, E. Biodegradable polymeric matrices for bioartificial implants. *Int. J. Artif. Organs* 2002;**25**(5):434–440.
14. Gaspar, R. S. and R. Duncan. Polymeric carriers: Preclinical safety and the regulatory implications for design and development of polymer therapeutics. *Adv. Drug Deliv. Rev.* 2009;**61**(13):1220–1231.
15. Kiick, K. L. Materials science. Polymer therapeutics. *Science* 2007;**317**(5842):1182–1183.
16. Benagiano, G., H. Gabelnick, and M. Farris. Contraceptive devices: Subcutaneous delivery systems. *Expert Rev. Med. Devices* 2008;**5**(5):623–637.
17. Dash, A. K. and G. C. Cudworth II. Therapeutic applications of implantable drug delivery systems. *J. Pharmacol. Toxicol. Methods.* 1998;**40**(1):1–12.
18. Nair, L. S. and C. T. Laurencin. Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* 2007;**32**(8–9):762–798.
19. Malafaya, P. B., G. A. Silva, and R. L. Reis. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):207–233.
20. Dang, J. M. and K. W. Leong. Natural polymers for gene delivery and tissue engineering. *Adv. Drug Deliv. Rev.* 2006;**58**(4):487–499.
21. Stacey, M. In *Natural & Synthetic Polymers an Introduction*, Vol. 17. H. I. Bolker (ed.). Marcel Dekker: New York, 1976, pp. 456–456.
22. Reinert, K. H., J. P. Carbone, J. Sven Erik, and F. Brian. Synthetic polymers. In *Encyclopedia of Ecology*. Academic: Oxford, 2008, pp. 3461–3472.
23. Thompson, H. Biomaterials: We have the technology. 2005 (cited September 2009); available from: http://www.devicelink.com/mddi/archive/05/05/024.html.
24. Schlechter, M. Biodegradable polymers (PLS025B). 2005 (cited September 2009); available from: http://www.bccresearch.com/report/PLS025B.html.
25. Navarro, M., A. Michiardi, O. Castano, and J. A. Planell. Biomaterials in orthopaedics. *J. R. Soc. Interface* 2008;**5**(27):1137–1158.
26. Holland, T. A. and A. G. Mikos. Biodegradable polymeric scaffolds. Improvements in bone tissue engineering through controlled drug delivery. *Adv. Biochem. Eng. Biotechnol.* 2006;**102**:161–185.
27. Liu, X. and P. X. Ma. Polymeric scaffolds for bone tissue engineering. *Ann. Biomed. Eng.* 2004;**32**(3):477–486.
28. Borenstein, J. T., G. Yogesh, T. Osamu, and Z. Hans. *Tissue engineering*. In *Comprehensive Microsystems*. 2008, Elsevier: Oxford, 2008, pp. 541–583.
29. Carlos Rodríguez-Cabello, J., J. Reguera, A. Girotti, M. Alonso, and A. M. Testera. Developing functionality in elastin-like polymers by increasing their molecular complexity: The power of the genetic engineering approach. *Prog. Polym. Sci.* 2005;**30**(11):1119–1145.
30. El-Amin, S. F., H. H. Lu, Y. Khan, J. Burems, J. Mitchell, R. S. Tuan, and C. T. Laurencin. Extracellular matrix production by human osteoblasts cultured on biodegradable polymers applicable for tissue engineering. *Biomaterials* 2003;**24**(7): 1213–1221.
31. Ma, P. X. Biomimetic materials for tissue engineering. *Adv. Drug Deliv. Rev.* 2008;**60**(2):184–198.
32. Pêgo, A. P., A. A. Poot, D. W. Grijpma, and J. Feijen. Biodegradable elastomeric scaffolds for soft tissue engineering. *J. Control. Release* 2003;**87**(1–3):69–79.

33. Sheridan, M. H., L. D. Shea, M. C. Peters, and D. J. Mooney. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. *J. Control. Release* 2000;**64**(1–3):91–102.

34. van Dijkhuizen-Radersma, R., L. Moroni, A. v. Apeldoorn, Z. Zhang, D. Grijpma, B. Clemens van, T. Peter, L. Anders, H. Jeffrey, F. W. David, C. Ranieri, D. d. B. Joost, and S. Jérôme. Degradable polymers for tissue engineering. In *Tissue Engineering*. Academic: Burlington, 2008, pp. 193–221.

35. Gomes, M., H. Azevedo, P. Malafaya, S. Silva, J. Oliveira, G. Silva, R. Sousa, J. Mano, R. Reis, B. Clemens van, T. Peter, L. Anders, H. Jeffrey, F. W. David, C. Ranieri, D. d. B. Joost, and S. Jérôme. Natural polymers in tissue engineering applications. In *Tissue Engineering*. Academic: Burlington, 2008, pp. 145–192.

36. Lee, S.-H. and H. Shin. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):339–359.

37. Malafaya, P. B., G. A. Silva, and R. L. Reis. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):207–233.

38. Deng, J. S., L. Li, Y. Tian, M. Meisters, H. C. Chang, D. Stephens, S. Chen, and D. Robinson. The relationship between structures and in vitro properties of a polyanhydride implant containing gentamicin sulfate. *Pharm. Dev. Technol.* 2001;**6**(4):541–549.

39. Dittrich, M., P. Solich, L. Opletal, A. J. Hunt, and J. D. Smart. 20-Hydroxyecdysone release from biodegradable devices: The effect of size and shape. *Drug Dev. Ind. Pharm.* 2000;**26**(12):1285–1291.

40. Park, E.-S., M. Maniar, and J. Shah. Water uptake in to polyanhydride devices: Kinetics of uptake and effects of model compounds incorporated, and device geometry on water uptake. *J. Control. Release* 1996;**40**(1–2):55–65.

41. Ju, X. J., R. Xie, L. Yang, and L. Y. Chu. Biodegradable "intelligent" materials in response to chemical stimuli for biomedical applications. *Expert Opin. Ther. Pat.* 2009;**19**(5):683–696.

42. Bawa, P., V. Pillay, Y. E. Choonara, and L. C. du Toit. Stimuli-responsive polymers and their applications in drug delivery. *Biomed. Mater.* 2009;**4**(2):22001.

43. Meng, F., Z. Zhong, and J. Feijen, Stimuli-responsive polymersomes for programmed drug delivery. *Biomacromolecules* 2009;**10**(2):197–209.

44. Onaca, O., R. Enea, D. W. Hughes, and W. Meier. Stimuli-responsive polymersomes as nanocarriers for drug and gene delivery. *Macromol. Biosci.* 2009;**9**(2):129–139.

45. Mendes, P. M. Stimuli-responsive surfaces for bio-applications. *Chem. Soc. Rev.* 2008;**37**(11):2512–2529.

46. Stoop, R. Smart biomaterials for tissue engineering of cartilage. *Injury* 2008;**39**(Suppl 1):S77–87.

47. Dayananda, K., C. He, D. K. Park, T. G. Park, and D. S. Lee. pH- and temperature-sensitive multiblock copolymer hydrogels composed of poly(ethylene glycol) and poly(amino urethane). *Polymer* 2008;**49**(23):4968–4973.

48. Qiu, Y. and K. Park. Environment-sensitive hydrogels for drug delivery. *Adv. Drug Deliv. Rev.* 2001;**53**(3):321–339.

49. Sershen, S. and J. West, Implantable, polymeric systems for modulated drug delivery. *Adv. Drug Deliv. Rev.* 2002;**54**(9):1225–1235.

50. Iconomopoulou, S. M., J. K. Kallitsis, and G. A. Voyiatzis. Incorporation of small molecular weight active agents into polymeric components. *Recent Pat. Drug Deliv. Formul.* 2008;**2**(2):94–107.
51. Jain, J. P., S. Modi, A. J. Domb, and N. Kumar. Role of polyanhydrides as localized drug carriers. *J. Control. Release* 2005;**103**(3):541–563.
52. Park, J. H., M. Ye, and K. Park. Biodegradable polymers for microencapsulation of drugs. *Molecules* 2005;**10**(1):146–161.
53. Gopferich, A. and J. Tessmar. Polyanhydride degradation and erosion. *Adv. Drug Deliv. Rev.* 2002;**54**(7):911–931.
54. Jain, J. P., D. Chitkara, and N. Kumar. Polyanhydrides as localized drug delivery carrier: An update. *Expert Opin. Drug Deliv.* 2008;**5**(8):889–907.
55. Tamada, J. and R. Langer. The development of polyanhydrides for drug delivery applications. *J. Biomater. Sci. Polym. Ed.* 1992;**3**(4):315–353.
56. Bala, I., S. Hariharan, and M. N. Kumar. PLGA nanoparticles in drug delivery: The state of the art. *Crit. Rev. Ther. Drug Carrier Syst.* 2004;**21**(5): 387–422.
57. Campolongo, M. J. and D. Luo. Drug delivery: Old polymer learns new tracts. *Nat. Mater.* 2009;**8**(6):447–448.
58. Dhiman, N., M. Dutta, and G. K. Khuller. Poly (DL-lactide-co-glycolide) based delivery systems for vaccines and drugs. *Indian J. Exp. Biol.* 2000;**38**(8):746–752.
59. Jain, R., N. H. Shah, A. W. Malick, and C. T. Rhodes. Controlled drug delivery by biodegradable poly(ester) devices: Different preparative approaches. *Drug Dev. Ind. Pharm.* 1998;**24**(8):703–727.
60. Jain, R. A. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* 2000;**21**(23):2475–2490.
61. Mundargi, R. C., V. R. Babu, V. Rangaswamy, P. Patel, and T. M. Aminabhavi. Nano/micro technologies for delivering macromolecular therapeutics using poly(D, L-lactide-co-glycolide) and its derivatives. *J. Control. Release* 2008;**125**(3):193–209.
62. Vert, M. Aliphatic polyesters: Great degradable polymers that cannot do everything. *Biomacromolecules* 2005;**6**(2):538–546.
63. Coombes, A. G., S. C. Rizzi, M. Williamson, J. E. Barralet, S. Downes, and W. A. Wallace. Precipitation casting of polycaprolactone for applications in tissue engineering and drug delivery. *Biomaterials* 2004;**25**(2):315–325.
64. Dhanaraju, M. D., D. Gopinath, M. R. Ahmed, R. Jayakumar, and C. Vamsadhara. Characterization of polymeric poly(ε-caprolactone) injectable implant delivery system for the controlled delivery of contraceptive steroids. *J. Biomed. Mater. Res. A* 2006;**76**(1):63–72.
65. Huatan, H., J. H. Collett, D. Attwood, and C. Booth. Preparation and characterization of poly(ε-caprolactone) polymer blends for the delivery of proteins. *Biomaterials* 1995;**16**(17):1297–1303.
66. Jia, W., Y. Gu, M. Gou, M. Dai, X. Li, B. Kan, J. Yang, Q. Song, Y. Wei, and Z. Qian. Preparation of biodegradable polycaprolactone/poly(ethylene glycol)/ polycaprolactone (PCEC) nanoparticles. *Drug Deliv.* 2008;**15**(7):409–416.
67. Juni, K. and M. Nakano. Poly(hydroxy acids) in drug delivery. *Crit. Rev. Ther. Drug Carrier Syst.* 1987;**3**(3):209–232.
68. Luciani, A., V. Coccoli, S. Orsi, L. Ambrosio, and P. A. Netti. PCL microspheres based functional scaffolds by bottom-up approach with predefined microstructural properties and release profiles. *Biomaterials* 2008;**29**(36):4800–4807.

69. Mei, L., H. Sun, and C. Song. Local delivery of modified paclitaxel-loaded poly(ε-caprolactone)/pluronic F68 nanoparticles for long-term inhibition of hyperplasia. *J. Pharm. Sci.* 2009;**98**(6):2040–2050.

70. Sheikh, F. A., N. A. Barakat, M. A. Kanjwal, S. Aryal, M. S. Khil, and H. Y. Kim. Novel self-assembled amphiphilic poly(ε-caprolactone)-grafted-poly(vinyl alcohol) nanoparticles: Hydrophobic and hydrophilic drugs carrier nanoparticles. *J. Mater. Sci. Mater. Med.* 2009;**20**(3):821–831.

71. Zhu, K. J., Y. Li, H. L. Jiang, H. Yasuda, A. Ichimaru, K. Yamamoto, P. Lecomte, and R. Jerome. Preparation, characterization and in vitro release properties of ibuprofen-loaded microspheres based on polylactide, poly(ε-caprolactone) and their copolymers. *J. Microencapsul.* 2005;**22**(1):25–36.

72. Ashammakhi, N. and P. Rokkanen. Absorbable polyglycolide devices in trauma and bone surgery. *Biomaterials* 1997;**18**(1):3–9.

73. Hutmacher, D., M. B. Hurzeler, and H. Schliephake. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. *Int. J. Oral. Maxillofac. Implants* 1996;**11**(5): 667–678.

74. Lu, J. M., X. Wang, C. Marin-Muller, H. Wang, P. H. Lin, Q. Yao, and C. Chen. Current advances in research and clinical applications of PLGA-based nanotechnology. *Expert Rev. Mol. Diagn.* 2009;**9**(4):325–341.

75. Miller, N. D. and D. F. Williams. The in vivo and in vitro degradation of poly (glycolic acid) suture material as a function of applied strain. *Biomaterials* 1984;**5** (6):365–368.

76. Pulapura, S. and J. Kohn. Trends in the development of bioresorbable polymers for medical applications. *J. Biomater. Appl.* 1992;**6**(3):216–250.

77. Shum, A. W. T. and A. F. T. Mak. Morphological and biomechanical characterization of poly(glycolic acid) scaffolds after in vitro degradation. *Polym. Degrad. Stab.* 2003;**81**(1):141–149.

78. Gupta, B., N. Revagade, and J. Hilborn. Poly(lactic acid) fiber: An overview. *Prog. Polym. Sci.* 2007;**32**(4):455–482.

79. Hyon, S. H., Biodegradable poly(lactic acid) microspheres for drug delivery systems. *Yonsei Med. J.* 2000;**41**(6):720–734.

80. Miyajima, M., A. Koshika, J. i. Okada, and M. Ikeda. Mechanism of drug release from poly(-lactic acid) matrix containing acidic or neutral drugs. *J. Control. Release* 1999;**60**(2–3):199–209.

81. Saulnier, B., S. Ponsart, J. Coudane, H. Garreau, and M. Vert. Lactic acid-based functionalized polymers via copolymerization and chemical modification. *Macromol. Biosci.* 2004;**4**(3):232–237.

82. Steendam, R., M. J. van Steenbergen, W. E. Hennink, H. W. Frijlink, and C. F. Lerk. Effect of molecular weight and glass transition on relaxation and release behaviour of poly(-lactic acid) tablets. *J. Control. Release* 2001;**70**(1–2):71–82.

83. Tsuji, H. Poly(lactide) stereocomplexes: Formation, structure, properties, degradation, and applications. *Macromol. Biosci.* 2005;**5**(7):569–597.

84. Wang, S., W. Cui, and J. Bei. Bulk and surface modifications of polylactide. *Anal. Bioanal. Chem.* 2005;**381**(3):547–556.

85. Barham, P. J., P. Barker, and S. J. Organ. Physical properties of poly(hydroxybutyrate) and copolymers of hydroxybutyrate and hydroxyvalerate. *FEMS Microbiol. Lett.* 1992;**103**(2–4):289–298.

86. Chen, C., C. H. Yu, Y. C. Cheng, P. H. F. Yu, and M. K. Cheung. Biodegradable nanoparticles of amphiphilic triblock copolymers based on poly(3-hydroxybutyrate) and poly(ethylene glycol) as drug carriers. *Biomaterials* 2006;**27**(27):4804–4814.
87. Martin, D. P. and S. F. Williams. Medical applications of poly-4-hydroxybutyrate: A strong flexible absorbable biomaterial. *Biochem. Eng. J.* 2003;**16**(2):97–105.
88. Pouton, C. W. and S. Akhtar. Biosynthetic polyhydroxyalkanoates and their potential in drug delivery. *Adv. Drug Deliv. Rev.* 1996;**18**(2):133–162.
89. van der Walle, G. A., G. J. de Koning, R. A. Weusthuis, and G. Eggink. Properties, modifications and applications of biopolyesters. *Adv. Biochem. Eng. Biotechnol.* 2001;**71**:263–291.
90. Cai, Z.-Y., D.-A. Yang, N. Zhang, C.-G. Ji, L. Zhu, and T. Zhang. Poly(propylene fumarate)/(calcium sulphate/[β]-tricalcium phosphate) composites: Preparation, characterization and in vitro degradation. *Acta Biomater.* 2009;**5**(2):628–635.
91. Fisher, J. P., D. Dean, and A. G. Mikos. Photocrosslinking characteristics and mechanical properties of diethyl fumarate/poly(propylene fumarate) biomaterials. *Biomaterials* 2002;**23**(22):4333–4343.
92. Haesslein, A., H. Ueda, M. C. Hacker, S. Jo, D. M. Ammon, R. N. Borazjani, J. F. Kunzler, J. C. Salamone, and A. G. Mikos. Long-term release of fluocinolone acetonide using biodegradable fumarate-based polymers. J. Control. *Release* 2006;**114**(2):251–260.
93. He, S., M. J. Yaszemski, A. W. Yasko, P. S. Engel, and A. G. Mikos. Injectable biodegradable polymer composites based on poly(propylene fumarate) cross-linked with poly(ethylene glycol)-dimethacrylate. *Biomaterials* 2000;**21**(23): 2389–2394.
94. Jayabalan, M., K. T. Shalumon, and M. K. Mitha. Injectable biomaterials for minimally invasive orthopedic treatments. *J. Mater. Sci. Mater. Med.* 2009;**20** (6):1379–1387.
95. Kempen, D. H., L. Lu, C. Kim, X. Zhu, W. J. Dhert, B. L. Currier, and M. J. Yaszemski. Controlled drug release from a novel injectable biodegradable microsphere/scaffold composite based on poly(propylene fumarate). *J. Biomed. Mater. Res. A* 2006;**77**(1):103–111.
96. Kim, C. W., R. Talac, L. Lu, M. J. Moore, B. L. Currier, and M. J. Yaszemski. Characterization of porous injectable poly-(propylene fumarate)-based bone graft substitute. *J. Biomed. Mater. Res. A* 2008;**85**(4):1114–1119.
97. Mistry, A. S., Q. P. Pham, C. Schouten, T. Yeh, E. M. Christenson, A. G. Mikos, and J. A. Jansen. In vivo bone biocompatibility and degradation of porous fumarate-based polymer/alumoxane nanocomposites for bone tissue engineering. *J. Biomed. Mater. Res. A* 2009;**92**(2):451–462.
98. Wang, S., L. Lu, and M. J. Yaszemski. Bone-tissue-engineering material poly (propylene fumarate): Correlation between molecular weight, chain dimensions, and physical properties. *Biomacromolecules* 2006;**7**(6):1976–1982.
99. Yaszemski, M. J., R. G. Payne, W. C. Hayes, R. Langer, and A. G. Mikos. In vitro degradation of a poly(propylene fumarate)-based composite material. *Biomaterials* 1996;**17**(22):2127–2130.
100. Bai, W., D. Chen, Z. Zhang, Q. Li, D. Zhang, and C. Xiong. Poly(para-dioxanone)/inorganic particle composites as a novel biomaterial. *J. Biomed. Mater. Res. B Appl. Biomater.* 2009;**90**(2):945–951.

101. Hutmacher, D. W., J. C. Goh, and S. H. Teoh, An introduction to biodegradable materials for tissue engineering applications. *Ann. Acad. Med. Singapore* 2001;**30** (2):183–191.

102. Li, M.-X., R.-X. Zhuo, and F.-Q. Qu. Study on the preparation of novel functional poly(dioxanone) and for the controlled release of protein. *React. Funct. Polym.* 2003;**55**(2):185–195.

103. Lin, H. L., C. C. Chu, and D. Grubb. Hydrolytic degradation and morphologic study of poly-p-dioxanone. *J. Biomed. Mater. Res.* 1993;**27**(2):153–166.

104. Saito, N., N. Murakami, J. Takahashi, H. Horiuchi, H. Ota, H. Kato, T. Okada, K. Nozaki, and K. Takaoka. Synthetic biodegradable polymers as drug delivery systems for bone morphogenetic proteins. *Adv. Drug Deliv. Rev.* 2005;**57** (7):1037–1048.

105. Song, C. X., X. M. Cui, and A. Schindler. Biodegradable copolymers based on p-dioxanone for medical application. *Med. Biol. Eng. Comput.* 1993;**31**(Suppl): S147–151.

106. Vogt, S., S. Berger, I. Wilke, Y. Larcher, J. Weisser, and M. Schnabelrauch. Design of oligolactone-based scaffolds for bone tissue engineering. *Biomed. Mater. Eng.* 2005;**15**(1–2):73–85.

107. Wang, X.-L., K.-K. Yang, Y.-Z. Wang, D.-Y. Wang, and Z. Yang. Crystallization and morphology of a novel biodegradable polymer system: Poly(1,4-dioxan-2-one)/starch blends. *Acta Mater.* 2004;**52**(16):4899–4905.

108. Kluin, O. S., H. C. van der Mei, H. J. Busscher, and D. Neut. A surface-eroding antibiotic delivery system based on poly-(trimethylene carbonate). *Biomaterials* 2009;**30**(27):4738–4742.

109. Papenburg, B. J., S. Schüller-Ravoo, L. A. M. Bolhuis-Versteeg, L. Hartsuiker, D. W. Grijpma, J. Feijen, M. Wessling, and D. Stamatialis. Designing porosity and topography of poly(1,3-trimethylene carbonate) scaffolds. Acta Biomater. 2009;**5**(9): 3281–3294.

110. Timbart, L., M. Y. Tse, S. C. Pang, O. Babasola, and B. G. Amsden. Low viscosity poly(trimethylene carbonate) for localized drug delivery: Rheological properties and in vivo degradation. *Macromol. Biosci.* 2009;**9**(8):786–794.

111. Zhang, Z., D. W. Grijpma, and J. Feijen. Trimethylene carbonate-based polymers for controlled drug delivery applications. *J. Control. Release* 2006;**116**(2): e28–e29.

112. Zhang, Z., R. Kuijer, S. K. Bulstra, D. W. Grijpma, and J. Feijen. The in vivo and in vitro degradation behavior of poly(trimethylene carbonate). *Biomaterials* 2006;**27**(9):1741–1748.

113. Zurita, R., L. Franco, J. Puiggalí, and A. Rodríguez-Galán. The hydrolytic degradation of a segmented glycolide-trimethylene carbonate copolymer (Maxon (TM)). *Polym. Degrad. Stab.* 2007;**92**(6):975–985.

114. Andriano, K. P., Y. Tabata, Y. Ikada, and J. Heller. In vitro and in vivo comparison of bulk and surface hsydrolysis in absorbable polymer scaffolds for tissue engineering. *J. Biomed. Mater. Res.* 1999;**48**(5):602–612.

115. Barr, J., K. W. Woodburn, S. Y. Ng, H.-R. Shen, and J. Heller. Post surgical pain management with poly(ortho esters). *Adv. Drug Deliv. Rev.* 2002;**54**(7): 1041–1048.

116. Chia, H. H., Y. Y. Yang, T. S. Chung, S. Ng, and J. Heller. Auto-catalyzed poly (ortho ester) microspheres: A study of their erosion and drug release mechanism. J. Control. *Release* 2001;**75**(1–2):11–25.

117. Einmahl, S., S. Capancioni, K. Schwach-Abdellaoui, M. Moeller, F. Behar-Cohen, and R. Gurny. Therapeutic applications of viscous and injectable poly(ortho esters). *Adv. Drug Deliv. Rev.* 2001;**53**(1):45–73.

118. Heller, J. Development of poly(ortho esters): A historical overview. *Biomaterials* 1990;**11**(9):659–665.

119. Heller, J. Development of poly(ortho esters): A historical overview. *Biomaterials* 1990;**11**(9):659–665.

120. Heller, J. and J. Barr. Biochronomer technology. *Expert Opin. Drug Deliv.* 2005;**2** (1):169–183.

121. Heller, J., J. Barr, S. Ng, H.-R. Shen, R. Gurny, K. Schwach-Abdelaoui, A. Rothen-Weinhold, and M. van de Weert. Development of poly(ortho esters) and their application for bovine serum albumin and bupivacaine delivery. *J. Control. Release* 2002;**78**(1–3):133–141.

122. Heller, J., J. Barr, S. Y. Ng, K. S. Abdellauoi, and R. Gurny. Poly(ortho esters): Synthesis, characterization, properties and uses. *Adv. Drug Deliv. Rev.* 2002;**54** (7):1015–1039.

123. Heller, J., J. Barr, S. Y. Ng, H. R. Shen, K. Schwach-Abdellaoui, S. Einmahl, A. Rothen-Weinhold, and R. Gurny. Poly(ortho esters)—their development and some recent applications. *Eur. J. Pharm. Biopharm.* 2000;**50**(1):121–128.

124. Heller, J. and K. J. Himmelstein, Poly(ortho ester) biodegradable polymer systems. *Methods Enzymol.* 1985;**112**:422–436.

125. Heller, J., Y. F. Maa, P. Wuthrich, S. Y. Ng, and R. Duncan. Recent developments in the synthesis and utilization of poly (ortho esters). *J. Control. Release.* 1991;**16**(1–2):3–13.

126. Merkli, A., J. Heller, C. Tabatabay, and R. Gurny. Synthesis and characterization of a new biodegradable semi-solid poly(ortho ester) for drug delivery systems. *J. Biomater. Sci. Polym. Ed.* 1993;**4**(5):505–516.

127. Shih, C., S. Lucas, and G. M. Zentner. Acid catalyzed poly(ortho ester) matrices for intermediate term drug delivery. *J. Control. Release* 1991;**15**(1):55–63.

128. Sintzel, M. B., J. Heller, S. Y. Ng, M. S. Taylor, C. Tabatabay, and R. Gurny. Synthesis and characterization of self-catalyzed poly(ortho ester). *Biomaterials* 1998;**19**(7–9):791–800.

129. Sintzel, M. B., A. Merkli, J. Heller, C. Tabatabay, and R. Gurny. Synthesis and analysis of viscous poly(ortho-ester) analogs for controlled drug release. *Int. J. Pharm.* 1997;**155**(2):263–269.

130. Tang, R., R. N. Palumbo, W. Ji, and C. Wang. Poly(ortho ester amides): Acid-labile temperature-responsive copolymers for potential biomedical applications. *Biomacromolecules* 2009;**10**(4):722–777.

131. van de Weert, M., M. J. van Steenbergen, J. L. Cleland, J. Heller, W. E. Hennink, and D. J. Crommelin. Semisolid, self-catalyzed poly(ortho ester)s as controlled-release systems: Protein release and protein stability issues. *J. Pharm. Sci.* 2002;**91** (4):1065–1074.

132. Wang, C., Q. Ge, D. Ting, D. Nguyen, H. R. Shen, J. Chen, H. N. Eisen, J. Heller, R. Langer, and D. Putnam. Molecularly engineered poly(ortho ester) microspheres for enhanced delivery of DNA vaccines. *Nat. Mater.* 2004;**3**(3):190–196.

133. Yu, J., Z. Lu, H. Zheng, and R. Zhuo. Synthesis and characterization of new ointment-like poly(ortho esters). *Eur. Polym. J.* 2002;**38**(5):971–975.

134. Alla, A., A. Rodríguez-Galán, A. Martínez de llarduya, and S. Muñoz-Guerra. Degradable poly(ester amide)s based on L-tartaric acid. *Polymer* 1997;**38**(19):4935–4944.

135. Armelin, E., N. Paracuellos, A. Rodríguez-Galán, and J. Puiggalí. Study on the degradability of poly(ester amide)s derived from the α-amino acids glycine, and -alanine containing a variable amide/ester ratio. *Polymer* 2001;**42**(19):7923–7932.

136. Bettinger, C. J., J. P. Bruggeman, J. T. Borenstein, and R. S. Langer. Amino alcohol-based degradable poly(ester amide) elastomers. *Biomaterials* 2008;**29**(15):2315–2325.

137. Edlund, U. and A. C. Albertsson. Polyesters based on diacid monomers. *Adv. Drug Deliv. Rev.* 2003;**55**(4):585–609.

138. Guo, K. and C.-C. Chu. Biodegradation of unsaturated poly(ester-amide)s and their hydrogels. *Biomaterials* 2007;**28**(22):3284–3294.

139. Yamanouchi, D., J. Wu, A. N. Lazar, K. Craig Kent, C.-C. Chu, and B. Liu. Biodegradable arginine-based poly(ester-amide)s as non-viral gene delivery reagents. *Biomaterials* 2008;**29**(22):3269–3277.

140. Yeol Lee, S., J. W. Park, Y. T. Yoo, and S. S. Im. Hydrolytic degradation behaviour and microstructural changes of poly(ester-co-amide)s. *Polym. Degrad. Stab.* 2002;**78**(1):63–71.

141. Dahiyat, B. I., E. M. Posadas, S. Hirosue, E. Hostin, and K. W. Leong. Degradable biomaterials with elastomeric characteristics and drug-carrier function. *React. Polym.* 1995;**25**(2–3):101–109.

142. Dahiyat, B. I., M. Richards, and K. W. Leong. Controlled release from poly (phosphoester) matrices. *J. Control. Release* 1995;**33**(1):13–21.

143. Koseva, N., A. Bogomilova, K. Atkova, and K. Troev. New functional polyphosphoesters: Design and characterization. *React. Funct. Polym.* 2008;**68**(5):954–966.

144. Li, Q., J. Wang, S. Shahani, D. D. N. Sun, B. Sharma, J. H. Elisseeff, and K. W. Leong. Biodegradable and photocrosslinkable polyphosphoester hydrogel. *Biomaterials* 2006;**27**(7):1027–1034.

145. Mao, H. Q. and K. W. Leong. Design of polyphosphoester-DNA nanoparticles for non-viral gene delivery. *Adv. Genet.* 2005;**53**:275–306.

146. Wang, S., A. C. Wan, X. Xu, S. Gao, H. Q. Mao, K. W. Leong, and H. Yu. A new nerve guide conduit material composed of a biodegradable poly(phosphoester). *Biomaterials* 2001;**22**(10):1157–1169.

147. Zhao, Z., J. Wang, H.-Q. Mao, and K. W. Leong. Polyphosphoesters in drug and gene delivery. *Adv. Drug Deliv. Rev.* 2003;**55**(4):483–499.

148. Chaubal, M. V., A. S. Gupta, S. T. Lopina, and D. F. Bruley. Polyphosphates and other phosphorus-containing polymers for drug delivery applications. *Crit. Rev. Ther. Drug Carrier Syst.* 2003;**20**(4):295–315.

149. Crommen, J., J. Vandorpe, and E. Schacht. Degradable polyphosphazenes for biomedical applications. *J. Control. Release* 1993;**24**(1–3):167–180.

150. Gunatillake, P. A. and R. Adhikari. Biodegradable synthetic polymers for tissue engineering. *Eur. Cell Mater.* 2003;**5**:1–16; discussion 16.

151. Lakshmi, S., D. S. Katti, and C. T. Laurencin. Biodegradable polyphosphazenes for drug delivery applications. *Adv. Drug Deliv. Rev.* 2003;**55**(4):467–482.

152. Lora, S., G. Palma, R. Bozio, P. Caliceti, and G. Pezzin. Polyphosphazenes as biomaterials: Surface modification of poly(bis(trifluoroethoxy)phosphazene) with polyethylene glycols. *Biomaterials* 1993;**14**(6):430–436.

153. Potin, P. and R. De Jaeger. Polyphosphazenes: Synthesis, structures, properties, applications. *Eur. Polym. J.* 1991;**27**(4–5):341–348.

154. Bourke, S. L. and J. Kohn. Polymers derived from the amino acid-tyrosine: Polycarbonates, polyarylates and copolymers with poly(ethylene glycol). *Adv. Drug Deliv. Rev.* 2003;**55**(4):447–466.

155. Deming, T. J. Synthetic polypeptides for biomedical applications. *Prog. Polym. Sci.* 2007;**32**(8–9):858–875.

156. Katchalski, E., J. T. E. M. L. Anson, and B. Kenneth. Poly-α-amino acids. In *Advances in Protein Chemistry*. Academic: New York, 1951, pp. 123–185.

157. Lavasanifar, A., J. Samuel, and G. S. Kwon. Poly(ethylene oxide)-block-poly(-amino acid) micelles for drug delivery. *Adv. Drug Deliv. Rev.* 2002;**54**(2): 169–190.

158. Sela, M., E. Katchalski, J. M. L. A. K. B. C. B. Anfinsen, and T. E. John. *Biological properties of poly-α-amino acids*. In *Advances in Protein Chemistry*. Academic: New York, 1959, pp. 391–478.

159. Kumar, N., R. S. Langer, and A. J. Domb. Polyanhydrides: An overview. *Adv. Drug Deliv. Rev.* 2002;**54**(7):889–910.

160. Domb, A. Synthesis and characterization of biodegradable aromatic anhydride copolymers. *Macromolecules* 1992;**25**(1):12–17.

161. Kim, M. S., K. S. Seo, H. S. Seong, S. H. Cho, H. B. Lee, K. D. Hong, S. K. Kim, and G. Khang. Synthesis and characterization of polyanhydride for local BCNU delivery carriers. *Biomed. Mater. Eng.* 2005;**15**(3):229–238.

162. Lesniak, M. S., U. Upadhyay, R. Goodwin, B. Tyler, and H. Brem. Local delivery of doxorubicin for the treatment of malignant brain tumors in rats. *Anticancer Res.* 2005;**25**(6B):3825–3831.

163. Domb, A. J. and M. Maniar. Absorbable biopolymers derived from dimer fatty acids. *J. Polym. Sci: Part A: Polym. Chem.* 1993;**31**(5):1275–1285.

164. Domb, A. J. and M. Maniar. *Fatty Acid Terminated Polyanhydride*. 1994; US Patent 5317079.

165. Domb, A. J. and R. Nudelman, Biodegradable polymers derived from natural fatty acids. *J. Polym. Sci. Part A: Polym. Chem.* 1995;**33**(4):717–725.

166. Jain, J. P., S. Modi, and N. Kumar. Hydroxy fatty acid based polyanhydride as drug delivery system: Synthesis, characterization, in vitro degradation, drug release, and biocompatibility. *J. Biomed. Mater. Res. A* 2007;**84**(3):740–752.

167. Jain, J. P., M. Sokolsky, N. Kumar, and A. J. Domb. Fatty acid based biodegradable polymer. *Polym. Rev.* 2008;**48**(1):156–191.

168. Kumar, N., M. Krishnan, T. Azzam, A. Magora, M. N. V. Ravikumar, D. R. Flanagan, and A. J. Domb. Analysis of fatty acid anhydrides and polyanhydrides. *Anal. Chim. Acta* 2002;**465**(1–2):257–272.

169. Teomim, D. and A. J. Domb. Fatty acid terminated polyanhydrides. *J. Poly. Sci. Part A: Polym. Chem.* 1999;**37**(16):3337–3344.

170. Teomim, D., A. Nyska, and A. J. Domb. Ricinoleic acid-based biopolymers. *J. Biomed. Mater. Res.* 1999;**45**(3):258–267.

171. Arias, J. L., M. A. Ruiz, M. López-Viota, and Á. V. Delgado. Poly(alkylcyanoacrylate) colloidal particles as vehicles for antitumour drug delivery: A comparative study. *Colloids Surf. B* 2008;**62**(1):64–70.

172. Graf, A., A. McDowell, and T. Rades. Poly(alkylcyanoacrylate) nanoparticles for enhanced delivery of therapeutics—Is there real potential? *Expert Opin. Drug Deliv.* 2009;**6**(4):371–387.

173. Vauthier, C., C. Dubernet, E. Fattal, H. Pinto-Alphandary, and P. Couvreur. Poly (alkylcyanoacrylates) as biodegradable materials for biomedical applications. *Adv. Drug Deliv. Rev.* 2003;**55**(4):519–548.

174. Vauthier, C., D. Labarre, and G. Ponchel, Design aspects of poly(alkylcyanoacrylate) nanoparticles for drug delivery. *J. Drug Target.* 2007;**15**(10):641–663.

175. Alves, C. M., Y. Yang, D. L. Carnes, J. L. Ong, V. L. Sylvia, D. D. Dean, C. M. Agrawal, and R. L. Reis. Modulating bone cells response onto starch-based biomaterials by surface plasma treatment and protein adsorption. *Biomaterials* 2007;**28**(2):307–315.

176. Azevedo, H. S., F. M. Gama, and R. L. Reis. In vitro assessment of the enzymatic degradation of several starch based biomaterials. *Biomacromolecules* 2003;**4**(6):1703–1712.

177. Cascone, M. G., N. Barbani, C. Cristallini, P. Giusti, G. Ciardelli, and L. Lazzeri. Bioartificial polymeric materials based on polysaccharides. *J. Biomater. Sci. Polym. Ed.* 2001;**12**(3):267–281.

178. Chandra, R. and R. Rustgi. Biodegradable polymers. *Prog. Polym. Sci.* 1998;**23**:1273–1335.

179. Elvira, C., J. F. Mano, J. San Roman, and R. L. Reis. Starch-based biodegradable hydrogels with potential biomedical applications as drug delivery systems. *Biomaterials* 2002;**23**(9):1955–1966.

180. Gomes, M. E., A. S. Ribeiro, P. B. Malafaya, R. L. Reis, and A. M. Cunha. A new approach based on injection moulding to produce biodegradable starch-based polymeric scaffolds: Morphology, mechanical and degradation behaviour. *Biomaterials* 2001;**22**(9):883–889.

181. Marques, A. P., R. L. Reis, and J. A. Hunt. The biocompatibility of novel starch-based polymers and composites: In vitro studies. *Biomaterials* 2002;**23**(6):1471–1478.

182. Mayer, J. M., G. R. Elion, C. M. Buchanan, B. K. Sullivan, S. D. Pratt, and D. L. Kaplan. Biodegradable blends of cellulose acetate and starch: Production and properties. *J. Macromol. Sci. Pure Appl. Chem.* 1995;**32**(4):775–785.

183. Czaja, W. K., D. J. Young, M. Kawecki, and R. M. Brown. The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules* 2007;**8**(1):1–12.

184. Fricain, J. C., P. L. Granja, M. A. Barbosa, B. de Jéso, N. Barthe, and C. Baquey. Cellulose phosphates as biomaterials. In vivo biocompatibility studies. *Biomaterials* 2002;**23**(4):971–980.

185. Klemm, D., B. Heublein, H. P. Fink, and A. Bohn. Cellulose: Fascinating biopolymer and sustainable raw material. *Angew. Chem. Int. Ed. Engl.* 2005;**44** (22):3358–3393.

186. Müller, F. A., L. Müller, I. Hofmann, P. Greil, M. M. Wenzel, and R. Staudenmaier. Cellulose-based scaffold materials for cartilage tissue engineering. *Biomaterials* 2006;**27**(21):3955–3963.

187. Prabaharan, M. and J. F. Mano. Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. *Macromol. Biosci.* 2006;**6**(12):991–1008.

188. Svensson, A., E. Nicklasson, T. Harrah, B. Panilaitis, D. L. Kaplan, M. Brittberg, and P. Gatenholm. Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. *Biomaterials* 2005;**26**(4):419–431.

189. Thomas, S., A review of the physical, biological and clinical properties of a bacterial cellulose wound dressing. *J. Wound Care* 2008;**17**(8):349–352.

190. Alves, N. M. and J. F. Mano. Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. *Int. J. Biol. Macromol.* 2008;**43**(5):401–414.

191. Arca, H. C., M. Gunbeyaz, and S. Senel. Chitosan-based systems for the delivery of vaccine antigens. *Expert Rev. Vaccines* 2009;**8**(7):937–953.

192. Bowman, K. and K. W. Leong. Chitosan nanoparticles for oral drug and gene delivery. *Int. J. Nanomed.* 2006;**1**(2):117–128.

193. Jayakumar, R., N. Nwe, S. Tokura, and H. Tamura. Sulfated chitin and chitosan as novel biomaterials. *Int. J. Biol. Macromol.* 2007;**40**(3):175–181.

194. Jiang, T., S. G. Kumbar, L. S. Nair, and C. T. Laurencin. Biologically active chitosan systems for tissue engineering and regenerative medicine. *Curr. Top. Med. Chem.* 2008;**8**(4):354–364.

195. Khor, E. and L. Y. Lim. Implantable applications of chitin and chitosan. *Biomaterials* 2003;**24**(13):2339–2349.

196. Kim, I. Y., S. J. Seo, H. S. Moon, M. K. Yoo, I. Y. Park, B. C. Kim, and C. S. Cho. Chitosan and its derivatives for tissue engineering applications. *Biotechnol. Adv.* 2008;**26**(1):1–21.

197. Kumar, M. N., R. A. Muzzarelli, C. Muzzarelli, H. Sashiwa, and A. J. Domb. Chitosan chemistry and pharmaceutical perspectives. *Chem. Rev.* 2004;**104** (12):6017–6084.

198. Masotti, A. and G. Ortaggi. Chitosan micro- and nanospheres: Fabrication and applications for drug and DNA delivery. *Mini. Rev. Med. Chem.* 2009;**9** (4):463–469.

199. Mourya, V. K. and N. N. Inamdar. Trimethyl chitosan and its applications in drug delivery. *J. Mater. Sci. Mater. Med.* 2009;**20**(5):1057–1079.

200. Panos, I., N. Acosta, and A. Heras. New drug delivery systems based on chitosan. *Curr. Drug Discov. Technol.* 2008;**5**(4):333–341.

201. Prabaharan, M. Review paper: Chitosan derivatives as promising materials for controlled drug delivery. *J. Biomater. Appl.* 2008;**23**(1):5–36.

202. Shahidi, F. and R. Abuzaytoun. Chitin, chitosan, and co-products: Chemistry, production, applications, and health effects. *Adv. Food Nutr. Res.* 2005;**49**: 93–135.

203. Shi, C., Y. Zhu, X. Ran, M. Wang, Y. Su, and T. Cheng. Therapeutic potential of chitosan and its derivatives in regenerative medicine. *J. Surg. Res.* 2006;**133** (2):185–192.

204. Vinsova, J. and E. Vavrikova. Recent advances in drugs and prodrugs design of chitosan. *Curr. Pharm. Des.* 2008;**14**(13):1311–1326.

205. Yilmaz, E. Chitosan: A versatile biomaterial. *Adv. Exp. Med. Biol.* 2004;**553**:59–68.

206. Hillier, K., M. Rakkar, S. J. Enna, and B. B. David. Alginic acid. *In xPharm: The Comprehensive Pharmacology Reference*. Elsevier: New York, 2007, pp. 1–3.

207. Draget, K. I., G. Skjåk Bræk, and O. Smidsrød. Alginic acid gels: The effect of alginate chemical composition and molecular weight. *Carbohydr. Polym.* 1994;**25** (1):31–38.

208. Tonnesen, H. H. and J. Karlsen. Alginate in drug delivery systems. *Drug Dev. Ind. Pharm.* 2002;**28**(6):621–630.

209. Beasley, K. L., M. A. Weiss, and R. A. Weiss. Hyaluronic acid fillers: A comprehensive review. *Facial Plast. Surg.* 2009;**25**(2):86–94.

210. Brown, M. B. and S. A. Jones. Hyaluronic acid: A unique topical vehicle for the localized delivery of drugs to the skin. *J. Eur. Acad. Dermatol. Venereol.* 2005;**19** (3):308–318.

211. Iavazzo, C., S. Athanasiou, E. Pitsouni, and M. E. Falagas. Hyaluronic acid: An effective alternative treatment of interstitial cystitis, recurrent urinary tract infections, and hemorrhagic cystitis? *Eur. Urol.* 2007;**51**(6):1534–1540; discussion 1540–1541.

212. Kogan, G., L. Soltes, R. Stern, and P. Gemeiner. Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol. Lett.* 2007;**29**(1):17–25.

213. Liao, Y. H., S. A. Jones, B. Forbes, G. P. Martin, and M. B. Brown. Hyaluronan: Pharmaceutical characterization and drug delivery. *Drug Deliv.* 2005;**12** (6):327–342.

214. Migliore, A. and M. Granata. Intra-articular use of hyaluronic acid in the treatment of osteoarthritis. *Clin. Interv. Aging* 2008;**3**(2):365–369.

215. Price, R. D., M. G. Berry, and H. A. Navsaria. Hyaluronic acid: The scientific and clinical evidence. *J. Plast. Reconstr. Aesthet. Surg.* 2007;**60**(10):1110–1119.

216. Volpi, N., J. Schiller, R. Stern, and L. Soltes. Role, metabolism, chemical modifications and applications of hyaluronan. *Curr. Med. Chem.* 2009;**16** (14):1718–1745.

217. Yadav, A. K., P. Mishra, and G. P. Agrawal. An insight on hyaluronic acid in drug targeting and drug delivery. *J. Drug. Target.* 2008;**16**(2):91–107.

218. Chourasia, M. K. and S. K. Jain. Polysaccharides for colon targeted drug delivery. *Drug Deliv.* 2004;**11**(2):129–148.

219. Hennink, W. E., O. Franssen, W. N. E. van Dijk-Wolthuis, and H. Talsma. Dextran hydrogels for the controlled release of proteins. *J. Control. Release* 1997;**48**(2–3):107–114.

220. Larsen, C. Dextran prodrugs—Structure and stability in relation to therapeutic activity. *Adv. Drug Deliv. Rev.* 1989;**3**(1):103–154.

221. Mehvar, R. Dextrans for targeted and sustained delivery of therapeutic and imaging agents. J. Control. *Release* 2000;**69**(1):1–25.

222. Van Tomme, S. R. and W. E. Hennink. Biodegradable dextran hydrogels for protein delivery applications. *Expert Rev. Med. Devices* 2007;**4**(2):147–164.

223. Bana, G., B. Jamard, E. Verrouil, and B. Mazieres. Chondroitin sulfate in the management of hip and knee osteoarthritis: An overview. *Adv. Pharmacol.* 2006;**53**:507–522.

224. Brandl, N., J. Holzmann, R. Schabus, and M. Huettinger. Effects of chondroitin sulfate on the cellular metabolism. *Adv. Pharmacol.* 2006;**53**:433–447.

225. Campo, G. M., A. Avenoso, S. Campo, A. M. Ferlazzo, and A. Calatroni. Antioxidant activity of chondroitin sulfate. *Adv. Pharmacol.* 2006;**53**:417–431.

226. Handley, C. J., T. Samiric, and M. Z. Ilic. Structure, metabolism, and tissue roles of chondroitin sulfate proteoglycans. *Adv. Pharmacol.* 2006;**53**:219–232.

227. Lamari, F. N. The potential of chondroitin sulfate as a therapeutic agent. *Connect. Tissue Res.* 2008;**49**(3):289–292.

228. Lauder, R. M. Chondroitin sulphate: A complex molecule with potential impacts on a wide range of biological systems. Complement. *Ther. Med.* 2009;**17**(1):56–62.

229. Morris, J. D. and K. M. Smith. Chondroitin sulfate in osteoarthritis therapy. *Orthopedics* 2009;**32**(4).

230. Yamada, S. and K. Sugahara. Potential therapeutic application of chondroitin sulfate/dermatan sulfate. *Curr. Drug Discov. Technol.* 2008;**5**(4):289–301.

231. Keppeler, S., A. Ellis, and J. C. Jacquier. Cross-linked carrageenan beads for controlled release delivery systems. *Carbohydr. Polym.* 2009;**78**(4):973–977.

232. Kranz, H., K. Jürgens, M. Pinier, and J. Siepmann. Drug release from MCC- and carrageenan-based pellets: Experiment and theory. *Eur. J. Pharm. Biopharm.* 2009;**73**(2):302–309.

233. Thomson, A. W. and E. F. Fowler. Carrageenan: A review of its effects on the immune system. *Agents Actions* 1981;**11**(3):265–273.

234. Valenta, C. The use of mucoadhesive polymers in vaginal delivery. *Adv. Drug Deliv. Rev.* 2005;**57**(11):1692–1712.

235. Ehrenfreund-Kleinman, T., T. Azzam, R. Falk, I. Polacheck, J. Golenser, and A. J. Domb. Synthesis and characterization of novel water soluble amphotericin B-arabinogalactan conjugates. *Biomaterials* 2002;**23**(5):1327–1335.

236. Ehrenfreund-Kleinman, T., Z. Gazit, D. Gazit, T. Azzam, J. Golenser, and A. J. Domb. Synthesis and biodegradation of arabinogalactan sponges prepared by reductive amination. *Biomaterials* 2002;**23**(23):4621–4631.

237. Prescott, J. H., P. Enriquez, C. Jung, E. Menz, and E. V. Groman. Larch arabinogalactan for hepatic drug delivery: Isolation and characterization of a 9 kDa arabinogalactan fragment. *Carbohydr. Res.* 1995;**278**(1):113–128.

238. Seifert, G. J. and K. Roberts. The biology of arabinogalactan proteins. *Annu. Rev. Plant Biol.* 2007;**58**:137–161.

239. Chivers, R. A. In vitro tissue welding using albumin solder: Bond strengths and bonding temperatures. *Int. J. Adhes. Adhes.* 2000;**20**(3):179–187.

240. Chuang, V. T., U. Kragh-Hansen, and M. Otagiri. Pharmaceutical strategies utilizing recombinant human serum albumin. *Pharm. Res.* 2002;**19**(5):569–577.
241. Gradishar, W. J. Albumin-bound paclitaxel: A next-generation taxane. *Expert. Opin. Pharmacother.* 2006;**7**(8):1041–1053.
242. Gupta, P. K. and C. T. Hung. Albumin microspheres. I: Physico-chemical characteristics. *J. Microencapsul.* 1989;**6**(4):427–462.
243. Gupta, P. K. and C. T. Hung. Albumin microspheres. II: Applications in drug delivery. *J. Microencapsul.* 1989;**6**(4):463–472.
244. Kratz, F. Albumin as a drug carrier: Design of prodrugs, drug conjugates and nanoparticles. *J. Control. Release* 2008;**132**(3):171–183.
245. Perez, E. A. Novel enhanced delivery taxanes: An update. *Semin. Oncol.* 2007;**34**(3) (suppl):1–5.
246. Abraham, L. C., E. Zuena, B. Perez-Ramirez, and D. L. Kaplan. Guide to collagen characterization for biomaterial studies. *J. Biomed. Mater. Res. B Appl. Biomater.* 2008;**87**(1):264–285.
247. Friess, W., Collagen—biomaterial for drug delivery. *Eur. J. Pharm. Biopharm.* 1998;**45**(2):113–136.
248. Lee, C. H., A. Singla, and Y. Lee. Biomedical applications of collagen. *Int. J. Pharm.* 2001;**221**(1–2):1–22.
249. Miyata, T., T. Taira, and Y. Noishiki, Collagen engineering for biomaterial use. *Clin. Mater.* 1992;**9**(3–4):139–148.
250. Olsen, D., C. Yang, M. Bodo, R. Chang, S. Leigh, J. Baez, D. Carmichael, M. Perala, E. R. Hamalainen, M. Jarvinen, and J. Polarek. Recombinant collagen and gelatin for drug delivery. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1547–1567.
251. Paul, R. G. and A. J. Bailey. Chemical stabilisation of collagen as a biomimetic. *Scient. World J.*2003;**3**:138–155.
252. Rao, K. P., Recent developments of collagen-based materials for medical applications and drug delivery systems. *J. Biomater. Sci. Polym. Ed.* 1995;**7**(7):623–645.
253. Ruszczak, Z. and W. Friess. Collagen as a carrier for on-site delivery of antibacterial drugs. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1679–1698.
254. Stenzel, K. H., T. Miyata, and A. L. Rubin. Collagen as a biomaterial. *Annu. Rev. Biophys. Bioeng.* 1974;**3**(0):231–253.
255. Stol, M., K. Smetana, P. Korbelá, and M. Adam. Poly(HEMA)-collagen composite as a biomaterial for hard tissue replacement. *Clin. Mater.* 1993;**13**(1–4):19–20.
256. Esposito, E., R. Cortesi, and C. Nastruzzi. Gelatin microspheres: Influence of preparation parameters and thermal treatment on chemico-physical and biopharmaceutical properties. *Biomaterials* 1996;**17**(20):2009–2020.
257. Sakai, S., K. Hirose, K. Taguchi, Y. Ogushi, and K. Kawakami. An injectable, in situ enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. *Biomaterials* 2009;**30**(20):3371–3377.
258. Young, S., M. Wong, Y. Tabata, and A. G. Mikos. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *J. Control. Release* 2005;**109** (1–3):256–274.
259. des Rieux, A., A. Shikanov, and L. D. Shea. Fibrin hydrogels for non-viral vector delivery in vitro. *J. Control. Release* 2009;**136**(2):148–154.

260. Eyrich, D., F. Brandl, B. Appel, H. Wiese, G. Maier, M. Wenzel, R. Staudenmaier, A. Goepferich, and T. Blunk. Long-term stable fibrin gels for cartilage engineering. *Biomaterials* 2007;**28**(1):55–65.

261. San-Galli, F., G. Deminière, J. Guérin, and M. Rabaud. Use of a biodegradable elastin—fibrin material, Neuroplast®, as a dural substitute. *Biomaterials* 1996;**17** (11):1081–1085.

262. Silver, F. H., M.-C. Wang, and G. D. Pins. Preparation and use of fibrin glue in surgery. *Biomaterials* 1995;**16**(12):891–903.

263. Wozniak, G. Fibrin sealants in supporting surgical techniques: The importance of individual components. *Cardiovas. Surg.* 2003;**11**(Suppl 1):17–21.

264. Ijntema, K., W. J. M. Heuvelsland, C. A. M. C. Dirix, and A. P. Sam. Hydroxyapatite microcarriers for biocontrolled release of protein drugs. *Int. J. Pharm.* 1994;**112**(3):215–224.

265. Matsumoto, T., M. Okazaki, A. Nakahira, J. Sasaki, H. Egusa, and T. Sohmura. Modification of apatite materials for bone tissue engineering and drug delivery carriers. *Curr. Med. Chem.* 2007;**14**(25):2726–2733.

266. Paul, W. and C. P. Sharma. Ceramic drug delivery: A perspective. *J. Biomater. Appl.* 2003;**17**(4):253–264.

267. Sopyan, I., M. Mel, S. Ramesh, and K. A. Khalid. Porous hydroxyapatite for artificial bone applications. *Sci. Technol. Adv. Mater.* 2007;**8**(1–2):116–123.

268. Wahl, D. A. and J. T. Czernuszka. Collagen-hydroxyapatite composites for hard tissue repair. *Eur. Cell Mater.* 2006;**11**:43–56.

269. Yoshikawa, H. and A. Myoui. Bone tissue engineering with porous hydroxyapatite ceramics. *J. Artif. Organs* 2005;**8**(3):131–136.

270. Yoshikawa, H., N. Tamai, T. Murase, and A. Myoui. Interconnected porous hydroxyapatite ceramics for bone tissue engineering. *J. Rog. Soc. Interface* 2009;**6** (Suppl 3):S341–348.

271. Tamada, J. A. and R. Langer, Erosion kinetics of hydrolytically degradable polymers. *Proc. Natl. Acad. Sci. U. S. A.* 1993;**90**(2):552–556.

272. Gopferich, A. Erosion of composite polymer matrices. *Biomaterials* 1997;**8** (5):397–403.

273. Gopferich, A. and R. Langer. Modeling of polymer erosion. *Macromolecules* 1993;**6**:4105–4112.

274. Langer, R. Drug delivery and targeting. *Nature* 1998;**392**(6679 Suppl):5–10.

275. Langer, R., D. Lund, K. Leong, and J. Folkman. Controlled release of macromolecules: Biological studies. *J. Control. Release* 1985;**2**:331–341.

276. Mathiowitz, E., J. S. Jacob, Y. S. Jong, G. P. Carino, D. E. Chickering, P. Chaturvedi, C. A. Santos, K. Vijayaraghavan, S. Montgomery, M. Bassett, and C. Morrell. Biologically erodible microspheres as potential oral drug delivery systems. *Nature* 1997;**386**:410–414.

277. Gorna, K. and S. Gogolewski. In vitro degradation of novel medical biodegradable aliphatic polyurethanes based on ε-caprolactone and Pluronics® with various hydrophilicities. *Polym. Degrad. Stab.* 2002;**75**(1):113–122.

278. Lao, L. L., S. S. Venkatraman, and N. A. Peppas. Modeling of drug release from biodegradable polymer blends. *Eur. J. Pharma. Biopharm.* 2008;**70**(3): 796–803.

279. Reed, A. M. and D. K. Gilding. Biodegradable polymers for use in surgery—poly (glycolic)/poly(Iactic acid) homo and copolymers: 2. In vitro degradation. *Polymer* 1981;**22**(4):494–498.

280. Barbanti, S. H., A. R. Santos, Jr., C. A. Zavaglia, and E. A. Duek. Porous and dense poly(L-lactic acid) and poly(D,L-lactic acid-*co*-glycolic acid) scaffolds: In vitro degradation in culture medium and osteoblasts culture. *J. Mater. Sci. Mater. Med.* 2004;**15**(12):1315–1321.

281. Djemai, A., L. F. Gladden, J. Booth, R. S. Kittlety, and P. R. Gellert. MRI investigation of hydration and heterogeneous degradation of aliphatic polyesters derived from lactic and glycolic acids: A controlled drug delivery device. *Magn. Reson. Imaging* 2001;**19**(3–4):521–523.

282. Li, S. and S. McCarthy. Further investigations on the hydrolytic degradation of poly (dl-lactide). *Biomaterials* 1999;**20**(1):35–44.

283. Siepmann, J., K. Elkharraz, F. Siepmann, and D. Klose. How autocatalysis accelerates drug release from PLGA-based microparticles: A quantitative treatment. *Biomacromolecules* 2005;**6**(4):2312–2319.

284. Wang, Z., S. Wang, R. Guidoin, Y. Marois, and Z. Zhang. In vitro homogeneous and heterogeneous degradation of poly(-caprolactone/polyethylene glycol/l-lactide): The absence of autocatalysis and the role of enzymes. *J. Biomed. Mater. Res. A* 2006;**79**(1):6–15.

285. Belbella, A., C. Vauthier, H. Fessi, J.-P. Devissaguet, and F. Puisieux. In vitro degradation of nanospheres from poly(d,l-lactides) of different molecular weights and polydispersities. *Int. J. Pharm.* 1996;**129**(1–2):95–102.

286. Braunecker, J., M. Baba, G. E. Milroy, and R. E. Cameron. The effects of molecular weight and porosity on the degradation and drug release from polyglycolide. *Int. J. Pharm.* 2004;**282**(1–2):19–34.

287. Farrar, D. F. and R. K. Gillson. Hydrolytic degradation of polyglyconate B: The relationship between degradation time, strength and molecular weight. *Biomaterials* 2002;**23**(18):3905–3912.

288. Park, T. G. Degradation of poly(d,l-lactic acid) microspheres: Effect of molecular weight. *J. Control. Release* 1994;**30**(2):161–173.

289. Saha, S. K. and H. Tsuji. Effects of molecular weight and small amounts of d-lactide units on hydrolytic degradation of poly(l-lactic acid)s. *Polym. Degrad. Stab.* 2006;**91**(8):1665–1673.

290. Yoon, J.-S., H.-J. Jin, I.-J. Chin, C. Kim, and M.-N. Kim. Theoretical prediction of weight loss and molecular weight during random chain scission degradation of polymers. *Polymer* 1997;**38**(14):3573–3579.

291. Allen, N. S., M. Edge, M. Mohammadian, and K. Jones. Hydrolytic degradation of poly(ethylene terephthalate): Importance of chain scission versus crystallinity. *Eur. Polym. J.* 1991;**27**(12):1373–1378.

292. Chan, C.-K. and I. M. Chu. In vitro degradation of poly(sebacic anhydride-*co*-ethylene glycol). *Mater. Chem. Phys.* 2004;**88**(1):59–66.

293. Miyajima, M., A. Koshika, J. i. Okada, M. Ikeda, and K. Nishimura. Effect of polymer crystallinity on papaverine release from poly (L-lactic acid) matrix *J. Control. Release* 1997;**49**(2–3):207–215.

294. Montaudo, G. and P. Rizzarelli. Synthesis and enzymatic degradation of aliphatic copolyesters. *Polym. Degrad. Stab.* 2000;**70**(2):305–314.

295. Schliecker, G., C. Schmidt, S. Fuchs, R. Wombacher, and T. Kissel. Hydrolytic degradation of poly(lactide-*co*-glycolide) films: Effect of oligomers on degradation rate and crystallinity. *Int. J. Pharm.* 2003;**266**(1–2):39–49.

296. Tsuji, H. and S. Miyauchi. Poly(L-lactide): VI. Effects of crystallinity on enzymatic hydrolysis of poly(L-lactide) without free amorphous region. *Polym. Degrad. Stab.* 2001;**71**(3):415–424.

297. Zilberman, M. Dexamethasone loaded bioresorbable films used in medical support devices: Structure, degradation, crystallinity and drug release. *Acta Biomater.* 2005;**1**(6):615–624.

298. Hurrell, S. and R. E. Cameron. The effect of initial polymer morphology on the degradation and drug release from polyglycolide. *Biomaterials* 2002;**23** (11):2401–2409.

299. Klose, D., F. Siepmann, K. Elkharraz, and J. Siepmann. PLGA-based drug delivery systems: Importance of the type of drug and device geometry. *Int. J. Pharm.* 2008;**354**(1–2):95–103.

300. Shen, E., M. J. Kipper, B. Dziadul, M. K. Lim, and B. Narasimhan. Mechanistic relationships between polymer microstructure and drug release kinetics in bioerodible polyanhydrides *J. Control. Release* 2002;**82**(1):115–125.

301. Witt, C., K. Mäder, and T. Kissel. The degradation, swelling and erosion properties of biodegradable implants prepared by extrusion or compression moulding of poly(lactide-co-glycolide) and ABA triblock copolymers. *Biomaterials* 2000;**21**(9):931–938.

302. Andrianov, A. K. and A. Marin. Degradation of polyaminophosphazenes: Effects of hydrolytic environment and polymer processing. *Biomacromolecules* 2006;**7** (5):1581–1586.

303. Gogolewski, S., M. Jovanovic, S. M. Perren, J. G. Dillon, and M. K. Hughes. The effect of melt-processing on the degradation of selected polyhydroxyacids: Polylactides, polyhydroxybutyrate, and polyhydroxybutyrate-*co*-valerates. *Polym. Degrad. Stab.* 1993;**40**(3):313–322.

304. Reich, G. Ultrasound-induced degradation of PLA and PLGA during microsphere processing: Influence of formulation variables. *Eur. J. Pharm. Biopharm.* 1998;**45** (2):165–171.

305. Södergård, A. and M. Stolt. Properties of lactic acid based polymers and their correlation with composition. *Prog. Polym. Sci.* 2002;**27**(6):1123–1163.

306. Yasin, M., S. J. Holland, and B. J. Tighe. Polymers for biodegradable medical devices: V. Hydroxybutyrate-hydroxyvalerate copolymers: Effects of polymer processing on hydrolytic degradation. *Biomaterials* 1990;**11**(7):451–454.

307. Johnson, L. R. and N. H. Stoller. Rationale for the use of Atridox therapy for managing periodontal patients. *Compend. Contin. Edu. Dent.* 1999;**20**(4 Suppl):19–25; quiz 35.

308. Page, R. C. The microbiological case for adjunctive therapy for periodontitis. *J. Int. Acad. Periodontol.* 2004;**6**(4 Suppl):143–149.

309. Southard, G. L., R. L. Dunn, and S. Garrett. The drug delivery and biomaterial attributes of the ATRIGEL technology in the treatment of periodontal disease. *Expert. Opin. Investig. Drugs* 1998;**7**(9):1483–1491.

310. Jonat, W. Goserelin (Zoladex)—its role in early breast cancer in pre- and perimenopausal women. *Br. J. Cancer*.2001;**85**(Suppl 2):1–5.

311. Mitchell, H. Goserelin ('Zoladex')—offering patients more choice in early breast cancer. *Eur. J. Oncol. Nurs.* 2004;**8**(Suppl 2):S95–103.
312. Schlaff, W. D. Extending the treatment boundaries: Zoladex and add-back. *Int. J. Gynaecol. Obstet.* 1999;**64**(Suppl 1):S25–31.
313. Tsukagoshi, S. A new LH-RH agonist for treatment of prostate cancer, 3-month controlled-release formulation of goserelin acetate (Zoladex LA 10.8 mg depot)—outline of pre-clinical and clinical studies. *Gan To Kagaku Ryoho.* 2002;**29** (9):1675–1687.
314. Agarwal, N., D. Fletcher, and J. Ward. Obesity and treatment of prostate cancer: What is the right dose of Lupron Depot? *Clin. Cancer Res.* 2007;**13**(13):4027.
315. Dlugi, A. M., J. D. Miller, and J. Knittle. Lupron depot (leuprolide acetate for depot suspension) in the treatment of endometriosis: A randomized, placebo-controlled, double-blind study. *Lupron Study Group. Fertil Steril.* 1990;**54** (3):419–427.
316. Machluf, M., A. Orsola, and A. Atala. Controlled release of therapeutic agents: Slow delivery and cell encapsulation. *World J. Urol.* 2000;**18**(1):80–83.
317. Anonymus. Product Portfolio. Available from: http://www.takeda.com/about-takeda/product-portfolio/article_73.html; 2009.
318. Richtering, W., J. Siepmann, and F. Siepmann. Microparticles used as drug delivery systems. In *Smart Colloidal Materials*. Springer Berlin/Heidelberg, 2006, pp. 15–21.
319. Anonymus, Eligard. Tiny implant fights prostate cancer. *Nursing* 2002;**32**(5):18.
320. Sartor, O. Eligard: Leuprolide acetate in a novel sustained-release delivery system. *Urology* 2003;**61**(2 Suppl 1):25–31.
321. Sartor, O. Eligard® 6: A new form of treatment for prostate cancer. *Eur. Urol. Supple.* 2006;**5**(18):905–910.
322. Flogstad, A. K., J. Halse, S. Bakke, I. Lancranjan, P. Marbach, C. Bruns, and J. Jervell. Sandostatin LAR in acromegalic patients: Long-term treatment. *J. Clin. Endocrinol. Metab.* 1997;**82**(1):23–28.
323. Grass, P., P. Marbach, C. Bruns, and I. Lancranjan. Sandostatin LAR (micro-encapsulated octreotide acetate) in acromegaly: Pharmacokinetic and pharmacodynamic relationships. *Metabolism* 1996;**45**(8 Suppl 1):27–30.
324. Heijckmann, C. A., P. P. Menheere, J. P. Sels, E. A. Beuls, and B. H. Wolffenbuttel. Clinical experience with Sandostatin LAR in patients with acromegaly. *Neth. J. Med.* 2001;**59**(6):286–291.
325. Lancranjan, I., C. Bruns, P. Grass, P. Jaquet, J. Jervell, P. Kendall-Taylor, S. W. Lamberts, P. Marbach, H. Orskov, G. Pagani, M. Sheppard, and L. Simionescu. Sandostatin LAR: A promising therapeutic tool in the management of acromegalic patients. *Metabolism* 1996;**45**(8 Suppl 1):67–71.
326. Cook, D. M., B. M. Biller, M. L. Vance, A. R. Hoffman, L. S. Phillips, K. M. Ford, D. P. Benziger, A. Illeperuma, S. L. Blethen, K. M. Attie, L. N. Dao, J. D. Reimann, and P. J. Fielder. The pharmacokinetic and pharmacodynamic characteristics of a long-acting growth hormone (GH) preparation (nutropin depot) in GH-deficient adults. *J. Clin. Endocrinol. Metab.* 2002;**87**(10):4508–4514.
327. Silverman, B. L., S. L. Blethen, E. O. Reiter, K. M. Attie, R. B. Neuwirth, and K. M. Ford. A long-acting human growth hormone (Nutropin Depot): Efficacy

and safety following two years of treatment in children with growth hormone deficiency. *J. Pediatr. Endocrinol. Metab.* 2002;**15**(Suppl 2):715–722.

328. Anonymus. Gliadel wafers for treatment of brain tumors. *Med. Lett. Drugs Ther.* 1998;**40**(1035):92.
329. Attenello, F. J., D. Mukherjee, G. Datoo, M. J. McGirt, E. Bohan, J. D. Weingart, A. Olivi, A. Quinones-Hinojosa, and H. Brem. Use of Gliadel (BCNU) wafer in the surgical treatment of malignant glioma: A 10-year institutional experience. *Ann. Surg. Oncol.* 2008;**15**(10):2887–2893.
330. Brem, H. and P. Gabikian. Biodegradable polymer implants to treat brain tumors. *J. Control. Release* 2001;**74**(1–3):63–67.
331. Perry, J., A. Chambers, K. Spithoff, and N. Laperriere. Gliadel wafers in the treatment of malignant glioma: A systematic review. *Curr. Oncol.* 2007;**14**(5):189–194.
332. Wang, C. C., J. Li, C. S. Teo, and T. Lee. The delivery of BCNU to brain tumors. *J. Control. Release* 1999;**61**(1–2):21–41.
333. Li, L. C., J. Deng, and D. Stephens. Polyanhydride implant for antibiotic delivery—from the bench to the clinic. *Adv. Drug. Deliv. Rev.* 2002;**54**(7):963–986.
334. Harrison, T. S. and K. L. Goa. Long-acting risperidone: A review of its use in schizophrenia. *CNS Drugs* 2004;**18**(2):113–132.
335. Keith, S. Use of long-acting risperidone in psychiatric disorders: Focus on efficacy, safety and cost-effectiveness. *Expert Rev. Neurother.* 2009;**9**(1):9–31.
336. Knox, E. D. and G. L. Stimmel. Clinical review of a long-acting, injectable formulation of risperidone. *Clin. Ther.* 2004;**26**(12):1994–2002.
337. Lessem, J. and A. Hanlon. A post-marketing study of 2805 patients treated for periodontal disease with Arestin. *J. Int. Acad. Periodontol.* 2004;**6**(4 Suppl):150–153.
338. Van Dyke, T. E., S. Offenbacher, L. Braswell, and J. Lessem. Enhancing the value of scaling and root-planing: Arestin clinical trial results. *J. Int. Acad. Periodontol.* 2002;**4**(3):72–76.
339. Anonymus. Capronor. *Hypotenuse* 1985;2–5.
340. Darney, P. D., C. M. Klaisle, S. E. Monroe, C. E. Cook, N. R. Phillips, and A. Schindler. Evaluation of a 1-year levonorgestrel-releasing contraceptive implant: Side effects, release rates, and biodegradability. *Fertil. Steril.* 1992;**58**(1):137–143.
341. Darney, P. D., S. E. Monroe, C. M. Klaisle, and A. Alvarado. Clinical evaluation of the Capronor contraceptive implant: Preliminary report. *Am. J. Obstet. Gynecol.* 1989;**160**(5 Pt 2):1292–1295.
342. Gettig, J., J. P. Cummings, and K. Matuszewski. H.P. Acthar gel and cosyntropin review: Clinical and financial implications. *P. T.* 2009;**34**(5):250–257.
343. Miller, M. A. and H. E. Bass. Effect of Acthar-c (ACTH) in sarcoidosis. *Ann. Intern. Med.* 1952;**37**(4):776–784.
344. Kim, S. C., D. W. Kim, Y. H. Shim, J. S. Bang, H. S. Oh, S. Wan Kim, and M. H. Seo. In vivo evaluation of polymeric micellar paclitaxel formulation: Toxicity and efficacy. *J. Control. Release* 2001;**72**(1–3):191–202.
345. Kim, T. Y., D. W. Kim, J. Y. Chung, S. G. Shin, S. C. Kim, D. S. Heo, N. K. Kim, and Y. J. Bang. Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free,

polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin. Cancer Res.* 2004;**10**(11):3708–3716.

346. Lee, K. S., H. C. Chung, S. A. Im, Y. H. Park, C. S. Kim, S. B. Kim, S. Y. Rha, M. Y. Lee, and J. Ro. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. *Breast Cancer Res. Treat.* 2008;**108**(2):241–250.
347. Lim, W. T., E. H. Tan, C. K. Toh, S. W. Hee, S. S. Leong, P. C. Ang, N. S. Wong, and B. Chowbay. Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol(R)-PM) in patients with solid tumors. *Ann. Oncol.* 2009;**21**(2):382–388.
348. Fu, Q., J. Sun, W. Zhang, X. Sui, Z. Yan, and Z. He. Nanoparticle albumin-bound (nab) technology is a promising method for anti-cancer drug delivery. *Recent Pat. Anticancer Drug Discov.* 2009;**4**(3):262–272.
349. Green, M. R., G. M. Manikhas, S. Orlov, B. Afanasyev, A. M. Makhson, P. Bhar, and M. J. Hawkins. Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer. *Ann. Oncol.* 2006;**17**(8):1263–1268.
350. Henderson, I. C. and V. Bhatia. Nab-paclitaxel for breast cancer: A new formulation with an improved safety profile and greater efficacy. *Expert Rev. Anticancer Ther.* 2007;**7**(7):919–943.
351. McIlwain, C., S. J. Enna, and B. B. David. *Abraxane. In xPharm: The Comprehensive Pharmacology Reference*. Elsevier: New York, 2008, pp. 1–5.
352. Miele, E., G. P. Spinelli, F. Tomao, and S. Tomao. Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. *Int. J. Nanomed.* 2009;**4**:99–105.
353. Anonymus. Dabur pharma introduces Nanoxel in India. Available from http://www.daburpharma.com/pdf/nanoinindia.pdf, 2007.
354. Krishnan, G. S. Big problem, nano solution. *Businessworld* 2007;**44**:1–2.
355. Cirkel, U., H. Ochs, and H. P. Schneider. A randomized, comparative trial of triptorelin depot (D-Trp6-LHRH) and danazol in the treatment of endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 1995;**59**(1):61–69.
356. Dal Prato, L., A. Borini, M. Cattoli, M. A. Bonu, E. Sereni, and C. Flamigni. GnRH analogs: Depot versus short formulations. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2004;**115**(Suppl 1):S40–S43.
357. Filicori, M., C. Flamigni, G. Cognigni, P. Dellai, R. Arnone, A. Falbo, and M. Capelli. Comparison of the suppressive capacity of different depot gonadotropin-releasing hormone analogs in women. *J. Clin. Endocrinol. Metab.* 1993;**77**(1):130–133.
358. Heger, S., C. J. Partsch, and W. G. Sippell. Long-term outcome after depot gonadotropin-releasing hormone agonist treatment of central precocious puberty: Final height, body proportions, body composition, bone mineral density, and reproductive function. *J. Clin. Endocrinol. Metab.* 1999;**84**(12):4583–4590.
359. Schneider, F., B. Heleil, H. Alm, H. Torner, F. Becker, T. Viergutz, G. Nurnberg, and W. Kanitz. Endocrine, morphological, and cytological effects of a depot GnRH agonist in bovine. *Anim. Reprod. Sci.* 2006;**92**(1–2):9–28.
360. Anonymus. Naltrexone (vivitrol)—a once-monthly injection for alcoholism. *Med. Lett. Drugs Ther.* 2006;**48**(1240):63–64.

361. Johnson, B. A. Naltrexone long-acting formulation in the treatment of alcohol dependence. *Ther. Clin. Risk Manag.* 2007;**3**(5):741–749.

362. Mannelli, P., K. Peindl, P. S. Masand, and A. A. Patkar. Long-acting injectable naltrexone for the treatment of alcohol dependence. *Expert Rev. Neurother.* 2007;**7** (10):1265–1277.

363. Aro, J., M. Ruutu, H. Juusela, E. Hansson, and J. Permi. Polyestradiol phosphate (160 mg/month) or LHRH analog (buserelin depot) in the treatment of locally advanced or metastasized prostatic cancer. *The Finnprostate Group. Ann. Chir. Gynaecol. Suppl.* 1993;**206**:5–8.

364. Schliecker, G., C. Schmidt, S. Fuchs, A. Ehinger, J. Sandow, and T. Kissel. In vitro and in vivo correlation of buserelin release from biodegradable implants using statistical moment analysis. *J. Control. Release* 2004;**94**(1):25–37.

365. Waxman, J., J. Sandow, H. Thomas, N. James, and G. Williams. A pharmacological evaluation of a new 3-month depot preparation of buserelin for prostatic cancer. *Cancer Chemother. Pharmacol.* 1989;**25**(3):219–220.

366. Waxman, J. H., J. Sandow, P. Abel, N. Farah, E. P. O'Donoghue, J. Fleming, J. Cox, K. Sikora, and G. Williams. Two-monthly depot gonadotropin releasing hormone agonist (buserelin) for treatment of prostatic cancer. *Acta Endocrinol. (Copenh.)* 1989;**120**(3):315–318.

367. Waxman, J. H., J. Sandow, A. Man, M. J. Barnett, W. F. Hendry, G. M. Besser, R. T. Oliver, and P. J. Magill. The first clinical use of depot buserelin for advanced prostatic carcinoma. *Cancer Chemother. Pharmacol.* 1986;**18**(2):174–175.

368. Anonymus. Abarelix: Abarelix-depot-F, abarelix-depot-M, abarelix-L, PPI 149, R 3827. *Drugs R. D* 2003;**4**(3):161–166.

369. Hogle, W. P. Abarelix (plenaxis). *Clin. J. Oncol. Nurs.* 2004;**8**(6):663–665.

370. Reddy, G. K. Abarelix (Plenaxis): A gonadotropin-releasing hormone antagonist for medical castration in patients with advanced prostate cancer. *Clin. Prostate Cancer* 2004;**2**(4):209–211.

371. Angelova, N. and D. Hunkeler. Rationalizing the design of polymeric biomaterials. *Trends Biotechnol.* 1999;**17**:409–421.

372. Kohn, J. and R. Langer. Bioresorbable and bioerodible materials in biomaterials science. In *An Introduction to Materials in Medicine*. Ratner, B. D., A. S. Hoffman, F. J. Schoen, and J. E. Lemons (Eds.). Academic Press: New York, 1996, pp. 64–72.

373. Pillai, O. and R. Panchagnula. Polymers in drug delivery. *Curr. Opin. Chem. Biol.* 2001;**5**:447–451.

PART II

BIODEGRADABLE POLYMERS OF NATURAL ORIGIN: PROTEIN-BASED POLYMERS

CHAPTER 2

COLLAGEN

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CONTENTS

Biodegradable Polymers in Clinical Use and Clinical Development, First Edition. Edited by Abraham J. Domb, Neeraj Kumar, and Aviva Ezra
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2.1 INTRODUCTION

The name *collagen* comes from Greek meaning "glue producer." When collagen is heated in water, it gradually breaks down to produce soluble derived protein, that is, gelatin or animal glue. It is the most abundant protein in the animal kingdom and is a major component of the extracellular matrix and connective tissues. It has a unique ability to form insoluble fibers that provide strength. There are more than 30 collagens and collagen-related proteins but the most abundant are collagens I and II [1].

2.2 OCCURRENCE

Collagens are the major structural elements of all connective tissues and are also found in the interstitial tissue of virtually all parenchymal organs, where they contribute to the stability of tissues and organs and maintain their structural integrity. Its type and organization is dictated by the structural role it plays in a particular organ [2]. It is found in tissues such as tendons, cartilage, organic matrix of bone, and the cornea of the eye. In some tissues, collagen may be dispersed as a gel that gives support to the structure, as in the extracellular matrix or the vitreous humor of the eye. In other tissues, collagen may be bundled in tight, parallel fibers that provide great strength, as in tendons. In the cornea of the eye, collagen is stacked so as to transmit light with a minimum of scattering. In bone, collagen forms a framework for the deposition of calcium phosphate crystals, acting like the steel cables in reinforced concrete so as to resist mechanical shear from any direction.

2.3 FUNCTIONS

Collagens are the structural building blocks of the body. They are the most abundant group of organic macromolecules in an organism and serve important

mechanical functions within the body, particularly in connective tissues. They surround the cells and give each tissue its characteristic structure, texture, and shape. In bone, tendon, fascia, and articular cartilage, fibrillar collagens provide most of the biomechanical properties essential for their functioning [2]. In tendons, collagen has the strength equal to light steel wire. Collagen in the cornea is transparent, in heart valves it is fatigue resistant, and in renal glomeruli it provides an excellent filtration system [3]. Second, collagens also exert important functions in the cellular microenvironment and are involved in the storage and release of cellular mediators (growth factors, cytokines, etc.) [4, 5].

2.4 STRUCTURE

A typical collagen molecule is a long, rigid structure in which three polypeptides (referred to as α chains) are wound around one another in a ropelike triple helix (Fig. 2.1) with a length of approximately 300 nm and a diameter of 1.5 nm [6]. Fibers made up of collagen have a high tensile strength. The collagen helix is left handed and has three amino acid residues per turn. The tight wrapping of the α chains in the collagen triple helix provides tensile strength greater than that of a steel wire of equal cross section.

2.4.1 Amino Acid Sequence

Each α-chain peptide is composed of about 1050 amino acid residues, which consist of approximately 33% glycine, 25% proline, and 25% hydroxyproline, and a relative abundance of lysine [3]. Proline and glycine are important in the formation of the triple-stranded helix. Proline facilitates the formation of the helical conformation of each α chain because its ring structure causes "kinks" in the peptide chain. Glycine, the smallest amino acid, is found in every third position of the polypeptide chain. This position is crucial as glycine is the only amino acid that is small enough to fit in the narrow core of the triple helix [7]. The amino acid

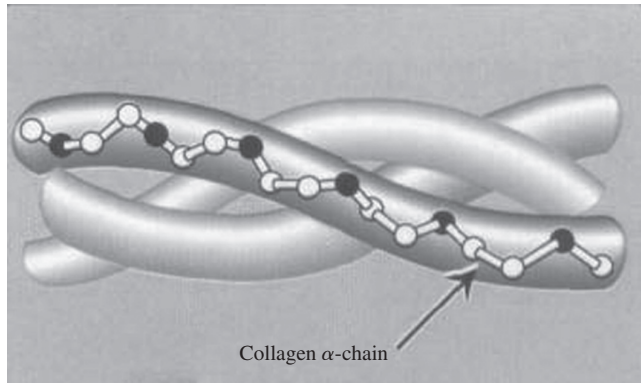

FIGURE 2.1 Triple-stranded helix of collagen.

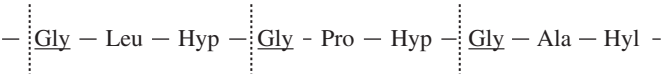

FIGURE 2.2 Primary amino acid sequence of a portion of α1 chain of collagen (Hyp is hydroxyproline and Hyl is hydroxylysine).

sequence can be represented by a repeating tripeptide unit, Gly–X–Y, where Gly is glycine, X is often proline, and Y is often hydroxyproline or hydroxylysine [8]. Thus, most of the α chain can be regarded as a polytripeptide whose sequence can be represented as $(-\text{Gly}-\text{X}-\text{Y}-)_{333}$ (Fig. 2.2).

2.4.2 Triple-Helical Structure

Collagen is also a coiled coil, that is, three separate polypeptides, called α chains are supertwisted about each other (Fig. 2.3). The three α chains are held together by interchain hydrogen bonds. The superhelical twisting is right handed in collagen, opposite in sense to the left-handed helix of its constituent α chains [9, 10]. For example, type I collagen triplex is a heteropolymer consisting of two α1 chains and one α2 chain of over 1000 residues in length [11].

Unlike most globular proteins that are folded into compact structures, collagen, a fibrous protein, has an elongated structure that places many of its amino acid side chains on the surface of the triple-helical molecule. (This allows bond formation between the exposed R groups of neighboring collagen monomers, resulting in their aggregation into long fibers). The triple helices are staggered by 67 nm with an additional gap of 40 nm between succeeding molecules (Fig. 2.4). Seen through an electron microscope, the linear polymers of fibrils have banding patterns, reflecting the regular staggered packing of the individual collagen molecules in the fibril (Fig. 2.5).

2.4.3 Hydroxyproline and Hydroxylysine

Collagen contains hydroxyproline (Hyp) and hydroxylysine (Hyl), which are not present in most other proteins. These residues result from the hydroxylation of some of the proline and lysine residues after their incorporation into polypeptide chains (Fig. 2.6). This hydroxylation is a result of posttranslational modification. Hydroxyproline is important in stabilizing the triple-helical structure of collagen because it maximizes interchain hydrogen bond formation.

FIGURE 2.3 Secondary left-handed helix and tertiary right-handed triple-helix structure showing two α1 chains (black colored) and one α2 chain (light shade colored) of collagen I molecule.

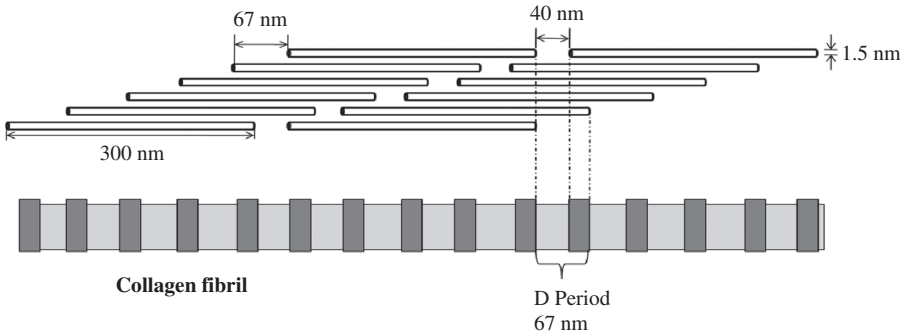

FIGURE 2.4 Schematic representation of staggered quaternary structure forming the collagen fibril (showing dark and light banding pattern of negatively stained isolated fibril).

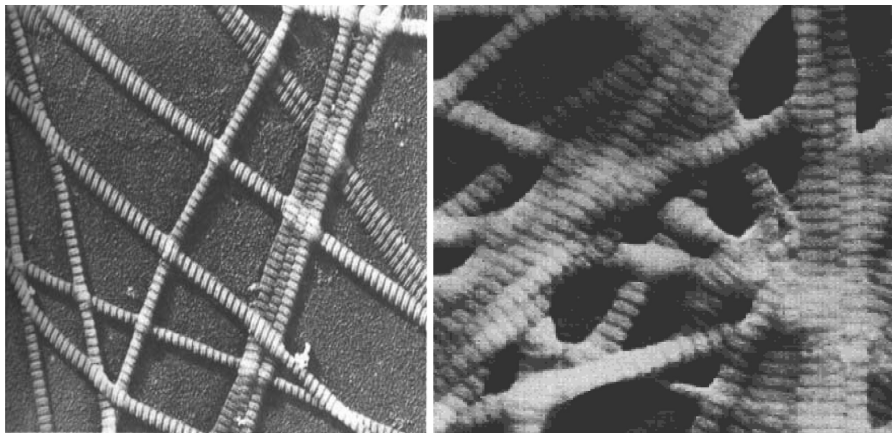

FIGURE 2.5 Staggered arrangement of collagen molecules causes striated appearance of fibrils as seen in electron micrographs of collagen fibers.

2.4.4 Glycosylation

The hydroxyl group of the hydroxylysine residues of collagen may be enzymatically glycosylated. Most commonly, glucose and galactose are sequentially attached to the polypeptide chain prior to triple-helix formation.

2.5 TYPES AND PROPERTIES

There are 29 collagen types, which differ in size, structure, and function (numbered I–XXIX with roman numerals in the order of their discovery; some with common names) [12]. Variations in the amino acid sequence of the α

TABLE 2.1 Most Abundant Types of Collagen

Collagen Type	Tissue Distribution
Fibril Forming	
Type I	Most connective tissues like skin, bone, dermis, tendon, ligaments, cornea, blood vessels
Type II	Hyaline cartilage, intervertebral disk, vitreous body
Type III	Blood vessels, fetal skin
Network Forming	
Type IV	Basement membranes
Type VII	Beneath stratified squamous epithelia
Fibril Associated	
Type IX	Cartilage
Type XII	Tendons, ligaments, some other tissues

chains result in structural components that are about the same size (approximately 1000 amino acids long) but with slightly different properties. These α chains are combined to form the various types of collagen found in tissues. The collagens can be organized into three groups, based on their location and functions in the body (Table 2.1).

2.5.1 Fibril-Forming Collagens

The most abundant and widespread family of collagens with about 90% of the total collagen is represented by the fibril-forming collagens [2]. In the form of fibers it acts to transmit forces, dissipate energy, and prevent premature mechanical failure in normal tissues [13]. Types I, II, and III are the fibrillar collagens and have the ropelike structure described above for a typical collagen molecule. Type I is the most abundant and diversely located member of the collagen family, found principally in fibril form [14].

Type I collagen fibers are found in supporting elements of high tensile strength, for example, tendon and cornea, whereas fibers formed from type II collagen molecules are restricted to cartilaginous structures and provide the tissue with its shock-absorbing properties and its resiliency to stress [15]. The fibrils derived from type III collagen are prevalent in more distensible tissues such as blood vessels.

2.5.2 Network-Forming Collagens

Types IV and VII form a three-dimensional mesh rather than distinct fibrils. For example, type IV molecules assemble into a sheet or meshwork that constitutes a major part of basement membranes, which separate cell types or divide cell layers from underlying connective tissue [16] and function as a semipermeable filtration barrier for macromolecules in organs such as the kidney and the lung.

2.5.3 Fibril-Associated Collagens (FACIT)

Types IX and XII bind to the surface of collagen fibrils, linking these fibrils to one another and to other components in the extracellular matrix. Despite the rather high structural diversity among the different collagen types, all members of the collagen family have one characteristic feature: a right-handed triple helix composed of three α chains. See Table 2.2.

TABLE 2.2 Collagen Family

Collagen Type	Structure	Tissue Distribution
Type I	Fibril-forming collagen	Most connective tissues like skin, bone, dermis, tendon, ligaments, cornea, blood vessels
Type II	Fibril-forming collagen	Hyaline cartilage, intervertebral disk, inner ear, vitreous body, nucleus pulposus
Type III	Fibril-forming collagen	Distensible and developing connective tissues, blood vessels, fetal skin
Type IV	Network-forming collagen	Basement membranes
Type V	Fibril-forming collagen	Tissues containing type I collagen, hyaline cartilage, nervous system
Type VI	Beaded filament-forming collagen	Bone, hyaline cartilage, soft connective tissues; blood vessels, skin, intervertebral disk, peripheral nerves
Type VII	Collagen of anchoring fibrils	Anchoring fibrils; skin, epidermal joints
Type VIII	Hexagonal network-forming collagen	Many tissues, especially endothelium
Type IX	FACIT[a] collagen	Hyaline cartilage, intervertebral disk, vitreous humor, cornea
Type X	Hexagonal network-forming collagen	Hypertrophic cartilage
Type XI	Fibril-forming collagen	Hyaline cartilage, intervertebral disk, inner ear
Type XII	FACIT collagen	Tissues containing type I collagen; fetal cartilage,tendons, ligaments
Type XIII	Collagen with a transmembrane domain	Many tissues like epidermis, hair follicle, nail root cells, endomysium, intestine, chondrocytes
Type XIV	FACIT collagen	Embryonic cartilage
Type XV	Endostatin-containing collagen	Many tissues in the basement membrane zone; muscle, kidney, pancreas
Type XVI	FACIT collagen	Many tissues; smooth muscle, fibroblasts, amnion, keratinocytes

(*Continued*)

TABLE 2.2 Continued

Collagen Type	Structure	Tissue Distribution
Type XVII	Collagen with a transmembrane domain	Hemidesmosomes; skin, dermal epidermal junctions
Type XVIII	Endostatin-containing collagen	Many tissues in the basement membrane zone
Type XIX	FACIT collagen	Many tissues in the basement membrane zone
Type XX	FACIT collagen	Embryonic skin, tendon, sterna cartilage, corneal epithelium
Type XXI	FACIT collagen	Many tissues (blood vessel walls)
Type XXII	FACIT collagen	Tissue junctions
Type XXIII	Collagen with a transmembrane domain	Heart, metastatic prostate cancer cells
Type XXIV	Fibril-forming collagen	Tissues containing type I collagen; developing bone, cornea
Type XXV	Collagen with a transmembrane domain	Brain, heart, testis, neurons
Type XXVI	Beaded filament-forming collagen	Testis, ovary
Type XXVII	Fibril-forming collagen	Embryonic cartilage, epithelial cell layers (stomach, lung, gonad, skin, cochlea, tooth)
Type XXVIII	Beaded filament-forming collagen	Basement membranes around Schwann cells in the peripheral nervous system
Type XXIX	Beaded filament-forming collagen	Epidermis

[a]FACIT, fibril-associated collagen with interrupted triple helices.

Source: From [2, 17–19].

2.6 BIOSYNTHESIS OF COLLAGEN

The polypeptide precursors of the collagen molecule are formed in fibroblasts (or in the related osteoblasts of bone and chondroblasts of cartilage) and are secreted into the extracellular matrix. Biosynthesis of collagens is a complex multistep process that starts with the transcription of genes within the nucleus to the aggregation of collagen heterotrimers into large fibrils [20, 21]. Collagens undergo extensive posttranslational modification in the endoplasmic reticulum prior to triple-helix formation. A number of enzymes and molecular chaperones assist in their correct folding and trimerization [8]. The collagen may be further modified to form intra- and intermolecular crosslinks, which aid in the formation of collagen fibers, fibrils, and then macroscopic bundles that are used to form tissue [22].

2.6.1 Formation of Pro-α-Chains

Collagen is one of many proteins that normally function outside of cells. Like most proteins produced for export, the newly synthesized polypeptide precursors of α chains contain a special amino acid sequence at their N-terminal ends. This acts as a signal that the polypeptide being synthesized is destined to leave the cell. The signal sequence facilitates the binding of ribosomes to the rough endoplasmic reticulum (RER) and directs the passage of the polypeptide chain into the cisternae of the RER. The signal sequence is rapidly cleaved in the endoplasmic reticulum to yield a precursor of collagen called a pro-α-chain.

2.6.2 Hydroxylation

The pro-α-chains are processed by a number of enzymatic steps within the lumen of the RER while the polypeptides are still being synthesized. Proline and lysine residues found in the Y position of the $-$Gly$-$X$-$Y$-$ sequence can be hydroxylated to form hydroxyl-proline and hydroxyl-lysine residues (Fig. 2.6). These hydroxylation reactions require molecular oxygen and the reducing agent vitamin C (ascorbic acid), without which the hydroxylating enzymes, *prolyl hydroxylase* and *lysyl hydroxylase,* are unable to function. Hydroxyproline serves to stabilize the collagen triple helix [23], its absence resulting in structurally unstable collagen [24, 25], which is not secreted from cells at a normal rate [10]. Hydroxylysine is necessary for formation of the intermolecular crosslinks in collagen [26]. In the case of ascorbic acid deficiency (and, therefore, a lack of prolyl and lysyl hydroxylation), collagen fibers cannot be crosslinked, greatly decreasing the tensile strength of the assembled fiber. One resulting deficiency disease is known as scurvy.

2.6.3 Glycosylation

Some hydroxylysine residues are modified by glycosylation with glucose or glucosyl-galactose. Glycosylation is required for the formation of a stable tertiary structure and is an alternative way of stabilizing the collagen triple helix that is independent of the presence of hyp [27].

2.6.4 Assembly and Secretion

After hydroxylation and glycosylation, pro-α-chains form procollagen, a precursor of collagen that has a central region of triple-helix flanked by the nonhelical amino- and carboxyl-terminal extensions called propeptides (Fig. 2.6). The formation of procollagen begins with the formation of interchain disulfide bonds between the C-terminal extensions of the pro-α-chains. This brings the three α chains into an alignment favorable for helix formation. The procollagen molecules are translocated to the Golgi apparatus, where they are packaged in secretory vesicles. The vesicles fuse with the cell membrane, causing the release of procollagen molecules into the extracellular space.

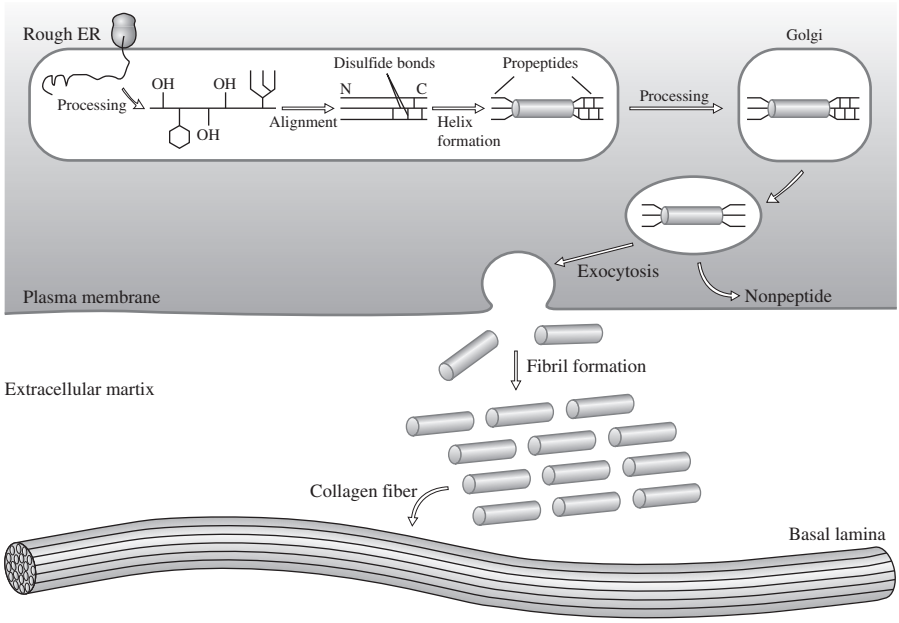

FIGURE 2.6 Major events in the biosynthesis of fibrous collagens. (From [28, 29]. Copyright © 2000 W. H. Freeman and Company.)

Modifications of the pro-α-chain polypeptide in the endoplasmic reticulum include hydroxylation, glycosylation, and disulfide-bond formation. Interchain disulfide bonds between the C-terminal propeptides of three pro-collagens align the chains in register and initiate formation of the triple helix. The process continues, zipper like, toward the N-terminus. All modifications occur in a precise sequence in the rough ER, Golgi complex, and the extracellular space, and allow lateral alignment and formation of the covalent cross-linkers that enable helices to pack into 50-nm-diameter fibrils. The α-helical region is colored red.

2.6.5 Extracellular Cleavage of Procollagen Molecules

After their release, the procollagen molecules are cleaved by *N*- and *C*-*procollagen peptidases,* which remove the terminal propeptides, releasing triple-helical collagen molecules.

2.6.6 Formation of Collagen Fibrils

Individual collagen molecules spontaneously associate to form fibrils. They form an ordered, overlapping, parallel array, with adjacent collagen molecules

arranged in a staggered pattern, each overlapping its neighbor by a length of approximately three-quarters of a molecule.

2.6.7 Crosslink Formation

The fibrillar array of collagen molecules serves as a substrate for *lysyl oxidase*. This extracellular enzyme oxidatively deaminates some of the lysyl and hydroxylysyl residues in collagen. The reactive aldehydes that result (allysine and hydroxyallysine) can condense with lysyl or hydroxylysyl residues in neighboring collagen molecules to form covalent crosslinks. (This crosslinking is essential for achieving the tensile strength necessary for proper functioning of connective tissue. Therefore, any mutation that interferes with the ability of collagen to form crosslinked fibrils almost certainly affects the stability of the collagen.) Sequence of intra and extracelluler collagen biosynthesis have been summarized in Fig. 2.7.

2.7 DEGRADATION

Normal collagens are highly stable molecules, having half-lives as long as several months. However, connective tissue is dynamic and is constantly being remodeled, often in response to growth or injury of the tissue. Breakdown of collagen fibrils in vivo is dependent on the proteolytic action of collagenases, which are part of a large family of matrix metalloproteinases [28]. For type I collagen the cleavage site is specific, generating three-quarter and one-quarter length fragments. These fragments are further degraded by other matrix proteinases to their constituent amino acids [29–31].

The triple helix is resistant to proteolytic cleavage by protein hydrolyzing enzymes such as pepsin, trypsin, and papain. *Clostridium histolyticum* produces

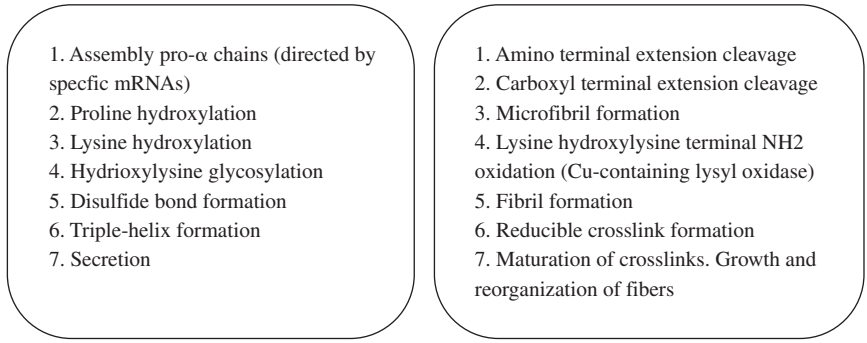

FIGURE 2.7 Summary of the different steps in collagen biosynthesis: (a) Sequence of intracellular collagen biosynthesis and (b) Sequence of extracellular collagen biosynthesis.

collagenases that cleave triple helices at numerous sites. The ability of collagens to resist cleavage by pepsin and trypsin, and their sensitivity to cleavage by bacterial collagenase, are used as research tools to identify and characterize collagens.

2.8 COLLAGEN DISORDERS

Collagens are the most abundant proteins in the body. Alterations in collagen structure resulting from abnormal genes or abnormal processing of collagen proteins results in numerous diseases. Defects in any one of the many steps in collagen fiber synthesis can result in a genetic disease involving an inability of collagen to form fibers properly. More than 1000 mutations have been identified in 22 genes coding for 12 of the collagen types. Some of the diseases that result from defective collagen synthesis include osteogenesis imperfecta, ehler danlos syndrome, lupus erythematosus, scleroderma, scurvy, polyarteritis nodosa, epidermolysis bullosa, rheumatoid arthritis, Crohn's disease, and Wegener's granulomatosis. Recent research suggests that collagens in the sclera also play an important role in the development of myopia [32].

2.9 SOURCE

Collagen has been, traditionally, isolated from the skins of land-based animals, such as cow and pig. Nondenatured collagens from these sources find applications in the cosmetics, biomedical, and pharmaceutical industries [33]. Denatured collagen, known as gelatin, finds applications in the food and biomedical industries.

2.10 CLINICAL APPLICATIONS OF COLLAGEN

Due to its excellent biocompatibility and safety, the use of collagen in biomedical applications has been rapidly growing and widely expanding to bioengineering areas. Collagen and collagen-based biomaterials have been widely used for a number of tissue engineering and medical applications (Fig. 2.8). Collagen sutures were used by Egyptian surgeons as far back as 3750 BC [3]. The main applications of collagen are collagen shields in ophthalmology, sponges for burns/wounds, minipellets for protein delivery, and as basic matrices for cell culture systems and in bone, tendon, and peripheral nerve repair [34]. Collagen can be processed into a number of forms such as sheets, films, tubes, rods, sponges, powders, injectable solutions, and dispersions [34, 35]. In most drug delivery systems made of collagen, in vivo absorption of collagen is controlled by the use of crosslinking agents, such as glutraldehyde. In order to render collagen suitable for tissue engineering applications, the mechanical strength of collagen must be enhanced. Also, the collagen-based biomaterial must be sterilized prior to use in tissue engineering applications. In recent years, studies of collagen and

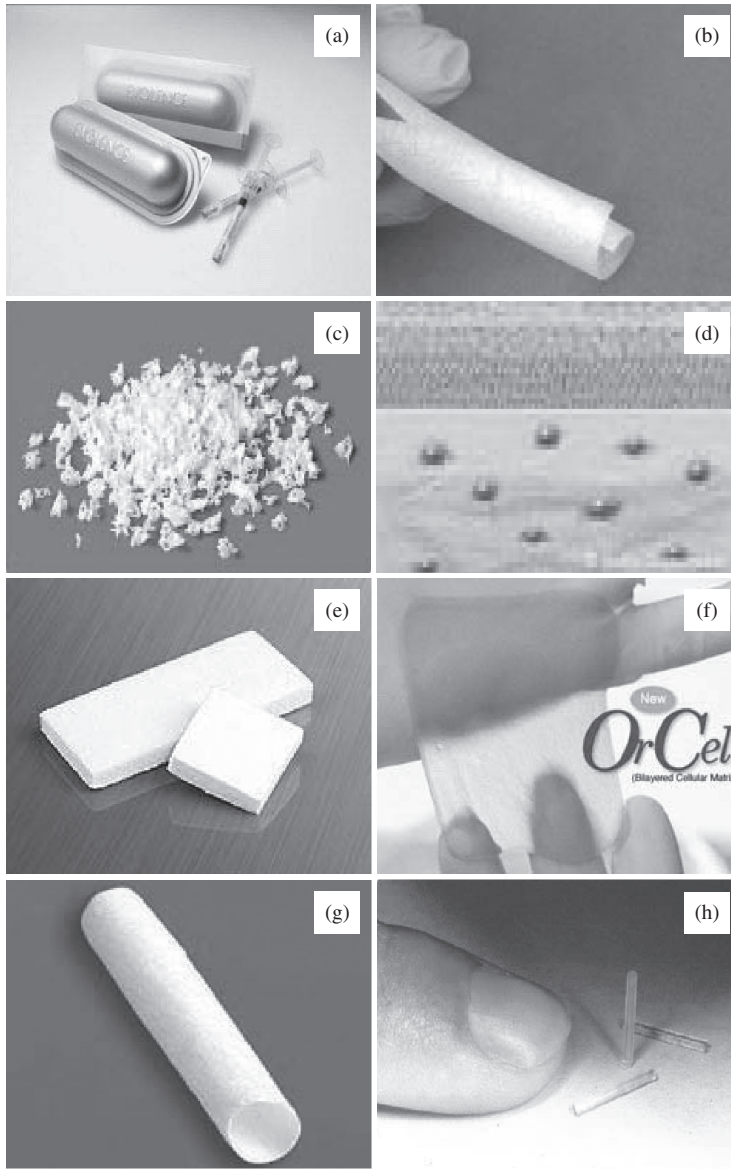

FIGURE 2.8 Different collagen marketed products: (a) Evolence, (b) TenoMend Collagen Tendon Wrap, (c) INFUSE Bone Graft, (d) Apligraf, (e) OssiMend Bone Graft Matrix, (f) OrCel, (g) NeuroMatrix/Neuroflex, and (h) Minipellets.

collagen-based materials have become an investigative hotspot especially in tissue engineering and drug delivery. Some new collagen-based drug delivery and engineered materials have been highlighted, which will promote their clinical applications [36].

2.10.1 Collagen Injections/Dermal Fillers

These are soft tissue fillers for injection into the mid to deep dermis for the correction of moderate to severe wrinkles and folds meant for restoring a smoother and younger appearance. The demands for dermal fillers have evolved dramatically during the past two decades. It has been over 25 years since the approval of the first dermal filler device, whereas the majority of the currently marketed dermal fillers were approved in the last 10 years [37]. Marketed wrinkle fillers are made up of various types of materials. The types of materials approved for dermal fillers vary from biologic to synthetic materials and absorbable to nonabsorbable compounds [38]. The ideal dermal filler should be nonpermanent but with a durable effect lasting between 1 and 2 years. Collagen was the first material to be approved by the U.S. Food and Drug Administration (FDA) for injection into facial scars, furrows, and lines in the form of dermal filler injections. Like collagen injections most of these wrinkle fillers are temporary because they are eventually absorbed by the body. Available literature strongly suggests that these dermal fillers are increasingly used these days to augment and contour tissues to improve the appearance of wrinkles. Discussion of some approved dermal fillers follows:

Zyderm Collagen Implant, Zyplast. They are bovine collagen implants approved by the FDA in 1981. Zyderm collagen implant is a sterile device composed of highly purified bovine dermal collagen (95% type I and 5% type III collagen) that is crosslinked with glutaraldehyde. It is dispersed in phosphate-buffered physiological saline containing 0.3% lidocaine. Lidocaine is used to numb the skin at the injection site. Zyderm collagen implant is available in two forms: Zyderm 1 collagen implant and Zyderm 2 collagen implant. Zyderm 2 collagen implant contains almost twice the collagen concentration of Zyderm 1 collagen implant [39].

CosmoDerm, CosmoPlast. Type I collagen loss in the dermis is one of the primary causes of wrinkles seen in aged skin. Dermal fillers using type I collagen derived from bioengineered skin are now being used with the trade names of CosmoDerm and CosmoPlast. Many aesthetic physicians are using them alone or in combination with hyaluronic acid fillers to replace both of these natural components of the skin [40]. CosmoDerm and CosmoPlast are sterile devices composed of highly purified human-based collagen that is dispersed in phosphate-buffered physiological saline containing 0.3% lidocaine. These are injected into the superficial papillary dermis for correction of soft tissue contour deficiencies such as wrinkles and acne scars.

Evolence Porcine Collagen Dermal Filler. Evolence is a new porcine-derived collagen gel based on the Glymatrix crosslinking technology, which results in a more natural and longer-lasting collagen product [41]. Evolence collagen filler is composed of 3.5% homogenous type I collagen that was extracted and purified from porcine tendons and suspended in phosphate-buffered saline and crosslinked with a ribose-mediated technology. Evolence is injected into the inner layers of facial skin (mid to deep dermis) in order to correct moderate to deep facial wrinkles and folds such as those around the nose and mouth [42, 43].

Injection of dermal fillers is one of the most commonly performed cosmetic procedures. Serious complications from fillers are rare but potentially devastating to patients and physicians. Granulomatous reactions to dermal fillers for tissue augmentation are rare but possible late complications occurring both with permanent (more frequent) and resorbable products. Skin necrosis, such as nasal alar necrosis, is one of the most feared serious complications of dermal fillers. Physicians should be aware of early intervention and treatment options should impending necrosis become apparent [44, 45].

2.10.2 Matrix Collagen Sponges

Matrix collagen sponge is an absorbent, porous collagen matrix intended for the management of moderately to heavily exudating wounds, for bone formation, and for drug delivery purpose [34].

Collagen Sponges for Wound Healing. The global wound care market was approximately $10 billion in 2007 and is projected to grow to $12.5 billion by 2012. The broad variety of wound types has resulted in a wide range of new wound dressings being frequently introduced into the market. The ideal dressing should achieve rapid healing at reasonable cost with minimal inconvenience to the patient. Pharmacological agents such as antibiotics, vitamins, minerals, and growth factors take active part in the healing process, hence, direct delivery of these agents to the wound site is desirable [46]. The development of actively hemostatic wound dressings for use in severe trauma is a major public health and military goal. Although some manufacturers claim that existing dressings activate platelets and/or blood coagulation, mechanistic evidence is often lacking [47].

Wound healing is an area in which there have been many recent advances using various different polymers. Collagen sponges have following properties making it suitable for purpose of wound healing.

1. Collagen sponges have the ability to easily absorb large quantities of tissue exudates preventing moist climate and secondary bacterial infections, few such marketed products include CollaStat, CollaCote, and Helistat absorbable collagen wound dressing and hemostatic products.

2. Coating of collagen sponge with growth factors further facilitates fast wound healing [48, 49].
3. Delivery of platelet-derived growth factor with sponges plays an important role in tissue regeneration and wound repair [50].
4. Collagen sponges are appropriate for delivery of antibiotics, such as gentamicin, to achieve high concentration of gentamicin at the local sites for treatment of implant-related infections [51] and for diabetic foot ulcers [52]. Ciprofloxacin-loaded sponges have also been proposed as an on-site delivery system [53].
5. Controlled delivery of silver sulfadiazine from collagen scaffold is achieved in infected deep burn wounds where dermis is lost [54].
6. For highly resilient activity, collagen sponges are combined with other materials such as elastin, fibronectin, or glycosaminoglycans [55].

A few marketed products include the following:

CellerateRx. CellerateRx is available for acute and chronic wounds, which include pressure ulcers of stage II–IV, diabetic ulcers, surgical wounds, ulcers due to arterial insufficiency, venous stasis ulcers, traumatic wounds, burns, and superficial wounds. It can also be used as an adjunct to current wound care therapies, helping to increase the efficacy of traditional and/or more expensive advanced wound care management.

Promogran. Promogran matrix wound dressing is a primary dressing comprised of 55% collagen and 45% oxidized regenerated cellulose. Promogran is indicated for the management of exuding wounds including diabetic ulcers, venous ulcers, ulcers caused by mixed vascular etiologies, full thickness and partial thickness wounds, donor sites and other bleeding surface wounds, abrasions, traumatic wounds healing by secondary intention, and dehisced surgical wounds [56, 57].

FloSeal Matrix. FloSeal Matrix is a new, two-component (collagen granules and thrombin) topical hemostatic sealant. FloSeal Matrix is an effective hemostasis adjunct in patients undergoing lacrimal surgery. It has the added benefits of ease of use and high patient satisfaction [58].

Collagen Sponges as Bone Substitutes. In the United States alone, approximately 500,000 patients annually undergo surgical procedures to treat bone fractures [59]. Favorable influence of collagen on cellular infiltration and wound healing is well known, subsequently, the matrix collagen sponge as an absorbent porous matrix is used for osteoinduction in the bone formation process. Bone serves as a powerful marker for regeneration, and its formation serves as a prototype model for tissue engineering based on morphogenesis.

Collagen in combination with other polymers or chemicals was also used for orthopedic defects. Collagen sponges containing bone morphogenetic protein

(BMP) were tested with and without antibiotic for the evaluation of the efficacy of BMP on promoting bone healing to induce bone formation [60, 61]. For optimal effect, BMP must be combined with an adequate matrix, which serves to prolong the residence time of the protein. Therefore, in clinical use recombinant human bone morphogenetic protein (rhBMP-2) was soaked onto an absorbable collagen sponge for bone regeneration [34, 59].

Reparation of bone defects remains a major clinical and economic concern, with more than 3 million bone grafts performed annually in the United States and Europe [62]. The search for alternatives to autologous bone grafting led to the approval by the U.S. FDA of an absorbable collagen carrier combined with rhBMP-2 for the treatment of certain bone diseases and fractures [62]. Demineralized bone collagen is also used as a bone graft material for the treatment of acquired and congenital orthopedic defects either by itself or in combination with hydroxyapatite.

Demineralized bone collagen in combination with hydroxyapatite is an excellent osteoinductive material and could be used as a bone substitute. In recent years, excipient systems have been used increasingly in biomedicine in reconstructive and replacement surgery, as bone cements, drug delivery vehicles, and contrast agents. The most bioefficient in terms of collagen formation and apatite nucleation materials are those that are able to provide soluble mineralizing species (Si, Ca, PO_4) at their implant sites and/or are doped or have been surface activated with specific functional groups [63].

A few marketed products include the following:

INFUSE Bone Graft. Local delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) as a bone graft substitute in spinal fusion was approved in July 2002 by the U.S. FDA as INFUSE Bone Graft. It consists of rhBMP-2 placed on an absorbable collagen sponge. INFUSE® Bone Graft induces new bone tissue at the site of implantation. This same product was FDA approved for a tibia long bone fresh fracture bone grafting application in August 2004, and for sinus elevation and alveolar defects associated with extraction sockets in March 2007. In addition, a new carrier is under clinical evaluation that will offer longer rhBMP-2 sustained release and compression resistance, further expanding the clinical utility of rhBMP-2 [64].

BIO-OSS K, BIO-OSS Blocks, and BIO-OSS Collagen. These are natural nonantigenic, porous bone mineral matrices, produced by the removal of all organic components from bovine bone. Due to their natural structure, BIO-OSS K, BIO-OSS Blocks, and BIO-OSS Collagen are physically and chemically comparable to the mineralized matrix of human bone. It is available as cortical granules and blocks [65, 66].

TenoMend Collagen Tendon Wrap. It is a resorbable type I collagen matrix that provides a nonconstricting encasement for injured tendons. TenoMend Collagen Tendon Wrap is designed to be an interface between the tendon and

tendon sheath or the surrounding tissue [67]. When hydrated, TenoMend Collagen Tendon Wrap is a conformable, nonfriable, self-curling collagen sheet designed for easy placement under, around, or over the injured tendon. TenoMend Collagen Tendon Wrap was successful in providing a protective environment for tendon repair.

OssiMend Bone Graft Matrix. OssiMend is an all-natural mineral–collagen composite bone graft material that, combined with autologous bone marrow, is used to fill bony voids or gaps of the skeletal system, including spine, extremities, and pelvis. OssiMend is ideal for use in spine fusion procedures; it is conformable, nonfriable, with high absorption capacity. OssiMend is provided as a sterile, dry material that is hydrated with autologous bone marrow at the point of use. OssiMend strips and pads can be cut into shapes and are designed to retain their shape and physical integrity following implantation into a bony site, while the granular form can be molded to fit the bone defect. The product is fully resorbed during the natural process of bone formation and remodeling.

Collagen Sponge Skin Replacement. Skin grafting is an important procedure to cover skin defects. Both artificial and natural polymers have been used to substitute connective tissue matrix and to reconstitute dermis. In the last decade, the main interest has been on the development of an in vitro reconstructed skin that can be transplanted directly to the wound bed and permanently replacing the missing tissue [68, 69]. Collagen is a natural substrate for cellular attachment, growth, and differentiation and is generally treated as a self-tissue by recipients into whom it is placed. Collagen has functional properties and certain sequences of the collagen fibrils are chemotactic and promote cellular proliferation and differentiation.

To constitute a composite skin substitute that can proliferate well with epidermal stem cells and fibroblasts on collagen sponge is needed [70]. Reconstituted type I collagen is suitable for skin replacement and burn wounds due to their mechanical strength and biocompatibility. In cultured skin substitutes, contracted collagen lattice has been used as a support for epithelial growth and differentiation to replace pathological skin. Allogenic cultured dermal substitute prepared by plating fibroblasts on to a collagen sponge matrix and subsequently freeze dried from a 1% aqueous solution of collagen provided a good environment for epithelialization [34]. Addition of selected antimicrobial drugs such as amikacin to the implantable collagen managed to control microbial contamination and increased healing of skin wounds. To address limitations such as deficient barrier function in vitro and delayed keratinization after grafting can be prevented by modifications of collagen-based systems by the combination of collagen with other proteins, such as glycosaminoglycans, fibrin, and biotin [34]. A few marketed products include the following:

Apligraf (Graftskin). Diabetic foot ulcerations can be a devastating complication of diabetes, causing prolonged hospitalization and significant morbidity.

Apligraf is a bioengineered living skin equivalent that consists of a dermal layer of allogenic human fibroblast cells, type I bovine collagen cells, and an epidermal layer of allogenic human keratinocyte cells. Apligraf is used for the treatment of noninfected partial and full thickness skin ulcers and for the treatment of neuropathic diabetic foot ulcers. Studies have demonstrated that Apligraf works through the delivery of growth factors and cytokines to the chronic wound environment [71].

OrCel. OrCel is a bilayered cellular matrix in which normal human allogeneic skin cells (epidermal keratinocytes and dermal fibroblasts) are cultured in two separate layers into a type I bovine collagen sponge. Extracellular secretion of cytokines and growth factors by the living cells in OrCel is a major contributing factor to the ability to accelerate wound healing. The product closes difficult wounds, such as skin ulcers in limbs or from diabetes, using a collagen matrix with embedded cells that form skin cells [72].

Collagen Sponges Repair Peripheral Nerve Damage. Approximately 300,000 people in the United States live with the effects of spinal cord injury and approximately 200,000 nerve repair procedures are performed annually in the United States. Peripheral nerve injuries have traditionally been the most challenging cases for surgeons. The treatment for long nerve injuries requires removing a donor nerve from a different location and using it to join together the severed nerve. Limitations of this method include requiring two incisions (for donor harvest and repair), creating donor site morbidity, and not always resulting in full functional recovery. To determine the rate of achieving electrophysiologically proved the functional recovery by autonomic nerve regeneration, with the aid of an artificial nerve conduit [73].

Recombinant human basic fibroblast growth factor (rhbFGF) is a peptide with many bioactivities such as promoting proliferation and migration of various cells. It plays an important role in neuroprotection and enhancement of nerve regeneration. To prolong the bioactivity of rhbFGF and to enhance its biological effects, absorbable collagen sponges were used to control the release of rhbFGF [74]. Thus, the goal of using collagen sponges is to produce biodegradable drug delivery scaffolds using collagen as nerve substitute for peripheral nerve repair to integrate with the damaged tissue and to promote consistent fully functional recovery following potential advantages:

- Clinical effectiveness in tensionless repair
- Eliminates donor site morbidity associated with autografts
- No second surgery required to harvest autograft
- Designed to be noninflammatory and resorbable

A few marketed products include the following:

NeuroMatrix/Neuroflex. Neuroflex and NeuroMatrix are safe, resorbable, collagen-based tubular matrices intended for use in the repair of severed

peripheral nerves. Both products are implanted using an efficient entubulation technique. The tubular nerve guide bridges the severed nerve and provides a protective environment to guide the regeneration of the nerve across the gap. These products are resorbable, semipermeable, collagen tubular matrix designed to create a conduit for axon growth across a nerve gap.

Collagen Sponges for Drug Delivery. Due to its biocompatibility and well-established safety profile, collagen represents a favorable matrix for on-site drug delivery. The applications covered includes: treatment and prophylaxis of bone and soft tissue infections, wound healing, as well as ophthalmic and periodontal treatment [75]. Based on the tissue repair and hemostyptic properties of collagen sponges, combinations of sponges with various drugs were developed. Advantages of drug-incorporated collagen particles have been described for the controlled delivery system for therapeutic actions.

A few marketed products include the following:

Collatamp G Topical and CollaRx® Bupivacaine Implant. The Collatamp G surgical implant is designed for site-specific delivery of pharmaceuticals, particularly antibiotics and anaesthetics. The topical application of Collatamp G comprises a gentamicin-impregnated collagen sponge that combines an active dressing with local antibiotic delivery for the treatment or prevention of infected skin ulcers, such as those caused on the legs and feet by diabetes. The CollaRx bupivacaine surgical implant is designed to provide local postoperative pain relief and thereby also reduce opioid dependency. The implant is a leave-behind collagen matrix loaded with the local anaesthetic bupivacaine, which has broad applicability across a wide range of general and orthopedic surgery [76].

2.10.3 Minipellets and Tablets for Protein Delivery

Minipellet is a matrix-type cylindrical solid collagen preparation for injection that has been developed for delivery of various compounds. Collagen minipellets have shown to be useful for controlled release of various protein drugs such as interferon, nerve growth factor, interleukin-2, fibroblast growth factor, and granulocyte colony-stimulating factor [35, 77]. Minipellets are also used to examine the effects of various additives on the profiles of rhBMP-2 release from minipellet, it is useful as a controlled-release formulation to release rhBMP-2 around the implanted site [78, 79]. Collagen pellets have also been studied extensively as a gene delivery carrier [80]. The advantages of the minipellet include [78] the following:

- Carrier material is a biodegradable natural protein collagen.
- Manufactured under mild processing conditions without any organic solvent or heating process, thus preventing denaturation of collagen and incorporated drug.

- Minipellet has sufficient mechanical strength for easily administration using conventional injection.
- Minipellet is small enough to be injected into the subcutaneous space through a syringe needle and yet spacious enough to contain large molecular weight protein drugs [34].
- A single subcutaneous injection of a minipellet causes prolonged retention of drugs and decreases its maximal concentration in the serum.
- Gradual slow release is obtained from collagen minipellet and exhibit less swelling as compared to sponge and film formulations.
- Minipellet was used as carrier for local delivery of various drugs including minocycline and lysozyme [34].

2.10.4 Collagen Gels

Hydrogels have been widely used as a drug carrier due to its ease of manufacturing and self-application. The production of a large and constant surface area is one of the major merits to be widely used for clinical and fundamental applications [34]. Collagen gels are flowable, suggesting the possibility of an easily injectable, biocompatible matrix for drug delivery [79] and tissue engineering [81]. These gels have been employed as scaffolds in tissue engineering [82] and as delivery matrices for cells and genes in gene therapy. They exhibit the following properties [79]:

- Good cell and tissue compatibility
- Drug release from matrices in a controlled manner
- Viscoelastic in nature, that is, semi-solid when at rest but can be induced to flow under, that is, stress
- No interference with normal function at the site of implantation and systemically

A few marketed products include the following:

Collatek Hydrogel. It is a sterile wound dressing consisting of polyacrylic acid and collagen. This dressing encourages healing by maintaining a moist environment at the wound site in dry to lightly exudating wounds. Collatek Hydrogel is intended for use on dry, light, and moderately exudating wounds of the following types: first- and second-degree burns, severe sunburns, abrasions, cuts, surgical wounds, pressure ulcers, venous stasis ulcers, ulcers caused by mixed etiologies, diabetic ulcers, donor sites, and grafts.

CollaWound Hydrogel. It is a collagen-based emulsion/liquid formulation comprising collagen derived from porcine hides. It is intended for the management of partial and full thickness wounds, pressure ulcers, diabetic ulcers,

venous ulcers, surgical wounds, first- and second-degree burns, superficial injuries, cuts, and abrasions.

2.10.5 Collagen in Ophthalmology

Successful treatment of eye diseases requires effective concentration of the drug in the eyes for a sufficient period of time. Conventional ocular drug delivery including eye drops, systemic administration, and ophthalmic ointments is no longer sufficient to combat ocular diseases [83]. Medical devices based on collagen have numerous applications in ophthalmology as grafts for corneal replacement, suture material, bandage lenses, or viscous solutions for use as vitreous replacements or protectants during surgery.

Inserts/Plugs. One of the most widely studied drug carrier applications of collagen are inserts and plugs for drug delivery to the corneal surface. The concept of using ocular collagen inserts to provide prolonged delivery of medication to the eye was initiated in the early 1970s [84]. Collagen plugs may be used as follows:

- As a diagnostic aid to determine the potential effectiveness of long-term lacrimal occlusion
- To prevent complications due to dry eyes after surgery
- To evaluate the dry component of ocular surface disease including conjunctivitis, corneal ulcer, pterygium, blepharitis, keratitis, red lid margins, recurrent corneal erosion, and filamentary keratitis
- To temporarily enhance the efficacy of topical medications. Release of pilocarpine can be extended for over 25 min, whereas when succinylated collagen insert was loaded with erythromycin estolate, effective concentration release was observed for more than 12 h [84].

A marketed product include the following:

UltraPlug Collagen Plugs. These collagen plugs are intended for temporary use with patients experiencing dry eye symptoms such as redness, burning, itching, or foreign body sensations, which can be relieved by blockage of the canaliculus. The UltraPlug collagen plug is composed of purified collagen, derived from the serosal layer of bovine intestines.

Shields. Collagen shields are manufactured from porcine or bovine collagen. The thin collagen films match the shape of the cornea when applied to the eye, provide sufficient oxygen transmission to allow corneal metabolism, and act as short-term bandage lenses. As the shields dissolve, they provide a layer of collagen solution that seems to lubricate the surface of the eye, minimize rubbing of the lids on the cornea, and faster epithelial healing [34]. The shields

come in a dehydrated form and have to be soaked with liquid prior to application. These devices could also be used to deliver ophthalmic medication when immersed in an aqueous drug solution/suspension immediately before placement in the eye. Collagen matrix may [84]

- Reversibly absorb drug molecules that were subsequently released.
- Act as a reservoir for drug and increasing the contact time between drug and cornea.
- Reduce the likelihood of systemic toxicity since dose reduction is possible [84, 85].

Presoaking the collagen shield in a pharmacological agent with adjunctive topical treatment represents the most efficacious method of utilizing collagen shields for drug delivery: for example, penetration of commercially available gatifloxacin and moxifloxacin into the anterior chamber of a rabbit eye was evaluated using collagen shields presoaked in the antibiotics. Results suggested that collagen shields can be more effective as a drug delivery system for these drugs [85].

An example of a marketed product follows:

Ocusert. It is an insoluble ophthalmic insert classified in the group of diffusional systems. It consists of a central reservoir of pilocarpine enclosed between two semipermeable membranes that allow the drug to diffuse from the reservoir at a precisely determined rate for a period of 7 days.

Hydrogels. The efficacy of ophthalmic semisolid hydrogels is mostly based on an increase of ocular residence time; this may be achieved by enhanced viscosity and mucoadhesive properties. Various formulations are reported using a variety of polymers that are initially liquids but gel after administration to the eye. When applied, the gels will remain in place in the cul-de-sac of the eye substantially longer than liquid formulations and will allow a sustained delivery of drugs or antibiotics [84]. Composite collagen hydrogel containing protein encapsulated alginate microspheres has been developed for ocular applications. The composite hydrogel supports human corneal epithelial cell growth and has adequate mechanical strength and excellent optical clarity for possible use as therapeutic lens for drug delivery or as corneal substitute for transplantation into patients who have corneal diseases [86].

Corneal Replacement Grafts. The need for corneas suitable for transplantation, combined with the decreasing supply, has fueled interest in the development of a corneal replacement [87]. Cornea is a fairly simple, sparsely populated, avascular, multilaminar structure and comprised of different cell types that require successful culturing techniques if a cornea is to be constructed by tissue engineering methods. In spite of initial success, no corneal construct has been produced so far that is appropriate for clinical use.

REFERENCES

1. Kadler, K. E., A. Hill, and E. G. Canty-Laird. Collagen fibrillogenesis: Fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr. Opin. in Cell Biol.* 2008;**20**(5):495–501.
2. Gelse, K., E. Pöschl, and T. Aigner. Collagens—structure, function, and biosynthesis. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1531–1546.
3. O'Grady, J. E. and D. M. Bordon. Global regulatory registration requirements for collagen-based combination products: Points to consider. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1699–1721.
4. Aigner, T. and J. Stöve. Collagens—major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1569–1593.
5. Kanematsu, A., A. Marui, S. Yamamoto, M. Ozeki, Y. Hirano, M. Yamamoto, O. Ogawa, M. Komeda, and Y. Tabata. Type I collagen can function as a reservoir of basic fibroblast growth factor. *J. Control. Release* 2004;**99**(2):281–292.
6. Kadler, K. E., D. F. Holmes, J. A. Trotter, and J. A. Chapman. Collagen fibril formation. *Biochem. J.* 1996;**316**(Pt 1):1.
7. Peter, H. B. and G. C. William. Osteogenesis imperfecta. In *Connective Tissue and Its Heritable Disorders: Molecular, Genetic, and Medical Aspects*, 2nd ed. P. M. Royce and B. Steinmann (Eds.). John Wiley & Sons: New Jersey, USA, 2003, pp. 385–430.
8. Kadler K. E., C. Baldock, J. Bella, and R. P. Boot-Handford. Collagens at a glance. *J. Cell Sci.* 2007;**120**(12):1955.
9. Traub, W., K. A. Piez, C. B. Anfinsen, and M. R. Frederic. *The Chemistry and Structure of Collagen. Advances in Protein Chemistry*. Academic Press: New York, 1971, pp. 243–352.
10. Prockop, D. J., R. A. Berg, K. I. Kivirikko, J. Uitto, G. N. Ramachandran, and A. H. Reddi. *Biochemistry of collagen*. G. N. Ramachandran and A. H. Reddi (Eds.). Plenum: New York, 1976, pp. 163–273.
11. Wess, T. J., and J. P. Orgel. Changes in collagen structure: Drying, dehydrothermal treatment and relation to long term deterioration. *Thermochim. Acta.* 2000;**365** (1–2):119–128.
12. Koopmans, G., B. Hasse, N. Sinis, G. Stefano, T. Pierluigi, and B. Bruno. The role of collagen in peripheral nerve repair. In *International Review of Neurobiology*. Academic Press: San Diego, 2009, pp. 363–379.
13. Pins, G. D., D. L. Christiansen, R. Patel, and F. H. Silver. Self-assembly of collagen fibers. Influence of fibrillar alignment and decorin on mechanical properties. *Biophys. J.* 1997;**73**(4):2164–2172.
14. Orgel, J. A. Miller, T. C. Irving, R. F. Fischetti, A. P. Hammersley, and T. J. Wess. The in situ supermolecular structure of type I collagen. *Structure* 2001; **9**(11):1061–1069.
15. Cao, H. and S. Y. Xu. Purification and characterization of type II collagen from chick sternal cartilage. *Food Chem.* 2008;**108**:439–445.
16. Barnard, K., L. J. Gathercole, and A. J. Bailey. Basement membrane collagen–evidence for a novel molecular packing. *FEBS Lett.* 1987;**212**(1):49–52.

17. Myllyharju, J. and K. I. Kivirikko. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet*. 2004;**20**(1):33–43.
18. Fox, M. A. Novel roles for collagens in wiring the vertebrate nervous system. *Curr. Opin. Cell Biol*. 2008;**20**(5):508–513.
19. Ihanamäki, T., L. J. Pelliniemi, and E. Vuorio. Collagens and collagen-related matrix components in the human and mouse eye. *Prog Retinal Eye Res*. 2004;**23**(4): 403–434.
20. Prockop, J. D. Collagens: Molecular biology, diseases, and potentials for therapy. *Ann. Rev. Biochem*. 1995;**64**(1):403–434.
21. Kietty, C. M. and M. E. Grant. The collagen family: Structure, assembly, and organization in the extracellular matrix. In P. M. Royce and B. Steinmann (Eds.). *Connective Tissue and Its Heritable Disorders: Molecular, Genetic, and Medical Aspects*, 2nd ed. John Wiley & Sons, New Jersey, USA, 2003, pp. 159–221.
22. Piez, K. A. Structure and assembly of the native collagen fibril. *Connect. Tissue Res*. 1982;**10**(1):25–36.
23. Ramachandran, G. N. and C. Ramakrishnan. Molecular structure. *Biochem. Collagen* 1972;**172**:45–84.
24. Berg, R. A. and D. J. Prockop. The thermal transition of a non-hydroxylated form of collagen. Evidence for a role for hydroxyproline in stabilizing the triple-helix of collagen. *Biochem. Biophy. Res. Commun*. 1973;**52**(1):115–120.
25. Jimenez, S., M. Harsch, and J. Rosenbloom. Hydroxyproline stabilizes the triple helix of chick tendon collagen. *Biochem. Biophys. Res. Commun*. 1973;**52**(1): 106–114.
26. Eyre, D. R. Collagen: Molecular diversity in the body's protein scaffold. *Science* 1980;**207**(4437):1315.
27. Bann, J. G., D. H. Peyton, and H. P. Bächinger. Sweet is stable: Glycosylation stabilizes collagen. *FEBS Lett*. 2000;**473**(2):237–240.
28. Stricklin, G. P., M. S. Hibbs, and M. E. Nimmi (Eds.). *Collagen*. 1988; CRC Press, Boca Ratan.
29. Everts V., E. van der Zee, L. Creemers, and W. Beertsen. Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling. *Histochem. J*. 1996;**28**(4):229–245.
30. Kucharz, E. J., and F. M. Pope (Eds.). *The Collagens: Biochemistry and Pathophysiology*. Springer-Verlag: Berlin, Germany, 1992.
31. Sabeh, F., I. Ota, K. Holmbeck, H. Birkedal-Hansen, P. Soloway, M. Balbin, C. Lopez-Otin, S. Shapiro, M. Inada, S. Krane, E. Allen, D. Chung, and S. J. Weiss. Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP. *J. Cell Biol*. 2004;**167**(4):769–781.
32. Yang, Y., X. Li, N. Yan, S. Cai, and X. Liu. Myopia: A collagen disease? *Med. Hypoth*. 2009;**73**(4):485–487.
33. Ogawa, M., R. J. Portier, M. W. Moody, J. Bell, M. A. Schexnayder, and J. N. Losso. Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (Pogonia cromis) and sheepshead seabream (Archosargus probatocephalus). *Food Chem*. 2004;**88**(4):495–501.

34. Lee, C. H., A. Singla, and Y. Lee. Biomedical applications of collagen. *Int. J. Pharm.* 2001;**221**(1–2):1–22.
35. Sano, A., T. Hojo, M. Maeda, and K. Fujioka. Protein release from collagen matrices. *Adv. Drug Deliv. Rev.* 1998;**31**(3):247–266.
36. Wang, B., K. Y. Wang, and Y. Ye. [Study status of collagen-based biomaterials in drug release and tissue engineering]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2004;**18**(2):112–114.
37. Beer, K. Dermal fillers and combinations of fillers for facial rejuvenation. *Dermatol. Clin.* 2009;**27**(4):427–432.
38. Smith, K. C. Reversible vs. nonreversible fillers in facial aesthetics: Concerns and considerations. *Dermatol. Online J.* 2008;**14**(8):3.
39. Cockerham, K. and V. J. Hsu. Collagen-based dermal fillers: past, present, future. *Facial Plast. Surg.* 2009;**25**(2):106–113.
40. Bauman, L. CosmoDerm/CosmoPlast (human bioengineered collagen) for the aging face. *Facial Plast. Surg.* 2004;**20**(2):125–128.
41. Monstrey, S. J., S. Pitaru, M. Hamdi, K. Van Landuyt, P. Blondeel, J. Shiri, A. Goldlust, and D. Shoshani. A two-stage phase I trial of Evolence30 collagen for soft-tissue contour correction. *Plast. Reconstr. Surg.* 2007;**120**(1):303–311.
42. Pitaru, S., M. Noff, L. Blok, E. Nir, S. Pitaru, A. Goldlust, and N. Savion. Long-term efficacy of a novel ribose-cross-linked collagen dermal filler: a histologic and histomorphometric study in an animal model. *Dermatol. Surg.* 2007;**33** (9):1045–1054; discussion 1054.
43. Smith, K.C. New fillers for the new man. *Dermatol. Ther.* 2007;**20**(6):388–393.
44. Grunebaum, L. D., I. Bogdan Allemann, S. Dayan, S. Mandy, and L. Baumann. The risk of alar necrosis associated with dermal filler injection. *Dermatol. Surg.* 2009;**35**(Suppl 2):1635–1640.
45. Hirsch, R. J. and M. Stier. Complications of soft tissue augmentation. *J. Drugs Dermatol.* 2008;**7**(9):841–845.
46. Boateng, J. S., K. H. Matthews, H. N. Stevens, and G. M. Eccleston. Wound healing dressings and drug delivery systems: A review. *J. Pharm. Sci.* 2008; **97**(8): 2892–2923.
47. Jesty, J., M. Wieland, and J. Niemiec. Assessment in vitro of the active hemostatic properties of wound dressings. *J. Biomed. Mater. Res. B Appl. Biomater.* 2009;**89B** (2):536–542.
48. Kanematsu, A., A. Marui, S. Yamamoto, M. Ozeki, Y. Hirano, M. Yamamoto, O. Ogawa, M. Komeda, and Y. Tabata. Type I collagen can function as a reservoir of basic fibroblast growth factor. *J. Control. Release* 2004;**99**(2):281–292.
49. Kanematsu, A., S. Yamamoto, M. Ozeki, T. Noguchi, I. Kanatani, O. Ogawa, and Y. Tabata. Collagenous matrices as release carriers of exogenous growth factors. *Biomaterials* 2004;**25**(18):4513–4520.
50. Sun, B., B. Chen, Y. Zhao, W. Sun, K. Chen, J. Zhang, Z. Wei, Z. Xiao, and J. Dai. Crosslinking heparin to collagen scaffolds for the delivery of human platelet-derived growth factor. *J. Biomed. Mater. Res. B Appl. Biomater.* 2009;**91**(1):366–372.
51. Swieringa, A. J., J. H. Goosen, F. G. Jansman, and N. J. Tulp. In vivo pharmacokinetics of a gentamicin-loaded collagen sponge in acute periprosthetic infection: serum values in 19 patients. *Acta Orthop.* 2008;**79**(5):637–642.

52. Griffis, C. D., S. Metcalfe, F. L. Bowling, A. J. Boulton, D. G. Armstrong. The use of gentamycin-impregnated foam in the management of diabetic foot infections: A promising delivery system? *Expert Opin. Drug Deliv.* 2009;**6**(6):639–642.
53. Sripriya, R., M. S. Kumar, M. R. Ahmed, and P. K. Sehgal. Collagen bilayer dressing with ciprofloxacin, an effective system for infected wound healing. *J. Biomater. Sci. Polym. Ed.* 2007;**18**(3):335–351.
54. Shanmugasundaram, N., T. S. Uma, T. S. Ramyaa Lakshmi, and M. Babu. Efficiency of controlled topical delivery of silver sulfadiazine in infected burn wounds. *J. Biomed. Mater. Res. A* 2009;**89**(2):472–482.
55. Wu, W. C., C. C. Lai, H. S. Chen, M. H. Sun, L. M. Lee, C. P. Shih, H. W. Lee, and W. C. Hsu. Efficacy and safety of biodegradable collagen-glycosaminoglycan polymer as a material for scleral buckling. *Invest. Ophthalmol. Vis. Sci.* 2008; **49**(6):2673–2678.
56. Barrett, S. A. and K. Moore. Use of Promogran to treat venous leg ulcers. *J. Wound Care* 2004;**13**(1):suppl 2–7.
57. Guarnera, G. and A. Restuccia. Promogran and complex surgical lesions: A case report. *J. Wound Care* 2004;**13**(6):237–239.
58. Durrani, O. M., A. I. Fernando, and T. Q. Reuser. Use of a novel topical hemostatic sealant in lacrimal surgery: a prospective, comparative study. *Ophthal. Plast. Reconstr. Surg.* 2007;**23**(1):25–27.
59. Geiger, M., R. H. Li, and W. Friess. Collagen sponges for bone regeneration with rhBMP-2. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1613–1629.
60. Glatt, V., F. N. Kwong, K. Park, N. Parry, D. Griffin, M. Vrahas, C. H. Evans, and M. Harris. Ability of recombinant human bone morphogenetic protein 2 to enhance bone healing in the presence of tobramycin: Evaluation in a rat segmental defect model. *J. Orthop. Trauma* 2009;**23**(10):693–701.
61. Tan, W., J. Xu, Y. Liu, J. Lin, and H. Wang. [Preparation and osteoinduction capacity of collagen/bone morphogenetic protein composites]. *Sheng Wu Gong Cheng Xue Bao* 2008;**24**(2):272–277.
62. Visser, R., P. M. Arrabal, J. Becerra, U. Rinas, and M. Cifuentes. The effect of an rhBMP-2 absorbable collagen sponge-targeted system on bone formation in vivo. *Biomaterials* 2009;**30**(11):2032–2037.
63. Hertz, A. and I. J. Bruce. Inorganic materials for bone repair or replacement applications. *Nanomed.* 2007;**2**(6):899–918.
64. McKay, B. Local sustained delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2). *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009;**1**:236–237.
65. Cardaropoli, D., S. Re, W. Manuzzi, L. Gaveglio, and G. Cardaropoli. Bio-Oss collagen and orthodontic movement for the treatment of infrabony defects in the esthetic zone. *Int. J. Periodontics Restorative Dent.* 2006;**26**(6):553–559.
66. Sun, J. and Q. Liu. [Bio-Oss collagen bone grafting in the treatment of endodontic-periodontic lesion]. *Nan Fang Yi Ke Da Xue Xue Bao* 2009;**29**(9):1905–1906.
67. Benjamin, M. and J. R. Ralphs. Fibrocartilage in tendons and ligaments—an adaptation to compressive load. *J. Anat.* 1998;**193**(Pt 4):481–494.
68. Windsor, M. L., M. Eisenberg, C. Gordon-Thomson, and G. P. Moore 2009. A novel model of wound healing in the SCID mouse using a cultured human skin substitute. *Australas. J. Dermatol.* 2009;**50**(1):29–35.

69. Morimoto, N., S. Takemoto, T. Kawazoe, S. Suzuki, K. Tomihata, and T. Taira. In vivo culturing of a bilayered dermal substitute with adipo-stromal cells. *J. Surg. Res.* 2008;**146**(2):246–253.
70. Hu, K. K., Y. C. Dai, J. Li, Y. Lei, J. D. Yuan, J. Li, and Q. Wu. [Construction of a tissue engineering skin with epidermal stem cells]. *Zhonghua Zheng Xing Wai Ke Za Zhi* 2007;**23**(1):25–28.
71. Dinh, T. L. and A. Veves. The efficacy of Apligraf in the treatment of diabetic foot ulcers. *Plast. Reconstr. Surg.* 2006;**117**(7 Suppl):152S–157S; discussion 158S–159S.
72. Still, J., P. Glat, P. Silverstein, J. Griswold, and D. Mozingo. The use of a collagen sponge/living cell composite material to treat donor sites in burn patients. *Burns* 2003;**29**(8):837–841.
73. Suzuki, K., A. Kawauchi, T. Nakamura, S. Itoi, T. Ito, J. So, O. Ukimura, A. Hagiwara, H. Yamagishi, and T. Miki. Histologic and electrophysiological study of nerve regeneration using a polyglycolic acid-collagen nerve conduit filled with collagen sponge in canine model. *Urology* 2009;**74**(4):958–963.
74. Yao, C. C., P. Yao, H. Wu, and Z. G. Zha. Absorbable collagen sponge combined with recombinant human basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve. *J. Mater. Sci. Mater. Med.* 2007;**18**(10):1969–1972.
75. Ruszczak, Z. and W. Friess. Collagen as a carrier for on-site delivery of antibacterial drugs. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1679–1698.
76. Singisetti, K. K. and G. P. Ashcroft. Intramedullary delivery of Collatamp in long bone infections: a simple innovative technique. *Ann. R. Coll. Surg. Engl.* 2009;**91**(5):437.
77. Maeda, M., S. Tani, A. Sano, and K. Fujioka. Microstructure and release characteristics of the minipellet, a collagen-based drug delivery system for controlled release of protein drugs. *J. Control. Release* 1999;**62**(3):313–324.
78. Maeda, H., A. Sano, and K. Fujioka. Controlled release of rhBMP-2 from collagen minipellet and the relationship between release profile and ectopic bone formation. *Int. J. Pharm.* 2004;**275**(1–2):109–122.
79. Wallace, D. G. and J. Rosenblatt. Collagen gel systems for sustained delivery and tissue engineering. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1631–1649.
80. Sano, A., M. Maeda, S. Nagahara, T. Ochiya, K. Honma, H. Itoh, T. Miyata, and K. Fujioka. Atelocollagen for protein and gene delivery. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1651–1677.
81. Usas, A., A. M. Ho, G. M. Cooper, A. Olshanski, H. Peng, and J. Huard. Bone regeneration mediated by BMP4-expressing muscle-derived stem cells is affected by delivery system. *Tissue Eng. Part A* 2009;**15**(2):285–293.
82. Zheng, L., J. Sun, X. Chen, G. Wang, B. Jiang, H. Fan, and X. Zhang. In vivo cartilage engineering with collagen hydrogel and allogenous chondrocytes after diffusion chamber implantation in immunocompetent host. *Tissue Eng. Part A* 2009;**15**(8):2145–2153.
83. Sultana, Y., R. Jain, M. Aqil, and A. Ali. Review of ocular drug delivery. *Curr. Drug Deliv.* 2006;**3**(2):207–217.
84. Friess, W. Collagen—biomaterial for drug delivery. *Eur. J. Pharm. Biopharm.* 1998;**45**(2):113–136.

85. Kleinmann, G., S. Larson, B. Hunter, S. Stevens, N. Mamalis, and R. J. Olson. Collagen shields as a drug delivery system for the fourth-generation fluoroquinolones. *Ophthalmologica* 2007;**221**(1):51–56.

86. Liu, W., M. Griffith, and F. Li. Alginate microsphere-collagen composite hydrogel for ocular drug delivery and implantation. *J. Mater. Sci. Mater. Med.* 2008;**19**(11):3365–3371.

87. Borene, M. L., V. H. Barocas, and A. Hubel. Mechanical and cellular changes during compaction of a collagen-sponge-based corneal stromal equivalent. *Ann. Biomed. Eng.* 2004;**32**(2):274–283.

CHAPTER 3

PROPERTIES AND HEMOSTATIC APPLICATION OF GELATIN

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3.1 INTRODUCTION

Gelatin is an important fibrous protein obtained from collagen by partial hydrolysis derived from mammalian, avian, and fish species. Today cattle bones, cattle hide, and pork skin serve as a principal raw material for gelatin production [1]. In addition to fish skin, fish bone and chicken bone can also serve as the source of raw material for gelatin manufacture. The preferred collagen source for producing high-quality gelatin is cow bone and hides [2].

For the last few decades the demand for gelatin has increased substantially worldwide. Recent reports show that the annual world production of gelatin is approximately 326,000 tons of which pig-skin-derived gelatin accounts for the highest (46%) amount followed by bovine hides (29.4%), bones (23.1%), and other sources (1.5%). The majority of gelatin is produced in western Europe with 40% output; the rest is produced in eastern Europe 2%, North America 20%, Latin America 17%, and 21% elsewhere [3].

However, though gelatin has such a wide range of applications, pessimism and strong concerns still persist among consumers with regard to its usage [4]. This is mainly due to religious sentiments (Judaism and Islam forbids the consumption of pork-related products while Hindus do not consume cow-related products) as well as the enhanced and stricter adherence to vegetarianism throughout the world [5]. In addition, there is an increasing concern among researchers about whether animal-tissue-derived collagens and gelatins are capable of transmitting pathogenic vectors such as prions [6]. However, studies conducted by various authorities have shown that the production process of gelatin is an effective barrier against possible bovine spongiform encephalopathy (BSE) prions.

In March 2003, the scientific steering committee of the European Union confirmed "the risk associated with bovine bone gelatin is close to zero." The European Food Safety Authority (EFSA) issued a statement on January 18, 2006, announcing that the residual BSE risk in bone-derived gelatin is regarded as being very small [7].

Significant amount of gelatin used in food and pharmaceutical industries is derived from either pig skin or cow hide and bone. The outbreak of zoonotic problems such as BSE and religious concerns have led to intensive research to find an alternative source for mammal-derived gelatin. Furthermore, the strong competition between manufacturers for the procurement of pig skin or other mammalian source resulted in increased demand and cost. However, today only a few alternatives are available, and as a result it has not been possible to eliminate gelatin derived from mammalian sources. Researchers from industry and academia are continuously searching for an alternative to either gelatin or mammalian gelatin, for example, gelatin from fish and poultry by-products. Poultry skin and bones are expected to yield gelatin in the near future, but commercial production is currently very low. Fish gelatin is also a better alternative to mammalian gelatin particularly with qualities such as lower melting point (resulting in faster dissolution). However, the production of fish

gelatin is also very low, accounting for just 1% of the annual world gelatin production [8].

3.2 GELATIN STRUCTURE

Gelatin is a protein derived from collagen, which is the major structural protein in the connective tissue of animal skin and bone. The fundamental unit of collagen is the tropocollagen rod, a triple-helical protein structure. Collagen and gelatin consist of varying amounts of 18 amino acids, of which glycine (Gly), proline (Pro), and hydroxyproline (Hyp) are the most abundant. The proline content is particularly important as it tends to promote formation of the polyproline II helix, which defines the tropocollagen trimer. The primary structure of gelatin closely resembles to that of collagen, whose triple-helical structure can have two different compositions. The triple helix of type I collagen from skin and bone is composed of two $\alpha 1$ (I) and one $\alpha 2$ (I) chains, while type III collagen from skin (not present in bone) is made up from three $\alpha 1$ (III) chains [9]. Collagen is composed of three α chains intertwined in such a way as to provide a three-dimensional structure that provides an ideal conformation for interchain hydrogen bonding [10]. The main difference between these three α-chain types is in the amino acid composition. The triple helix is approximately 300 nm in length and the chain has a molecular weight of approximately 10^5 kDa. The triple helix is stabilized by the interchain hydrogen bonds. Collagen denaturation causes separation of the rods and total or partial separation of the chains due to the destruction of hydrogen bonds causing loss of triple-helix conformation, and following the polymer exist in a coiled form. Industrial gelatins are mixtures of different components: α chains (one polymer chain), β chain (α-chain dimer) and γ chains (α-chain trimer, higher molecular weight), and some lower-molecular-weight fragments. Gelatin that contains more α chains would show higher gel strength. Table 3.1 gives the composition of an alkaline hide sample and an acid pig skin sample, both with very similar bloom and viscosity values [11].

Table 3.1 shows that the main differences between the samples are contents of α structure and β peptides. In general, the Bloom value is proportional to the sum of the α and β fractions, together with their larger peptides, while the

TABLE 3.1 Composition of Gelatin

Source	$<A$	A	α	β Peptide	β	γ	$\gamma - X$	1–4	Q	Bloom (g)	Viscosity (mPa s)
Limed hide	28	4	30	6	13	3	8	4	3	250	5.7
Acid pig skin	35	4	10	12	11	6	11	7	4	250	5.5

Source: From Johnston-Banks [11].

viscosity is proportional to the content of higher-molecular-weight fractions. The triple-helical structure of gelatin does not fit any of the common structural categories, such as the α helix, β sheet, or β turn found in most globular proteins.

3.3 USES OF GELATIN

Gelatin is a special and unique hydrocolloid serving multiple functions with a wide range of applications. Because of its unique functional and technological properties among which is the ability to form thermoreversible gel, it is widely used in food, pharmaceutical, cosmetic, and photographic applications.

Because of its nonimmunogenic, biocompatible, and biodegradable nature, it is used in the food industry: Gelatin is utilized in confectionaries, low-fat spreads, fat reduction and for mouth feel, dairy, baked goods, and meat products [7, 11]. In the pharmaceutical and medical fields, gelatin is also used as a matrix for implants [12], in injectable drug delivery microspheres [13] and in intravenous infusions [14]. Gelatin is used for biomedical applications such as tissue engineering. It is preferred for biomedical applications over collagen because collagen is known for antigenicity; gelatin does not exhibit antigenicity and it is one of the most convenient proteins for such application [15]. There are also reports in which live unattended viral vaccines used for immunization against measles, mumps, rubella, Japanese encephalitis, rabies, diphtheria, and tetanus toxin contain gelatin as a stabilizer [16]. In the pharmaceutical industry, gelatin also is widely used for the manufacture of soft and hard capsules, microspheres, plasma expanders, and in wound care (wound dressing material and wound healing agents) bone repairing matrices, ligament substitutes, and scaffolds for tissue engineering purpose.

Gelatin, being low in calories, is normally recommended for use in food-stuffs to enhance protein levels and is especially useful in body-building foods. In addition, it is also used to reduce carbohydrate levels in foods formulated for diabetic patients.

3.4 MANUFACTURING OF GELATIN

There are four different methods [17] used to manufacture gelatin:

1. Acid process
2. Alkali process
3. Heat pressure method
4. Enzymatic process (recombinant microbial process)

All gelatin manufacturing processes consist of three main stages, namely (a) pretreatment of the raw material, (b) extraction of the gelatin, and (c) purification and drying of gelatin.

Manufactured gelatin is often blended to produce trade-quality gelatin, with specific properties for specific applications [18]. Depending on the method in which collagens are pretreated, two different types of gelatin (each with different characteristics) can be produced. Type A gelatin (isoelectric point at pH 6–9) is produced from acid-treated collagen, and type B gelatin (isoelectric point at pH 5) is produced from alkali-treated collagen [19]. See Figure 3.1. Acid treatment is most suitable for the less covalently crosslinked collagens found in pig or fish skins, while alkaline treatments are more suitable for the

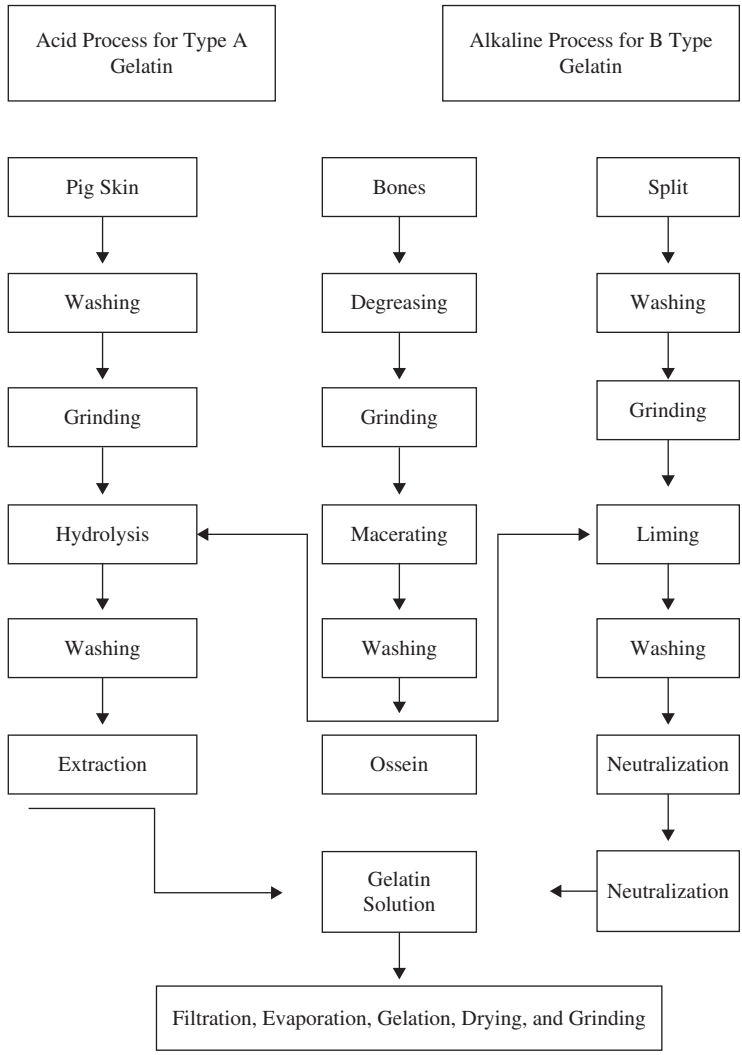

FIGURE 3.1 Manufacturing process for gelatin.

more complex collagens found in bovine hides. Among all of these processes, acid and alkaline processes are the best and the most widely used [20].

3.4.1 Acid Manufacturing Process

The acid process is mainly used for the preparation of gelatin from pig skin, fish skin, and sometimes for bones. In this method the following steps are performed in order to make material ready for the main step: size reduction, washing, removing hairs, and degreasing. After pretreatment the material is acidified to about a pH 4 and then heated stepwise from 50°C to boiling. This takes 10–40 h. Thereafter the denaturated collagen or gelatin has to be defatted and filtered to a high clarity. It is then concentrated either by vacuum evaporation or by membrane ultrafiltration to a reasonably high concentration of gelatin. The material is then dried by passing dry air grounded and blended as needed.

3.4.2 Alkali Manufacturing Process

This method is suitable for more complex collagen such as the one obtained from bovine hides where the animals are relatively old at slaughter. Compared to the acid process, the alkali process requires longer time, normally several weeks (4–6 weeks). After pretreatment, the raw material is digested with caustic soda. During this process the asparagine and glutamine side chains are quickly hydrolyzed to aspartic and glutamic acid, respectively, which affects their isoionic point (4.8–5.2). However, a shortened alkali treatment (7 days) may produce gelatin with an isoionic point as high as 6.

After alkali treatment the material is washed with water to make it free from alkali and acidified to a desired extraction pH. It is then denaturated by heating and demineralized by using ion exchange or ultrapurification, dried by vaccum evaporation, grinded, and blended.

3.4.3 Heat Pressure Method

The heat pressure process is used for gelatin extraction from bones. This process is relatively fast and requires no chemicals compared to acid and alkali processes, which usually take longer time and require chemicals and a large amount of water.

Typically, bones are crushed and washed with water after degreasing. The crushed bones are then treated in a high-pressure tank along with water at a ratio of 1 : 3 (bones : water) at 104°C and 1.2 atmospheric pressure for 5–6 h. They are then filtered in order to remove all the solid material. The solid is then defatted by phase separation followed by concentration, drying milling, and blending. The gelatin obtained by this method is of a limited quality and use, but the advantages are short processing time and no chemicals used.

3.4.4 Enzymatic Process

Recombinant microbial production offers the only known technology for the cost-effective manufacturing of gelatin with consistent quality that is free from animal components. It offers high productivity and consistent quality gelatin suitable for tissue engineering applications. For this method genetically engineered microorganisms such as yeast (*Pichia pastoris, Saccharomyces cerevisiae, Hansenula polymorpha*) and bacteria (*Escherichia coli, Bacillus brevis*) are used on synthetic media [21].

3.5 RHEOLOGICAL PROPERTIES OF GELATIN

Gelatin is categorized as a physical gel, that is, the interactions or bonds between the chains that physically make up the material in nature (van der Waal's interactions and hydrogen bond's with $E \approx 2$ K cal/mol). Some physical gels, such as alginate, are not thermally reversible. However, the bonding energy in gelatin is relatively week, and, as a result, gelatin is capable of forming thermoreversible gels. Apart from gelatin, casein (a milk protein that forms spherical aggregates approximately 20–300 nm in diatemer), agarose, pectin, and carrageens also form reversible gels [22].

Gel strength and gel melting point are the major physical properties of gelatin gels. These are governed by molecular weight, as well as complex interactions determined by the amino acid composition and the ratio of α/β chains present in the gelatin [23]. According to Schrieber and Garies [7] the gel strength mainly depends on the proportion of fractions having a molecular weight of approximately 100,000 g mol^{-1}. In addition, there is a strong correlation between gel strength and the α-chain content in gelatin. Gelatin containing more α chains would thus show higher gel strength. On the other hand a high ratio of peptides with molecular weight higher or lower than the α chains would decrease the gel strength [24]. The gel strengths of commercial gelatins are expressed using Bloom values. The Bloom value is the weight in grams that is required for a specified plunger to depress the surface of a standard thermostated gel to a defined depth under standard condition [7]. The gelling strength of commercial gelatins ranges from 100 to 300, but gelatins with Bloom values of 250–260 are the most desirable [25]. Table 3.2 shows the Bloom values, gelling temperatures, and melting points of gelatins obtained from bovine, porcine, and fish collagen as reported in the literature [3].

Fish gelatin typically has a Bloom value ranging from as low as 0 to 270 (tested under the conditions of the standard Bloom test) compared to the high Bloom values for bovine or porcine gelatin, which have Bloom values of 200–240. The wide range of Bloom values found for the various gelatins arises from the different sources and is associated with the amino acid composition and extraction conditions, which influence the physical properties of gelatins such as gelling point and gelling strength; for example, the use of high

TABLE 3.2 Gel Strength and Melting Point of Various Fish Gelatins

Type of Gelatin	Bloom Value (g)	Gelling Temp. (°C)	Melting Point (°C)
Sole fish gelatin	350	18–19	19.4
Tilapia fish gelatin	273	Not reported	25.4
Grass fish gelatin	267	19.5	26.8
Tuna fish gelatin	426	18.7	24.3
Cat fish gelatin	243–256	15–18	23–27
Porcine gelatin	220–240	22–25	31–35
Bovine gelatin	210–240	20–24	30–33.8

concentration of sulfuric acid, sodium hydroxide, and citric acid has been reported to result in low Bloom values [26], indicating that the gel-forming ability of the gelatin is sensitive to acid and alkali hydrolysis, as both affect the degree of crosslinking in the collagen.

The most remarkable characteristics of gelatin are its solubility in water and the ability to form thermoreversible gels. As a thermoreversible gel, gelatin gels will start melting when the temperature increases above a certain point, which is called the gel melting point and is usually lower than human body temperature. This melt-in-the mouth property has become one of the most important characteristics of gelatin gels and is widely exploited in the food and pharmaceutical industries. The rheological properties of thermoreversible gelatin gels are primarily a function of temperature (below the melting point of the gel) and concentration of gelatin for a given gelation type. The transformation of collagen to gelatin is interpreted as the disintegration of the helical structures into random coils. Upon cooling, the random coils undergo a coil-to-helix transition during which they attempt to reform the original structure. The resulting three-dimensional network is responsible for the strength and integrity of the gelatin gel [3].

Emulsifying and Foaming Properties of Gelatin. Gelatin and to some extent also collagen are used as foaming, emulsifying, and wetting agents in food, pharmaceutical, medical, and technical applications due to their surface-active properties. The hydrophobic areas on the peptide chain are responsible for giving gelatin its emulsifying and foaming properties. However, gelatin is generally a weaker emulsifier than other surface-active substances such as globular proteins and gum arabic. Therefore, when used alone, gelatin often produces relatively droplet sizes during homogenization, and it has to be either hydrophobically modified by the attachment of nonpolar side groups or used in conjunction with anionic surfactants to improve its effectiveness as an emulsifier.

3.5.1 Methods for Rheological Properties of Gelatin

Following are some methods used for the rheological characterization (viscoelasticity and gel strength) and chemical properties (amino acid composition, molecular weight distribution) of gelatin.

Amino Acid Analysis. For amino acid analysis typically dry gelatin is dissolved in distilled water and hydrolyzed in vaccum-sealed glass tubes at 108°C for 18 h in the presence of 5.7 N HCl containing 0.1% phenol and norleucin as internal standard. After hydrolysis the sample is vaccum dried, dissolved in a buffer, and analyzed for amino acid composition [27].

Determination of Total Protein Content. Although protein determination could be carried out by the Kjeldahl method, because of time and sample consumption it is not used for routine determination. For total protein determination Biuret and Lowry assays are commonly used, as both the methods are simple, rapid, and relatively more precise [1]. In this method the color response of gelatins in the Biuret assay is determined at 540 nm, while the color response of gelatin in the Lowry assay is determined at 650 nm. Bovine serum albumin is used as the reference protein for both assays. The concentration of bovine serum albumin is determined by using the absorbance at 320 nm serving as background scattering correction.

Electrophoretic Analysis. Gelatin is dissolved in distilled water at 60°C and then a two- or threefold concentration loading buffer containing β-mercaptoethanol is added. Samples are then heat denaturated for 5 min at 90°C and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using 4% stacking gels and 5% resolving gels. Protein bands are stained with Coomassie Brilliant Blue R-250. The protein bands stained on the gel are scanned; fetal calf skin is used as the marker for α, β, and γ components mobility [27].

Viscoelastic Properties. The rheometer is used for the determination of dynamic viscoelastic properties of gelatin. Dry gelatin and cold distilled water are mixed for 60 min and then placed in to a water bath held at 45°C. The gelatin solution is prepared by stirring the mixture for 20 min. The viscosity measurement of the sample is performed at a scan rate of 1°C/min, frequency 1 Hz, an oscillating applied stress of 3.0 Pa, and a gap of 0.15 mm. The gelation solution is cooled from 40 to 5°C, kept at 5°C for 10 min, and then heated from 5 to 40°C, viscoelastic properties of gelatin slices that maturated in low temperature are observed. The gel slices are heated and cooled on the rheometer. The elastic modulus (G'), viscosity modulus (G''), and phase angle (rad) values are represented as a function of temperature [24].

Gel Strength. The gel strength is determined by dissolving dry gelatin in distilled water (6.6% w/v) at 60°C. The solution of gelatin matures at 10°C for 16–18 h in a refrigerator. The gel strength test is then carried out on a texture analyzer with a 5-kN load cell equipped with a 1.27-cm diameter flat-faced cylindrical Teflon plunger. The maximum force (in *g*) is determined when the plunger has penetrated 4 mm in to the gelatin gel [24].

3.6 HEMOSTATIC APPLICATION OF GELATIN

Hemostatic agents are defined by the FDA as devices intended to produce hemostasis by accelerating the clotting process of blood [28]. Control of intraoperative bleeding is a big challenge during surgery, and postoperative hemorrhage is a significant risk; it increases operating room time and may require blood transfusion. In most cases, continuous bleeding impedes identification of eloquent or pathological structures in microsurgical approaches such as intraspinal surgery and affects clear visualization by the surgeon [29]. There are several conventional techniques in use to control bleeding and minimize loss of blood during surgical procedures. Manual pressure, tourniquet application, and ligatures are some of the techniques in use to stop the bleeding, but these methods are not very useful as they are laborious and often add time to the operation [30]. Other techniques to achieve hemostasis include electrical and chemical methods; the use of electrocauterization or laser has limited application as these methods result in thermal injury to adjacent vascular and neural structure, damage to wound edges, char, necrosis of tissue, which impairs wound healing and may cause infection [31]. In contrast manual pressure is safe, but it is not very effective to control bleeding from complex injuries wherein the area of bleeding is not easily accessible. Among all these methods, use of chemical hemostatic agents proved to be more useful under such conditions.

Hemostatic agents are often used in surgery as they help a patient's coagulation system to quickly develop an occlusive clot. These agents are used in intraoperative hemostatsis, hemorrhage control in dental extractions and odontostomatology, intestinal, orthopedic, and ear surgery, and in urological and for microcoil embolization in upper and lower gastrointestinal hemorrhage.

The biosaftey and compatibility of gelatin was evaluated in many studies before application, for instance, biocompatibility and genotoxicity of gelatin sponge was evaluated in vitro. Cytotoxicity was analyzed by neutral red uptake and amino acid black staining test, where genotoxicity was analyzed by the Ames test, Sister Chromatides Exchange (SCE), and chromosal aberrations. The undiluated extract of gelatin sponge was found to be cytotoxic, but cell viability was not affected by 1 : 2 and 1 : 10 diluted extract. The same material was also found to be nongenotoxic by all three assays [32].

There are several types of hemostatic agents available commercially with different configuration and combinations approved by the FDA for use as hemostatic agents during and after operative procedures. The following are different types of chemical hemostatic agents: collagen-based hemostat, albumin-based hemostat, cellulose-based hemostat, gelatin-based hemostat, polysaccharide-based hemostat, inorganic hemostat, fibrin-based hemostat, and polymeric hemostat. These hemostats act in a variety of ways, some of them stimulate formation of fibrin or inhibit fibrinolysis, whereas some others improve hemostasis, and some hemostats provide tamponade at the site of bleeding and activate coagulation. A topical hemostat is often necessary because of the choice of technique

used, and one has to take into account the site of surgical procedure, type of procedure, amount of bleeding, experience of application, cost, and preference of the surgeon [33].

The gelatin sponge was first introduced in the 1940s by Dr. Gray for use in a neurosurgical procedure. Commercial gelatin-based hemostats have been in use to stop bleeding since 1945 [34] and are available commercially under various names and configurations. These are water soluble and are widely used to stop bleeding. Though the mechanism of action of gelatin-based hemostats is not fully understood, it is believed that the hemostatic properties are the result of induction of hemostasis by hastening the development of thrombus and providing structural support to it. After application, gelatin-based hemostats are absorbed in 4–6 weeks [35]; when applied during nasal surgery, it liquefies within 2–5 days. It has been reported that gelatin induces a better quality of clot than collagen-based agents [36]. Following are some commercial gelatin-based hemostats:

- Gelfoam (Pharmacia & Upjohn): Porcine gelatin in powder form, non-antigenic, can be used as dry or with sterile saline.
- Surgifoam (Ethicon): Porcine gelatin as sponge (off-white porous), powder, or paste, more suitable for irregular cuts/wound in spinal, vascular, or spinal applications, can be applied as paste (spreading) or through applicator. (See Fig. 3.2.)
- Floseal Matrix (Baxter Healthcare): Bovine gelatin, human thrombin, viscous gel, through syringe application.

3.6.1 Gelfoam

Gelfoam is a water-insoluble hemostat available since 1945 and approved on July 8, 1983. It is an off-white, nonelastic, porous, pliable product prepared from pure gelatin. It is capable of absorbing up to 45 times its weight of whole blood [37]. It has been used widely in a number of clinical indications such as

FIGURE 3.2 Surgifoam: (a) gelatin powder, (b) gelatin sponge, and (c) gelatin paste.

gastrointestinal bleeding, trauma, postpartum hemorrhage, and in preoperative tumor embolization, retroperitonal and pelvic bleeding, and uterine artery embolization to reduce hepatic hypertrophy of one lobe before surgical resection of other lobe. When placed in soft tissues, it is usually absorbed completely within 4–6 weeks without inducing excessive scar. When applied to bleeding nasal, rectal, or vaginal mucosa, it liquefies within 2–5 days. Gelfoam is available in a wide range of formulations, for example, Gelfoam Plus, Gelfoam sponge, Gelfoam powder. It is effective in ebbing blood flow when manual pressure, ligature, and conventional procedures are impractical. It is not advisable to use Gelfoam in intravascular compartments or in the closure of skin incisions. As Gelfoam swells, it limits its application to be used within confined space such as the spinal formina where it can cause spinal cord nerve and brain compression. There are some reports of neurological deficits due to the intraspinal use of gelatin [38]. A study was carried out to assess the hemostatic application of gelatin paste and gelation sponge (soaked in thrombin) and microfibrillar collagen from bone bleeding. A total of 45 hip replacements were done with the use of the above-mentioned hemostatic agents to reduce bleeding from cancellous bone (femoral surface of trochanteric osteotomies) during hip replacement. Gelatin paste was more effective among these with 85% reduction, followed by gelatin sponge soaked in thrombin (75%) and microfibrillar collagen (47%) compare to control (11%, spontaneous bleeding reduction in eight control hips to which no agent was given) [39].

The efficacy of Gelfoam powder as a bone hemostat in cardiopulmonary surgery was evaluated by two randomized open-label clinical trials. The objectives of the study were to evaluate the efficacy of Gelfoam as a hemostatic agent in the treatment of sterna bone bleeding in cardiopulmonary bypass surgery, to identify deleterious effects, if any, on interference with bone healing, and to determine any systemic or local wound side effect from Gelfoam in situ. A total of 215 patients between 18 and 74 years old undergoing cardiopulmonary bypass surgery were enrolled and grouped into two groups: the Gelfoam group (108) and the control group (107). The Gelfoam paste was applied to the cut sternal surface immediately following sternotomy. The control group received no treatment to the cut surface. Blood loss was monitored both during bypass surgery and postoperatively. It was observed that the amount of blood loss in the Gelfoam group was significantly less than that of the control group. The mean blood loss in the Gelfoam group was 13.72 gm, whereas in the control group it was more than double, 27.71 gm. At hospital discharge, normal bone healing was reported for 105 patients (97%) in the Gelfoam group and 104 patients (97%) in the control group. At the 3-month follow-up 95% of patients in the Gelfoam group and 93% of patients in the control group were healed. A clinical study of gelfoam showed that the paste made from Gelfoam is safe and effective in treating intraoperative bleeding when applied to cut surface of cancellous bone.

Several reports of adverse reactions associated with the use of this material have been reported and show that Gelfoam may serve as a nidus for infection

and abscess formation and may potentiate bacterial growth. With the use of Gelfoam, the foreign body reaction, "encapsulation" of fluid and hematoma have been reported. When Gelfoam was used in laminectomy operations, multiple neurological events were noticed such as cauda equine syndrome, spinal stenosis, meningitis, headache, paresthesias, pain, bladder and bowel disfunction, and impotence. Toxic shock syndrome has also been reported in association with the use of Gelfoam in nasal surgery. Fever, failure of absorption, and hearing loss are also adverse effects associated with the use of Gelfoam in tympanoplasty [40].

3.6.2 Floseal

Floseal was approved on December 8, 1999, as an effective hemostat from Baxter Corp. It works on wet, actively bleeding tissues and conforms to irregular wound surfaces. It can be applied to both hard and soft tissues. It is absorbed within 6–8 weeks. Floseal is well suited for application to a heavy bleeding site [41]. Several studies have been carried out to compare Floseal with gelfoam in combination with thrombin. It has been observed that Floseal results in a faster and more effective hemostasis than Gelfoam in heparinized patients [42]. It is useful in ear, nose, and throat interventions.

The usefulness of Floseal in many surgical disciplines has been reported [43, 44]. In endoscopic neurosurgery and removal of tumor, the surgeon has to face oozing or focal bleeding from mucosa, vascular structure, or lesion, which results in reduction of the visibility for the surgeon and subsequently tumor removal and the patient's safety. Specific skills and techniques are required to stop intradural bleeding so as to preserve neurovascular structures and maintain the operating field clear. In such cases Floseal has been found useful.

When applied to the bleeding site, the Floseal granules adhere to the surface and swells, resulting in restriction of blood flow, whereas thrombin activates clot formation. After that it can be irrigated to make the operative field clear. After application of Floseal matrix, homeostasis was observed immediately. This matrix was found useful for both oozing and focal hemorrhage and effective for high flow bleeding [45]. In one controlled randomized clinical trial (93 cardiac patients) of the Floseal matrix versus thrombin-soaked Gelfoam as hemostatic agents in cardiac surgery [42], demonstrated that Floseal matrix was safe and effective with a faster time of coagulation and better efficacy (94%) than Gelfoam (60%). After application of the Floseal matrix at the bleeding site the gelatin particles swell and tamponade bleeding. The gelatin matrix–thrombin composite slows blood flow and provides exposure to a high concentration of thrombin, thus hastening clot formation.

Floseal has been found to be more effective in nasal packing during posterior epistaxis. Removal of nasal packing may lead to pain, infection, rhinorrhea, disturbance of breathing, necrosis, sleep apnea, and epistaxis. Several studies have shown that nasal packing such as tampons and gauze are often uncomfortable to remove [46]. In a histopathologic study on the sheep model a loss of

ciliated epithelial cells was shown on the removal of the cotton sponge [47]. Gelatin–thrombin matrix has been studied for its hemostatic effect after endoscopic sinus surgery in a multicenter, prospective, single-arm study. Thirty-five patients were enrolled (17 male, 13 female, age 48 ± 15 years). Patient satisfaction and postoperative healing were evaluated; 29 patients achieved hemostasis 10 min after application (96.7% success rate). No complications such as synechiae, adhesion, or infection were reported [48]. Floseal was also found to be more useful in percutaneous nephrolithotomy (PCNL). Two patients were treated with PCNL for evaluation of initial clinical use of Floseal followed by five patients. Gelatin matrix sealant was injected down the nephrostomy tract. The operative time was 75 and 180 min. After the procedure both patients had stable postoperative hemoglobin and no evidence of bleeding or obstruction on postoperative computerized tomography [49]. Safety and efficacy of the surgeon-prepared gelatin as hemostat was compared with Floseal for hemostasis in laproscopic partial nephrectomy (LPN). From August 2006 to July 2008 forty LPNs were performed at two different institutes by the same surgeon for this study. Estimated blood loss, operative time, transfusion rate, and complications were taken into consideration while comparing gelatin versus Floseal. The study demonstrated that the same efficacy was observed with surgeon-prepared gelatin and Floseal [50].

Care must be taken while applying this agent; it should not be injected into the blood vessels or allowed to enter the blood vessels. It should not be applied in the absence of active bleeding. Care should be taken while using this agent to stop wound bleeding, and it should not be used in closure of skin incisions as it can interfere with the healing of wound/skin edges. If the patient has a known history of allergies to material of bovine origin, the material should not be applied. As it swells 10–20% when it comes in contact with blood or body fluids, only an adequate amount of the material should be used, and excess of material must be removed from the site of application using mild water irrigation. This material should not be used along with methylmethacrylate or other acrylate adhesive.

3.6.3 Surgifoam

The Surgifoam absorbable gelatin sponge is a sterile water-insoluble, malleable, off-white porous sponge. In intraspinal surgery mechanical methods of hemostasis are not helpful because of depth; electrical methods such as use of bipolar forceps are also not suggestive as they may induce thermal injuries and most of the intraspinal procedures are associated with removal of bone (less or more). Blood starts oozing from the chipped bone, to stop this bleeding bone wax is used commonly. Bone wax has certain limitations such as difficulty in molding. In such cases Surgifoam powder has an advantage as it can form a paste that can be spreaded or shaped to the irregular surface of the chipped bone [51]. It was approved by the FDA as Surgifoam sponge for clinical use on September

30, 1999. It was approved in a powdered form as Surgifoam powder in 2002. In 2004 it was approved for clinical use as a mixture with thrombin for improved hemostasis. Surgifoam sponge was compared to a control sponge in unpublished trials of 281 patients and found to be effective without any adverse effects. No randomized, controlled clinical trials have been published to compare Surgifoam thrombin with other hemostatic agents. An open, randomized, controlled, multicenter, and unmasked clinical trial (281 patients) was designed to evaluate Surgifoam for hemostatic application and safety compared to absorbable gelatin sponge. Cardiovascular, general surgical, and orthopedic patients were enrolled for the study. The sponges were soaked with saline or used as dry, and patients were followed for 2 months after surgery to assess the safety of the study material. A total of 281 patients received treatment with the study material. The hemostasis data was collected immediately during surgery and the patients were examined at 2–4 weeks and again at 6–8 weeks in order to obtain safety data. Statistical analysis showed that Surgifoam and control sponge are equivalent in the ability to achieve hemostasis within 10 min [52].

A controlled, randomized, multicenter open clinical study was conducted to evaluate safety and efficacy of Surgifoam with absorbable gelatin sponge. 206 patients were enrolled: patients were followed for 2 months after surgery to assess the safety of the sponge. Hemostasis was achieved within 10 min. Patient sera were tested for the immune response (antiporcine collagen immunoglobulin). Sera were collected prior to surgery, at 2–4 weeks and 6–8 weeks following surgery. Only 1 of the tested patients had antibodies at baseline and 6 of the 206 patients had antibodies at the 6–8 weeks time point [53]. The safety and effectiveness for use in urological procedures has not been established through randomized clinical trial.

For less painful postoperative care use of absorbable nasal packing not only helps in easy removal but is also more comfortable. Surgiflo hemostat matrix with thrombin has gained popularity as nasal packing after sinus surgery. A study was carried out with the use of Surgiflo hemostat in combination with thrombin–JMI (Jones Pharma Inc., Bristol), when applied to a bleeding surface allows intimate tissue contact and conformation to an irregular wound bed and accelerates clot formation. The above mixture was effective in controlling bleeding in 96.7% of patients undergoing endoscopic sinus surgery and recommended for randomized controlled clinical trials.

Surgifoam can be stored at room temperature (15–30°C), the material is to be used as soon as the packet is opened, with the unused part discarded. The package should be opened and the material should be handled in sterile condition. Surgifoam can be cut with scissors to desired shape and size. A sponge of the required amount is to be used, manual compression of the sponge before applying to bleeding site, and held with moderate pressure until hemostasis is achieved should be followed. Excess sponge can be removed by irrigation with sterile saline.

3.7 CONCLUSION

For decades absorbable gelatin-based hemostatic agents have been used for hemostatsis. No hemostatic agent is entirely safe and all have more or less side effects. Though the safety of patient has first priority, knowledge, proper handling, and use of minimal/required amount of these agents make them more popular as hemosatats than other methods and are the first choice for surgeons in the operating room. These agents not only help in improving a patient's condition but also reduce complications and lower direct or indirect cost [54]. Few randomized clinical studies have been carried out but most compare one agent with another. More clinical data is needed. Safety and efficacy in many areas, for instance, clinical data on the use of these agents in gynecology surgery, is limited. In spite of these issues these agents are popular in surgical field and will remain.

Note: Editors/contributors to this chapter have no commercial or any other association with the companies that make/market the products summarized in this chapter.

REFERENCES

1. Zhou, P. and J. M. Regenstein. Determination of total protein content in gelatin solutions with Lowery or Biuret assay. *J. Food Sci.* 2006;**71**:C474–479.
2. Rowlands, A. G. and D. J. Burrows. Enzyme method of manufacturing gelatin. U. S. Patent, PN No 6, 100, 381, 2009.
3. Karim, A. A. and R. Bhat. Fish gelatin: Properties, challenges and prospectus as an alternative to mammalian gelatins. *Food hydrocoll.* 2009;**23**:563–576.
4. Asher, D. M. The transmissible spongiform encephalopathy agents: Concerns and responses of the United States regulatory agencies in maintaining the safety of biologics. *Develop. Biol. Standard.* 1999;**100**:103–108.
5. Karim, A. A. and R. Bhat. Gelatin alternatives for the food industry: Recent developments, challenges, and prospects. *Trends Food Sci. Tech.* 2008;**19**:644–656.
6. Wilesmith, J. W., J. B. M. Rayan, and M. J. Atkinson. Bovine spongiform encephalopathy: Epidemiological studies on the origin. *Vet. Record.* 1991;**128**: 199–203.
7. Schrieber, R. and H. Gareis. *Gelatin Handbook*, Wiley-VCH: Weinhem, 2007.
8. Arnesen, J. A. and A. Gildberg. Extraction of muscle proteins and gelatin from cod head. *Process Biochem.* 2006;**41**:697–700.
9. Estaca, J. G., P. Montero, F. F. Martin, A. Aveman, and M. C. G. Guillen. Physical & chemical properties of tuna-skin & bovine hide gelatin films with added aqueous aregano & rosemary extracts. *Food Hydrocoll.* 2009;**23**:1334–1341.
10. te Nijenhuis, K. Thermorevesible networks: Viscoelastic properties and structure of gels. *Adv. Polym. Sci.* 1997;**130**:1–267.

11. Johnston-Banks, F. A. *Gelatin*. In *Food Gels*. P. Harris (Ed.). Elsevier Science: Essex, 1990, pp. 233–289.
12. Pollack, S. V. Silicon, fibrel and collagen implantation for facial lines and wrinkles. *J. Dermatol. Surg. Oncol.* 1990;**16**:957–961.
13. Rao, K. P. Recent developments of collagen based materials for medical applications and drug delivery system. *J. Biomater. Sci. Polym. Ed.* 1995;**7**:623–645.
14. Saddler, J. M. and P. J. Horsey. The new generation gelatins: A review of their history, manufacture and properties, *Anesthia* 1987;**42**:257–262.
15. Ito, A., A. Mase, Y. Takizawa, M. Shinkai, H. Honda, K. I. Hata, M. Ueda, and T. Kobayashi. Transglutaminase-mediated gelatin matrices incorporating cell adhesion factors as a biomaterial for tissue adhesion. *J. Biosci. Bioeng.* 2003;**95**: 196–199.
16. Burke, C. T., T.-A. Hsu, and D. B. Volkin. Formation, stability and delivery line attenuated vaccines for hhuman use. *Crit. Rev. Therap. Drug Carrier Syst.* 1999;**10**:1–83.
17. Valipour, P., A. Talebian, and A. G. Ebadi. Gelatin preparation from cow's bone by heat-pressure method. *Asian J. Chem.* 2008;**20**:1337–1342.
18. de Wolf, F. A. Collagen and gelatin. *Prog. Biotech.* 2003;**23**:128–133.
19. Stainstby, G. *Gelatin gels*. In *Collagen as Food, Advances in Meat Research*. A. M. Pearson, T. R. Dutson, and A. J. Bailey (Eds.). Van Nostrand Reinhold: NewYork, 1987, Vol. 4, pp. 209–222.
20. Lu, L., C. A. Garcia, and A. G. Mikos. *J. Biomed. Mater. Res.* 1999;**46**:236.
21. Baez, J., D. Olsen, and J. W. Polarek. Recombinant microbial systems for the production of human collagen and gelatin. *Appl. Microbial. Biotechnol.* 2005;**69**:245–252.
22. Papon, P., J. Leblon, and P. H. E. Meijer. Gelation and transition in biopolymers. In *The Physics of Phase Transitions*. Springer: Berlin, 2007, pp. 189–213.
23. Cho, S. M., K. S. Kwak, D. C. Park, Y. S. Gu, C. I. Ji, and D. H. Jang. Processing optimization and functional properties of gelation from shark (*Isurus oxyrinchus*) cartilage. *Food Hydrocoll.* 2004;**18**:573–579.
24. Liu, H., D. Li, and S. Guo. Rheological properties of channel cat fish *(Ictalurus punctaus)* gelatin from fish skins preserved by different methods. *LWT–Food Sci. Tech.* 2008;**41**:414–419.
25. Holzer, D. Gelatin production. U.S. Patent, 5,484,888, 1996.
26. Gudmundsson, M. and H. Hafsteinsson. Gelatin from cod skins as affected by chemical treatment. *J. Food Sci.* 1997;**62**:37–47.
27. Gomez-Guillen, M. C., J. Turnay, M. D. Fernadez-Diaz, N. Uimo, M. A. Lizarbe, and P. Mantero. Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocoll.* 2002;**16**: 25–34.
28. Duenas, G., F. Omar, and J. M. Goldberg. Topical hemostatic agents in gynecologic surgery. *Obest. Gynecol. Surv.* 2008;**63**:389–394.
29. Sabel, M. and W. Stummer. The use of local agents: Surgical and surgifoam, *Eur. Spine J.* 2004;**13**:S97–S101.

30. Zwischenberger, J. B., R. Brunston, Jr., J-R. Swann, and V. R. Lonti. Comparison of two topical collagen based hemostatic sponges during cardiothoracic procedures. *J. Invest. Surg*. 1999;**12**:101–106.

31. Tan, S. R. and W. D. Tope. Effectiveness of microporous polysaccharide hemispheres for achieving hemostasis in mohs micrographic surgery. *Dermatol. Surg*. 2004;**30**:908–914.

32. Cenni, E., G. Ciapetti, S. Stea, A. Corradini, and F. Carozzi. Biocompatibility and performance in vitro of a hemostatic gelatin sponge. *J. Biomater. Sci. Polym. Edn*. 2004;**11**:685–699.

33. Seyednejad, H., M. Imani, T. Jamieson, and A. M. Seifalian. Review: Topical hemostatic agents. *Br. J. Surg*. 2008;**95**:1197–1225.

34. Hong, Y. M. and K. R. Loughlin. The use of hemostatic agents and sealents in urology. *J. Urol*. 2006;**176**:2367–2374.

35. Weaver, F. A., D. B. Hood, and M. Zatina. Gelatin-thrombin based hemostatic sealent for intraoperative bleeding in vascular surgery. *Ann. Vasc. Surg*. 2002;**16**:286–293.

36. Szpalski, M., R. Gunzburg, and B. Sztern. An overview of blood-sparking techniques used in spine surgery during perioperative period. *Eur. Spine J*. 2004;**13**:S18–S27.

37. Council on Pharmacy and Chemistry. Absorbable gelatin sponge-new and nonofficial remedies. *JAMA* 1947;**135**:921.

38. Friedman, J. and T. S. Whitecloud III. Lumbar cauda equine syndrome associated with the use of gelfoam: Case report. *Spine*. 2001;**26**:E485–487.

39. Harris, W. H., Crothers, O. D., Bernard, J. L., Moyen, and Bourene, R. B. Topical hemostatic agents for bone bleeding in humans. *J. Bone Joint Surg*. 1978;**60A**: 454–456.

40. Gelfoam Abosrbable gelatin powder. Package insert. Pharmacia & Upjohn, Kalamazoo, MI; 2003.

41. Oz, M. C., J. F. Rondinone, and N. S. Shargil. Floseal matrix: New generation topical hemostatic sealent. *J. Corrd. Surg*. 2003;**18**:486–493.

42. Oz, M. C., D. M. Cosgruve III, B. R. Budduke, J. D. Hill, M. R. Flaunery, and R. Palumbo. Controlled clinical trial of novel hemostatic agent in cardiac surgry. *Ann. Thorac. Surg*. 2000;**69**:1376–1382.

43. Durrani, O. M., A. I. Fernando, and T. Q. Reuser. Use of a novel topical hemostatic sealant in lacriminal surgery: A prospective comparative study. *Ophthal. Plast. Reconstr. Surg*. 2007;**23**:25–27.

44. Ellegala, D. B., N. F. Maartens, and E. R. Laws, Jr. Use of floseal hemostatic sealant in trans-sphenoidal pituitary surgery: Technical note. *Neurosurgery* 2002;**51**:513–515.

45. Cappabianca, P., F. Esposito, I. Esposito, L. M. Cavallo, and C. A. Leone. Use of a thrombin-gelatin hemostatic matrix in endoscopic endonasal extended approaches: Technical note. *Acta Neurochir*. 2009;**151**:69–77.

46. Neil L. Kao. Endoscopic sinus. *Arch. Otolaryngol. Head Neck Surg*. 1995;**121**: 814–815.

47. Show, C. L., R. B. Dymock, A. Couein et al. Effect of packing on nasal mucosa of sheep. *J. Laryngol. Otol*. 2002;**114**:506–509.

48. Bradford, A., M. D. Woodworth, R. K. Chandra, J. D. Lebenger, B. Ilie, and R. J. Schlosser. A gelatin-thrombin matrix for hemostasis after endoscopic sinus surgery. *Am. J. Otolaryngol. Head Neck Med. Surg*. 2009;**30**:49–53.

49. Lee, D. I., C. Uribe, L. Eichel, S. Khonsari, J. Basillote, H. K. Park, C. C. Li, E. M. Mcdougall, and R. V. Claman. Sealing percutaneous nephrolithotomy tracts with gelatin matrix hemostatic sealant: Initial clinical use. *J. Urol*. 2004;**171**:575–578.

50. Guzzo, T. J., R. A. Pollock, A. Forney, P. Aggarwal, B. R. Matlaga, and M. E. Allaf. Safety and efficacy of a surgeon-prepared gelatin hemostatic agent compared with floseal for hemostasis in laproscopic partial nephrectomy. *J. Endourol*. 2009;**23**:279–282.

51. Sabel, M. and W. Stummer. The use of local agents: Surgicel and Surgifoam. *Eur. Spine J*. 2004;**13**:S97–S101.

52. Ferschi, M. B. and M. D. Rollins. Thromboemboli, acute right heart failure & disseminated intravascular coagulation after intraoperative application of a topical hemostatic matrix. *Anesth. Analg*. 2009;**108**:434–436.

53. Surgifoam Absorbable gelatin sponge. Package insert. Johnson & Johnson, New Brunswick, NJ, 2008.

54. Tomizawa, Y. Clinical benefits & risk analysis of topical hemostats: A review. *J. Artif. Organs*. 2005;**8**:137–142.

PART III

BIODEGRADABLE POLYMERS OF NATURAL ORIGIN: POLYSACCHARIDES

CHAPTER 4

CHITOSAN AND ITS DERIVATIVES IN CLINICAL USE AND APPLICATIONS

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CONTENTS

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4.1 INTRODUCTION

Chitosan (poly 1,4-β-D-glucosamine) is a naturally occurring cationic polysaccharide and refers to a large group of polymers that differ in their molecular weights (50–2000 kDa) and degree of deacetylation (40–98%) [1]. It is derived by partial *N*-deacetylation of chitin from crustacean shells [2]. Chitosan possess a rigid crystalline structure with inter- and intramolecular hydrogen bonds (Fig. 4.1).

The pKa value of chitosan is around 6.2–7 and is insoluble in neutral and alkaline pH solutions [3]. Chitosan degrades by hydrolysis of glycosidic links of acetylated residues in the presence of lysozyme [4]. Thus the degradation rate mainly depends on the degree of deacetylation [2]. In acidic medium, the amine groups in chitosan get protonated, resulting in a positively charged soluble polysaccharide with a high charge density. Thus at a high degree of deacetylation, chitosan adopts a more extended conformation due to charge repulsion, whereas at a low degree of deacetylation, it becomes coiled [1].

Recently, chemical modifications to chitosan have received a great deal of attention since the modifications can impart new biological activities and improve the mechanical properties [2]. The presence of three kinds of functional groups—amino group at C-2 and primary and secondary hydroxyl groups at C-6 and C-3 positions, respectively, in chitosan—allows chemical modifications of chitosan, resulting in numerous functional derivatives with desirable properties. For example, the sulfation process can alter the properties from cationic, hemostatic, and insoluble at alkaline pH to anionic, anticoagulant, and water soluble material [5]. Similarly quaternization of chitosan improves the stability of polyplexes and transfection efficiency [6]. Chitosan and its derivatives that have been used for various biomedical applications are summarized in Table 4.1.

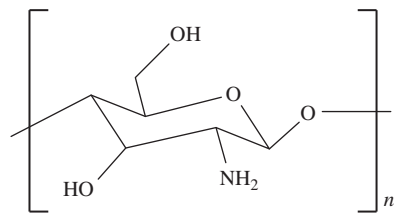

FIGURE 4.1 Structure of chitosan.

TABLE 4.1. Properties and Biomedical applications of various chitosan derivatives.

Derivatives	Properties	Applications	Reference
Sulfanilamide derivatives: (a) Chitosan sulfates (b) Sulfanilamide derivatives of chitosan	Reduces oxidative damage caused to cells	Antioxidant and free radical scavenging activity	7
Lauryl succinyl chitosan	Increases the residence time and bioavailability of drug and paracellular permeability	Oral peptide delivery	8
TMC–*N*-trimethyl chitosan chloride PEG–TMC	Widens the paracellular route for passage of macromolecular drugs	Permeability and uptake of hydrophilic molecules	9, 10
Carboxy methyl chitosan	Increases aqueous solubility and zwitter ion characters. Opens the tight junctions	Delivery systems	11
Chitosan acetate	Permeability across intestinal epithelia	Granulation binder for tablets. Sustained drug release and sustained release	12
Polyethyleneimine chitosan beads	Encapsulation by emulsion phase inversion	Increased mechanical stability and adsorption capacity and limits biodegradation	13
Deoxycholic acid conjugated chitosan oligosaccharide	Nonviral carriers		14
Chitosan N-betainates	Ability to condense gene into nanoparticles that can be endocytosed by cells and enters the nucleus	Gene delivery and high transfection efficiency	15
Chitosan–thioethylamidine	Increased mucoadhesion	Drug release	16
Carboxyl ethyl chitosan	Decrease the deoxyribonucleic acid (DNA) damage by scavenging the ROS and forms stable polymer radical	Antimutagenic (Protect E. gracilis from genotoxic actions of mutogens like acridine orange and ofloxacin)	17

The chief constituent in chitosan is glucosamine, a natural material that is present in the body and used to produce glycosaminoglycans, which form cartilage tissue [4]. Chitosan exhibits excellent properties such as biodegradability and biocompatibility and is amenable to chemical modification [18]. Moreover, it possesses microbicidal [1], antihypercholesterolemic [2], and immune stimulatory effects [19]. Chitosan promotes wound healing and has high availability, thus making it and its derivatives the most extensively investigated materials for pharmaceutical and biomedical applications [20]. This chapter presents an overview of recent research on various biomedical applications of chitosan and its derivatives, highlighting its role as biosensor, permeation enhancer, delivery vehicle, tissue engineering scaffold, and wound healing material.

4.2 CATIONIC NATURE OF CHITOSAN AND ITS IMPLICATIONS IN THERAPEUTICS

The cationic nature of chitosan is one of the key factors that render chitosan-based materials as versatile with a broad range of therapeutic applications [21]. Most of the bioactive properties of chitosan such as biocompatibility, biodegradability, microbicidal, and mucoadhesiveness exclusively depend on the cationic property of chitosan. The positively charged chitosan electrostatically interacts with negatively charged glycosaminoglycans and proteoglycans, which are linked with cytokines or growth factors [22]. This improves the cell adhesion and proliferation property of chitosan-based scaffolds [23]. Chitosan possesses a mucoadhesive property due to electrostatic interaction between positively charged chitosan and negatively charged sialic acid residues on mucosal surfaces [24]. The cationic property of chitosan has a high affinity toward the anions in bacterial cell wall, thereby preventing the mass transport across the cell wall. This microbicidal action of chitosan also plays a major role in the wound healing process [21].

In neutral or alkaline solutions, chitosan possesses free amino groups. The amine groups of chitosan aid in activation of macrophages and cytokines in mice models, thereby demonstrating their role in the wound healing process [18]. Moreover, chitosan has both thrombogenic and platelet adhesive properties, which also improve the wound healing process [18]. An antioxidant property has been identified in all kinds of sulfated chitosans [22]. The addition of the sulfanilamide group on chitosan and chitosan sulfates produce a stronger scavenging ability against superoxide and hydroxyl radicals [7]. 1,3,5-Thiadiazine-2-thione derivatives of chitosan have been shown to actively scavenge reactive oxygen species. Cationic chitosan shows the cytotoxic effects on various tumor cell lines such as HeLa (cervical cancer), HT1080 (human brosarcoma), A549 (lung cancer), and MRC-5 (human lung broblast) [25]. The antacid and antiulcer characteristics of chitosan were found to be more useful to prevent drug irritation in the stomach while delivering the drug [26].

4.3 SAFETY OF CHITOSAN

The toxicity of chitosan depends upon its route of administration, molecular weight, degree of deacetylation, and charge density [27]. The oral toxicity of chitosan shows a low lethal dose (LD_{50}) value of 16 g/kg in rats [28]. In rabbits, the oral administration of chitosan showed a significant amount being metabolized in the gut due to the presence of chitinolytic enzymes [18]. The intestinal absorption of radiolabeled chitosan was investigated in rats. The radiolabeled chitosan was digested into low-molecular-weight compounds in the gastrointestinal tract and distributed in tissues extensively [29]. Oral administration of chitosan up to 6.75 g in humans is reported as safe [18]. This may be attributed to the fact the chitosan does not accumulate in the body since it is converted into glucosamine derivatives, which are either excreted or used in amino sugar pool [18]. Baldrik et. al., demonstrated that 6–18% of chitosan was eliminated through feces after oral administration in dogs [18]. Moreover, the administration into the jejunum and colon showed about 40% loss of original weight [18]. Lee et al., evaluated the excretion rate of radiolabeled glucosamine in urine, feces, and expired air in rats after oral administration and observed that the clearance was found to be around 4, 17, and 54%, respectively. The remaining 25% was distributed into cartilage or connective tissue [30]. In vivo biodegradation of water-soluble chitosan in a mouse model reported 50% of deacetylated material was degraded and cleared readily, signifying no concerns of bioaccumulation [31].

Studies have also demonstrated the cytotoxic effects of high-molecular-weight chitosan when administered intravenously due to blood contact [18]. The intravenous administration of chitosan at low (4.5 mg/kg/day) and high doses (50 mg/kg/day) in rabbits for 10 days showed that at low dose no sign of abnormality was observed whereas a high dose caused death due to blood cell aggregation [18]. Chitosan administration through the subcutaneous route showed clinical signs such as vigor loss, anorexia, increased white blood cell count at 50 mg/kg/day, and death at 150 mg/kg/day [32]. As the charge density increases, the toxicity also increases [32]. Thus chemical modifications with little or no change in charge density produces little effect on toxicity beyond the unmodified one [33]. Alternatively, chemical modifications to mask the cationic charge may also lead to reduced toxicity of chitosan.

4.4 CHEMICAL MODIFICATIONS OF CHITOSAN

Although chitosan is soluble in aqueous dilute acids below pH 6.5, it is insoluble in water and most organic solvents [18]. Such limited solubility of chitosan brings special attention toward the modification of chitosan. Currently, the study of various modified chitosans or chitosan derivatives for biomedical applications are of more interest. The occurrence of reactive

functional groups in chitosan offers huge potential for chemical modification, which affords a wide range of derivatives such as *N*,*N*,*N*-trimethyl chitosan, thiolated chitosan, and sugar-bearing chitosan [34]. Generally, addition of side chain disrupts the crystal structure of the molecule and thereby increases the amorphous fraction. This event reduces the stiffness of the material and improves the solubility. Such chemical modifications of chitosan have been proved to impart desired physiochemical properties such as solubility, hydrophilicity, crystallinity, stability, charge density and the like into native chitosan. Hence, changes in the biological and physiochemical properties of chitosan depend on the nature of the side group. For example, *O*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (O-HTCC) possesses higher charge density than chitosan, resulting in a higher loading efficiency for model protein, bovine serum albumin [35]. Similarly, galactosylated chitosan has been found to be very effective to target asialoglycoprotein receptors in liver parenchymal cells for the treatment of liver-related disease [36]. Anticancer drugs require efficient intracellular delivery to kill the cancer cells. For example, the primary site of action for paclitaxel is the microtubule. Development of *N*-acetyl histidine conjugated glycol chitosan (NAcHis-GC) has been found to be proficient for the intracytoplasmic delivery of paclitaxel where histidine acts as pH-responsive fusogen [37]. Some widely used chitosan derivatives with specific properties are discussed in the following section.

4.5 HYDROPHOBICALLY MODIFIED CHITOSAN

Self-assembled nanoparticles or micelles have received a great deal of attention as a carrier molecule since it protects the drug from the environment. Chitosan derivative with amphiphilic property is essential for the formation of self-assembled nanoparticles, and the amphiphlicity can be introduced by the addition of hydrophobic moieties to chitosan [38]. Hydrophobic chitosan derivatives self-assemble into a micelle with a hydrophobic core in an aqueous media. Such a hydrophobic core of nanoparticles has been proved to be a reservoir for various drugs, and, since nanoparticles are small in size, they can be administered intravenously [34]. Additionally, conjugation of targeting moiety on such nanoparticles improves the therapeutic efficiency of chitosan-based material. Figure 4.2 shows the structures of the chitosan–linoleic acid conjugate and the chitosan–deoxycholic acid conjugate. Oleoyl-chitosan, which has a diameter of about 315.2 nm, has been evaluated as a carrier for hydrophobic antitumor drugs [38]. Moreover, its high encapsulation efficiency, sustained release profile, and no fibroblast cytotoxicity have also been proved. There are a variety of hydrophobically modified chitosans developed using moiety such as deoxycholic acid, linolenic acid, and linoleic acid [39–42]. These can form nanosized self-aggregates used to deliver hydrophobic drugs.

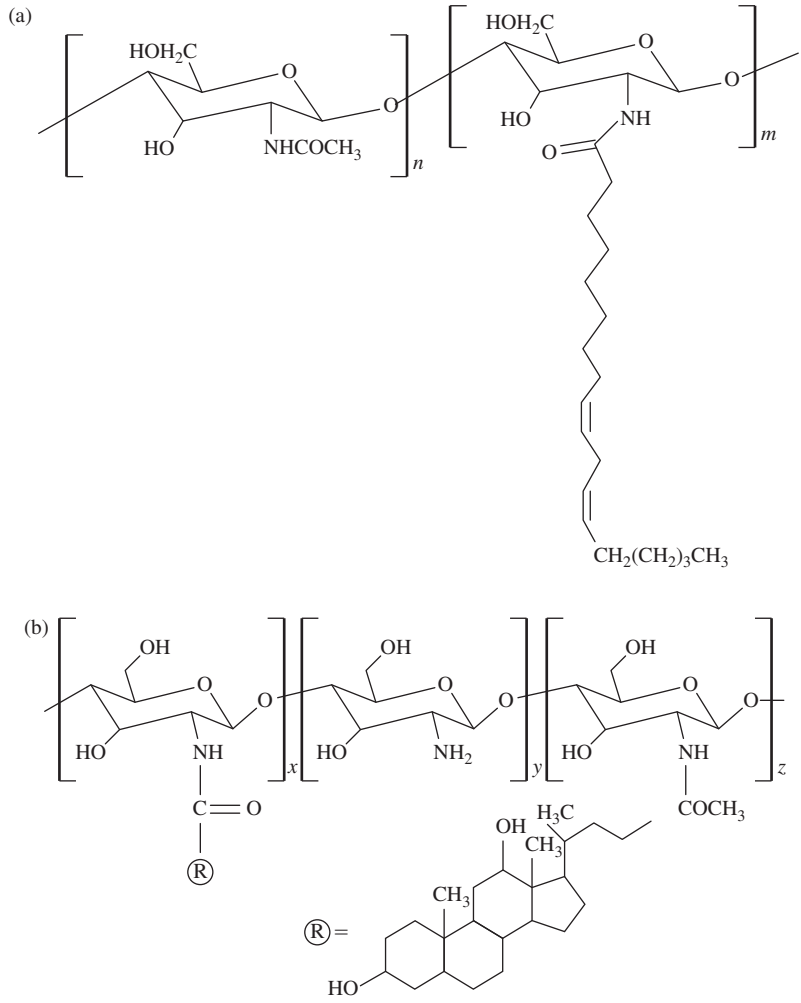

FIGURE 4.2 Structure of hydrophobically modified chitosan: (a) chitosan–linoleic acid conjugate and (b) chitosan–deoxycholic acid conjugate.

4.6 QUATERNIZATION OF CHITOSAN

4.6.1 Trimethyl Chitosan Chloride

Trimethyl chitosan chloride (TMC) is a derivative of chitosan that is completely soluble in neutral and basic pH at which native chitosan is not soluble, and the solubility of TMC mainly depends on the degree of methylation [43]. This can be used to increase the paracellular transport of hydrophilic mannitol

across the Caco-2 cells [44]. Moreover, the opening of a tight junction mainly depends upon the degree of substitution in TMC. Chitosan-HCl and TMC with varying degrees of deacetylation for enhancing the permeability of radiolabeled mannitol in Caco-2 intestinal epithelia at pH 7.2 was investigated [45]. TMC with 60% substitution increases the permeability of ^{14}C-labeled mannitol across Caco-2 monolayers where as chitosan-HCl and TMC with 12.8% methylation failed to permeate the cell membrane [45]. Chitosan may not be a suitable delivery system for targeting peptide drugs to jejunum or ileum since it gets aggregated at above pH 6.5 [46]. This can be overcome by the substitution of a primary amine with the methyl group, resulting in TMC. The investigation of cytotoxic effects of TMC on Caco-2 monolayers during permeability enhancement clearly stated that the TMC broadens the paracellular pathways without affecting the cell membrane [47]. Effect of various quaternized derivatives such as TMC, dimethylethylchitosan (DMEC), diethylmethylchitosan (DEMC), and triethylchitosan (TEC) on opening of a tight junction and transport of insulin across Caco-2 cells were observed in the order of TMC > DMEC > DEMC > TEC > chitosan [48] (Fig. 4.3). This may be due to steric hindrance of alkyl groups that shield cationic charge of quaternary amines. An in vivo study in rats on buserelin absorption with or without TMC60 at neutral pH indicated a significant increase in serum concentration of buserelin after the coadministration of the peptide drug with TMC60 [49]. Hence, this can be used as a safe permeability enhancer for the transmucosal delivery of peptide drugs.

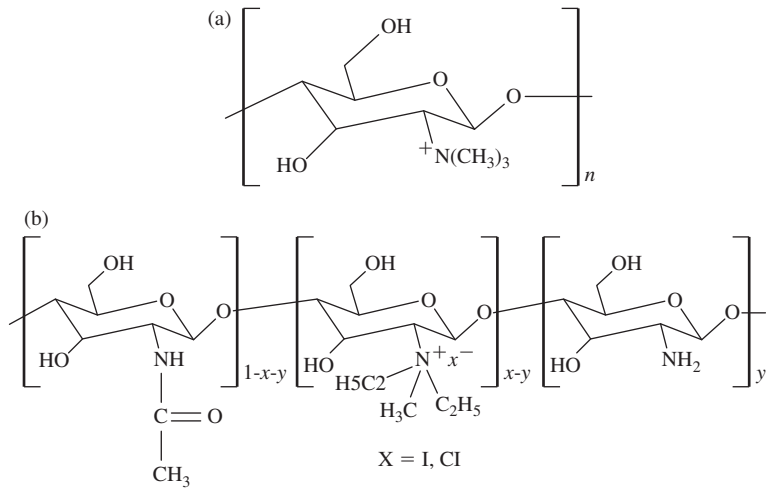

FIGURE 4.3 Structure of hydrophobically modified chitosan (a) trimethyl chitosan (TMC) and (b) diethylmethyl chitosan (DEMC).

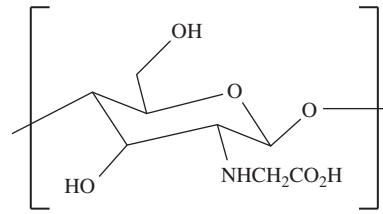

FIGURE 4.4 Monocarboxy methylated chitosan (MCC).

4.6.2 Monocarboxy Methylated Chitosan

Monocarboxy methylated chitosan (MCC) was synthesized by the chemical modification of amine groups with glyoxylic acid and sodium borohydride as the reducing agent (Fig. 4.4) [50]. The introduction of the carboxyl group bearing moiety on chitosan possesses polyampholytic or zwitter ionic characteristics [50]. This property is very useful to make clear gels or solutions even in the presence of polyanions (e.g., heparin) at neutral or alkaline pH [50]. However, chitosan and TMC were precipitated out of the solution in the presence of heparin, but MCC promotes aggregation in an acidic environment [50, 51]. Crosslinking of molecules can stabilize the chitosan derivative in acidic condition by preventing the cationization of amino groups. Both TMC and MMC found to have absorbtion enhancing properties [46, 52]. MMC has been found to enhance the paracellular transport of an anionic macromolecule through controlled, transient, and reversible opening of tight junctions without affecting the functional integrity of Caco-2 intestinal cell monolayers [46].

4.6.3 Thiolated Chitosan

Chitosan-thioglycolic acid is one of the derivatives used as a tissue engineering scaffold [53]. Thiolated chitosan is prepared by the modification of primary amine groups with cysteine, thioglycolic acid, and 2-iminothiolane (Fig. 4.5) [16]. Thiolation of chitosan augments its mucoadhesive property due to the formation of disulfide bonds with cysteine-rich subdomains of mucus glycoproteins [34]. This material is liquid below 25°C and has the ability to become a gel at 37°C [54]. The introduction of the thiol group makes the chitosan a more suitable candidate for being a scaffold in terms of wettability, degradation rate, and in situ gelling property [55]. The in vitro investigations of such conjugates using L-929 mouse fibroblast cells was found to be nontoxic [56].

4.6.4 PEGylated Chitosan

The limited solubility of chitosan due to its rigid crystalline structure restricts its use in the biomedical field [57]. Many researchers have tried to improve its solubility by grafting hydrophilic polymers onto chitosan [57, 58]. One such polymer is polyethyleneglycol (PEG), which improves the half-life of a drug in

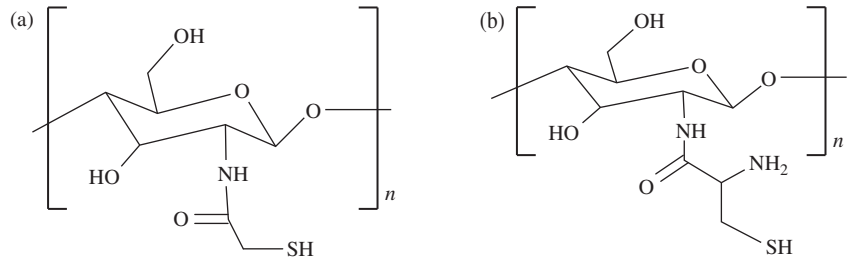

FIGURE 4.5 Structure of (a) chitosan–thioglycolic acid and (b) chitosan–cystein.

plasma circulation [59]. In addition, PEGylated trimethyl chitosan has improved the colloidal stability, biocompatibility, and transfection efficiency for gene delivery when compared to unmodified TMC [10]. Moreover, graft copolymerization onto chitosan effectively improves chitosan properties such as its chelating property, its adsorption, and its bacteriostatic properties [60].

4.7 CLINICAL APPLICATIONS OF CHITOSAN AND ITS DERIVATIVES

Chitosan has been extensively examined for biomedical applications in which attention has been focused on its absorption enhancing, mucoadhesive, and controlled-release properties [16]. Due to its linear unbranched structure and higher molecular weight, chitosan is an excellent viscosity-enhancing agent in acidic conditions [2]. This viscosity regulates biological properties such as wound healing, osteogenesis, and biodegradation [61]. The following sections deal with the applications of chitosan and its derivatives in the use as penetration enhancers in vaccine, drug, and gene delivery, antihyperlipidemic agents, for treatment of cancer, coating material on drugs, biosensor, and regenerative medicine.

4.8 PENETRATION ENHANCERS IN VACCINE AND DRUG DELIVERY

Chitosan at a high degree of deacetylation has been found to be very effective as permeation enhancer at both a high and a low molecular weight, whereas in a low degree of deacetylation it was only effective at a high molecular weight [62]. The influence of molecular weight and degree of deacetylation on the permeability of Caco-2 cells in intestinal monolayers was investigated [62]. By inhibiting the permeation effects of chitosan using anionic heparin, it has been demonstrated that the cationic charge of the chitosan only mediates the permeation effects [51]. The striking advantage of mucosal vaccination is the production of local antibodies at the sites when the pathogen enters the host. As vaccines alone are not sufficiently taken up after mucosal administration, it is necessary that it is

coadministered with penetration enhancers such as adjuvants or encapsulated particles. Bioavailability in an oral peptide drug is one of the major issues in vaccine delivery due to the presence of various barriers such as an acidic environment in the gastrointestinal tract, enzymatic barriers, and very limited absorption through the gut wall [63]. The mucoadhesive property of chitosan is very helpful to retain the peptide in the intestine for a longer period, thereby improving the bioavailability [64]. The combination of bioadhesion and opening of tight junctions between epithelial cells play a major role in chitosan-based mucosal delivery [65].

Rekha et al., developed insulin-loaded novel lauryl succinyl chitosan nanoparticles showing sustained release of insulin at a neutral pH [63]. Moreover, the low release at acidic pH (pH 1.2) reduces the insulin loss in the stomach, and the mucoadhesive property of such derivatives improved the retention time of insulin [63]. Ovalbumin-loaded chitosan microparticles was found to be easily taken up by the epithelium of murine Peyer's patches and released by the model antigen after the intracellular digestion in Peyer's patches [66].

Chitosan easily forms microparticles and nanoparticles that have the potential ability to encapsulate large amounts of antigens such as ovalbumin, diphtheria toxoid, or tetanus toxoid [66, 67]. It has been revealed that ovalbumin-loaded chitosan microparticles are taken up by Peyer's patches of the gut-associated lymphoid tissue (GALT) [66]. Coadministration of chitosan loaded with antigens in the nasal cavity, enhanced both mucosal and systemic immune responses. In contrast to oral administration, nasally administered vaccines have to be transported over a very small distance and are also not exposed to low pH values and degrading enzymes. Therefore, in the case of nasal delivery, the vaccine need not be incorporated into microparticles. Thus chitosan particles, powders, and solutions are promising candidates for mucosal and nasal vaccine delivery. Effective systemic and mucosal immune responses against the antigens were observed when *Bordedetella pertussis* filamentous hemaggulutinin and recombinant pertussis toxin were coadministrated with chitosan [67].

4.8.1 Carriers in Peptide Drug Delivery

The development of new delivery systems that could protect proteins from the environment, target to specific sites, and control the release for a prolonged period of time represents an enabling technology for treating various diseases and genetic disorders. Though the bioactive proteins and peptides are potential therapeutic agents, they cannot be administered orally. Oral administration of peptides is associated with the problems of very poor bioavailability due to the degradation by proteolytic enzymes in the gastrointestinal tract and of the impermeability to intestinal mucosa due to hydrophilicity, whereas parenteral route of administration requires repeated injections [63, 68]. Moreover, chitosan opens the tight junctions between the epithelial cells, which allows

the transport of oral vaccines and macromolecular drugs across the epithelial barrier [28, 52, 69]. Chitosan nanoparticles increase the nasal absorption of insulin to a larger extent in rabbits [25]. The coadministration of chitosan hydrochloride improves the intestinal absorption of peptide drugs in rats [70]. Microspheres prepared from chitosan and sodium alginate were found to release ibuprofen in a pH-dependent manner [47]. The cationic chitosan forms a complex through electrostatic attraction. When this ionic complex transferred to physiological pH, can dissociate the portion of polyanion, thereby releasing drugs in a pH- dependent manner. YIGSR-chitosan showed higher inhibitory activity against lung metastasis of B16Bl6 melanoma cells in mice than a peptide alone [71]. Recombinant human interleukin-2-loaded chitosan microspheres have been taken by model cells (HeLa, L-strain cells) and released the interleukin-2 for up to 3 months in a sustained manner [66].

4.8.2 Opthalmic Drug Delivery

The efforts in ophthalmic drug delivery have been devoted to increase the corneal penetration of drugs with the final goal of improving the efficiency of treatments of different ocular diseases [72]. The attempts include the use of colloidal drug delivery systems, such as liposomes, biodegradable nanoparticles, and nanocapsules [72]. The short residence time of these colloidal systems in the ocular mucosa represents a limitation in the therapy of extraocular diseases, such as keratoconjunctivitis sicca, or dry eye disease [72]. Chitosan nanoparticles have been used for site-specific delivery of drugs to the ocular mucosa, using the immunosuppressive peptide cyclosporin A (CyA) as a model drug [72]. The use of chitosan can also increase the corneal residence time of antibiotics and enhance the intraocular penetration of some specific drugs [73, 74].

The advantages of these systems in ocular drug delivery includes the ability to contact intimately with the corneal and conjunctival surfaces, thereby increasing delivery to external ocular tissues without compromising inner ocular structures and systemic drug exposure and providing these target tissues with long-term drug levels [72]. Consequently, these systems show great promise with regard to the circumvention of the present limitations in the management of external inflammatory or autoimmune ocular diseases, such as keratoconjunctivitis sicca, or dry eye disease [72].

4.9 GENE DELIVERY VEHICLE

One of the major challenges in gene delivery is to protect the DNA from the nuclease action. The cationic charge of chitosan is found to be very useful to interact ionically with negatively charged DNA, thereby protecting the DNA against the nuclease [5]. Chitosan and its derivatives have been used to mediate DNA delivery into various cell types such as B16 melanoma cells, COS-I, HeLa, human embryonic kidney (HEK) cells, human lung carcinoma cells, and mesenchymal stem cells [75]. Galactosylated chitosan–dextran–DNA was found to effectively transfect into liver cells [5]. Preparation of chitosan–plasmid DNA

complexes are cost effective and protect the DNA against DNases action without restricting plasmid size [76]. Moreover, the appropriate combination of the degree of deacetylation (DDA) and molecular weight determine the maximum transgene expression [76]. Similarly the gene transfection efficiency of quartenery chitosan was affected by the degree of quartenization [77, 78]. Germershaus et al., described the effect of physiochemical properties such as hydrodynamic diameter, condensation efficiency, and DNA release on transfection efficiency of chitosan-based nonviral vectors [6]. They found that quartenization of chitosan prevents polyplex aggregation and increases the transfection efficiency when compared to chitosan. Improvement of the colloidal stability of polyplexes and increased cellular uptake was observed in PEGylated TMC than in unmodified TMC [6]. Topical application of chitosan-based nanoparticles loaded with plasmid DNA has been utilized as a promising candidate for genetic immunization [5].

4.10 OTHER DELIVERY SYSTEMS

Corrigan et al., describe the development and characterization of salbutamol-sulfate-loaded spray-dried chitosan microparticles toward the delivery of drugs for treating asthma [79]. Chitosan has been found to be a promising carrier for colon drug delivery due to its colonic bacterial flora-mediated degradation [66]. Corticosteroid-loaded chitosan microspheres have been targeted to the colon for the treatment of colitisulcerosa [80]. Chitosan films prepared by solvent casting and evaporation technique have been investigated for the controlled release of riboflavin as a model drug and found to be efficient for use as carrier in stomach [81]. A chitosan-glycerol film was found to release colony-stimulating factor, GM-CSF [59]. Magnetic chitosan microspheres have been found to deliver the drug at the target site capillaries under the influence of external magnetic field [59]. Various forms of chitosan-based drug delivery systems such as tablets, capsules, microspheres, nanoparticles, beads, films, and gel are used to deliver the drugs such as salicylic acid, aspirin, paclitaxel prednisolone, doxorubicin, cyclosporin A, progesterone, and the like [65, 81, 82].

4.11 REGENERATIVE MEDICINE

Chitosan and its derivatives possess various special properties favorable to regenerate various tissues such as skin, bone, liver, cartilage, and nerve [83]. The antimicrobial is more effective in the regeneration of skin especially in the case of diabetic ulcer and burn victims [2, 81]. The incorporation of basic fibroblast growth factor in chitosan improves the wound-healing property [84]. Bilayer physical chitosan hydrogel improved the reconstruction of full skin for the treatment of third-degree burns [84]. A full thickness wound of 15 mm diameter on the back of a rat healed perfectly using water-soluble chitosan (WSC) complex ointment prepared from water-soluble chitosan and heparin [85]. Skin appendages such as hair follicles and sebaceous glands completely

regenerated similar to normal skin in WSC-complex-treated rat that cannot be repaired in full thickness wounds [85]. The application of a collagen–chitosan matrix as an implantable bioartificial liver was established due to its biological property and excellent blood compatibility [2]. The mechanical property of chitosan was increased threefold by the addition of alginate. The ionic interactions between chitosan and alginate contributed the improved mechanical property. This enables the mineral deposition in vitro and the formation of vascular tissue and calcified matrix in vivo [86]. Microsphere-based chitosan–PLAGA composite scaffolds showed excellent mechanical property similar to that of trabecular bone. Moreover, it has been observed that the presence of chitosan in chitosan–PLGA composite up-regulates the gene expression osteoblast-like MC3T3-E1 cells [87].

Electrospun nanofibers have received much attention because they mimic the extracellular matrix architecture. However, making electrospun chitosan nanofibers is a tough challenge due to their rigid backbone. In our laboratory, we have successfully spun a chitosan nanofiber mat for wound-healing applications (Fig. 4.6).

In vitro studies demonstrated that chitosan-based matrices support chondrogenic activity and also allow the expression of cartilage ECM proteins by chondrocytes [88]. Such matrices maintained the characteristics of differentiated chondrocyte phenotype such as round morphology, expressing type II collagen, aggregans and limited mitosis. Chitosan-gelatin, chitin/chitosan, and chitosan-polyglycolic acid have been proved effective to regenerate nerves [42]. Additionally, Jio et al., have found that chitosan-PGA grafts restore long-term delayed nerve defects (poor growth permisive environment due to the loss of schwann cell basement membrane) in rats [42].

The antimicrobial and thrombogenic properties of chitosan have generated considerable interest in wound-dressing applications [18]. The outer membrane of erythrocytes and platelets are negatively charged. Thus the cationic chitosan shows more affinity toward erythrocytes and platelets, resulting in platelet activation and clot formation in the absence of coagulation factors [18]. Celox is used to control the bleeding and maintain the hemostasis in animal models

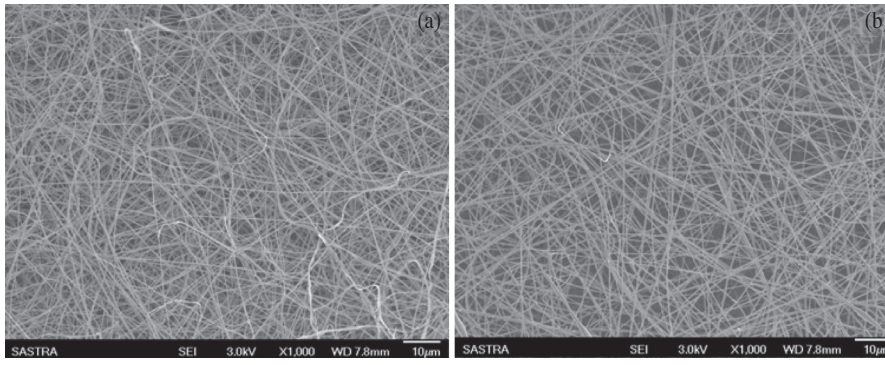

FIGURE 4.6 Electrospun nanofibers of (a) chitosan and (b) chitosan–gelatin.

[4]. Wound dressings made from chitosan-gelatin has showed its antibacterial property and reduces the wound area with minimal healing time compared to sterile vaseline guaze [89]. Meshes coated with triclosan-loaded chitosan gel were used to reduce bacterial count and to prevent graft infection in rats [90]. The sustained release of sulfadiazine from chitosan gel was found to be effective against infection in wound healing [91]. Chitosan-based wound dressings enhance the wound-healing process on multiple levels, which induce chemotaxis of macrophages and neutrophils, stimulate granulation tissue and reepithelization, carry growth factors, limit scar formation, possess antimicrobial activity, and enable sustained release of exogenous antimicrobial drugs to the site of infection [91, 92].

4.12 HYDROGELS

Currently, injectable in situ chitosan hydrogel systems have received a great deal of attention for the treatment of various cancers [94]. Subcutaneous injection of paclitaxel-loaded azide-chitosan-lactose hydrogel beneath the tumor was found to treat lung cancer by inhibiting tumor growth and tumor angiogenesis [93]. The intratumoral injection of chitosan/β-glycerophosphate hydrogel loaded with paclitaxol or doxorubicin or camptothecin were proved to be effective against the treatment of various cancers such as breast cancer and cervical cancer [93, 94]. An *N*-succinyl chitosan drug (e.g., mitomycin C) conjugates showed antitumor properties against various tumor cell lines (L1210, P388, or B16) in mice [68, 95]. Chitosan nanoparticles loaded with gadopentetic acid have been used as an intratumoral injectable device for gadolinium neutron-capture therapy [66].

4.13 CHITOSAN AS A COATING MATERIAL ON DRUGS

Chitosan coating of drugs can improve the bioadhesive property, drug payloads, and provide a sustained release of the drug [66]. The chitosan-coated polymeric microspheres of polyglycolic acid–polycaprolactone have been prepared to deliver the antiproliferative agents for the treatment of restenosis (narrowing of blood vessel due to smooth muscle cell proliferation). [66]. Similarly, chitosan coated with alginate microspheres and PLLA microspheres also has been found to release the drug over a period of time [66, 95].

4.14 BIOSENSOR

The exciting property of chitosan as a carrier molecule in delivery systems has been known for many years. In recent years, chitosan-based hybrid composites have received more attention for the development of biosensors [96]. The organic–inorganic hybrid nanobiocomposites improve the biosensor characteristics by surmounting the existing problem of aggregation and rapid degradation of metal oxide nanoparticles [96].

Wang et al., have developed the amperometric biosensor based on iron oxide–chitosan composite for the determination of ferritin [97]. Zinc oxide–chitosan nanobiocomposite has been utilized for the estimation of urea and cholesterol [98, 99]. Kaushik et al., have fabricated the chitosan–iron oxide (CH-Fe_3O_4) biosensor for glucose, urea, and ochratoxin [96, 100, 101]. Cross-linked chitosan matrix has been used as an amperometric biosensor for the estimation of hydrogen peroxide [102].

4.15 ANTIHYPERLIPIDEMIC EFFECT

Chitosan is also being investigated as an antihyperlipidemic agent [17]. The underlying hypothesis is that the positively charged chitosan may bind to free fatty acids and bile salt that disrupts lipid absorption in the gut. It has also been proposed that higher solubility of chitosan in the stomach emulsifies fat [18]. This fat entrapped gel in the intestine prevents the intestinal absorption of fat. Moreover, it has been found that the chitosan forms flocculus in duodenum, which can entrap dietary oil. Chitosan supplements in a diet have been found to reduce the cholesterol levels in rats [18]. However, the cholesterol-lowering effect of chitosan remains contentious with later findings and needs to be further evaluated [18].

4.16 CHALLENGES

Amazing systematic progress has been made on chitosan and its derivatives, demonstrating its huge applications in the biomedical field. The cationic nature of chitosan makes it a versatile material, not only for delivery applications but also as an extracellular matrix analog in regenerative medicine and as a versatile sensing element in biosensors. Moreover, all the applications mainly depend upon the degree of deacetylation and the molecular weight of chitosan. Additionally, the mucoadhesive property of chitosan appears to have potential as an absorption enhancer supporting drug uptake across the epithelial barrier. Extensive research has been carried out to demonstrate the safety of chitosan. Chitosan is capable of activating wound healing and tissue repair. Experiments are continuing on novel derivatives of chitosan to exert better biological activities for the improvement of human life.

REFERENCES

1. Hejazi, R. and M. Amiji. Chitosan-based gastrointestinal delivery systems. *J. Control. Release* 2003;**89**:151–165.
2. Wang, X. H., D. P. Li, W. J. Wang, Q. L. Feng, F. Z. Cui, Y. X. Xu, X. H. Song, and M. Werf. Cross linked collagen/chitosan matrix for articial livers. *Biomaterials* 2003;**24**:3213–3220.

3. Trapani, A., J. Sitterberg, U. Bakowsky, and T. Kissel. The potential of glycol chitosan nanoparticals as carrier for low water soluble drugs. *Int. J. Pharm.* 2009;**375**:97–106.
4. Wilson, B., M. Samanta, K. Santhi, K. P. Sampath Kumar, M. Ramasamy, and B. Suresh. Chitosan nanoparticles as a new delivery system for anti-Alzheimer drug tacrine. *Nanomed. Nanotech. Biol. Med.* 2010;**6**:144–152.
5. Senel, S. and S. J. Mc Clure. Potential applications of chitosan in veterinary medicine. *Adv. Drug Deliv. Rev.* 2004;**56**:1467–1480.
6. Germershaus, O., S. Mao, J. Sitterberg, U. Bakowsky, and T. Kissel. Gene delivery using chitosan, trimethyl chitosan or polyethylenglycol-graft-trimethyl chitosan block copolymers: Establishment of structure–activity relationships in vitro. *J. Control. Release* 2008;**125**:145–154.
7. Zhong, Z., X. Ji, R. Xing, S. Liu, Z. Guo, X. Chen, and P. Li. The preparation and antioxidant activity of the sulphanilamide derivatives of chitosan and chitosan sulfates. *Bioorg. Med. Chem.* 2007;**15**:3775–3782.
8. Rekha, M. R. and C. P. Sharma. Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption. *J. Control. Release* 2009;**135**:144–151.
9. Kotze, A. F., M. M. Thanou, H. L. Leuben, B. G. de Boer, V. J. Coos, and H. E. Junginger. Effect of the degree of quaternization of N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells. *Eur. J. Pharm. Bio. Pharm.* 1999;**47**:269–274.
10. Germershaus, O., S. Mao, J. Sitterberg, U. Bakowsky, and T. Kissel. Gene delivery using chitosan, trimethyl chitosan or polyethyleneglycol-graft-trimethyl chitosan block copolymers: Establishment of structure–activity relationships in vitro. *J. Control. Release* 2008;**125**:145–154.
11. Mphahlele, R. Drug release properties of cross linked N-carboxymethylated chitosan wafers. Mini dissertation. Tshwane University of Technology, South Africa, 2005.
12. Nunthanid, J., P. Sriamornsak, S. Limmatvapirat, S. Puttipipatkhachorn, L. Y. Lim, and E. Khor. Characterization of chitosan acetate as a binder for sustained release tablets. *J. Control. Release* 2004;**99**:15–26.
13. Chen, F., G. Luo, W. Yang, and Y. Wang. Preparation and adsorption ability of polysulfone microcapsules containing modified chitosan gels. *Tsinghua Sci. Tech.* 2005;**10**:535–541.
14. Chae, C. Y., S. Sohee, L. Minhyung, J. M. Kyeong, and N. J. Woon. Deoxycholic acid conjugated chitosan oligosaccharide nanoparticles for efficient gene carrier. *J. Control. Release* 2005;**109**:330–344.
15. Gao, Y., Z. Zhang, L. Chen, W. Gu, and Y. Li. Chitosan N betainates/DNA self-assembly nanoparticles for gene delivery: In vitro uptake and transfection efficiency. *Int. J. Pharm.* 2009;**71**:156–162.
16. Kafedjiiski, K., A. H. Krauland, M. H. Hoffer, and A. Bernkop-Schnurch. Synthesis and in vitro evaluation of a novel thiolated chitosan. *Biomaterials* 2005;**26**:819–826.
17. Kogana, G., Y. A. Skorik, I. Zitnanova, L. Krizkova, Z. Durackova, C. A. R. Gomes, Y.G. Yatluk, and J. Krajcovic. Antioxidant and antimutagenic activity of N-(2-carboxyethyl) chitosan. *Toxicol. Appl. Pharm.* 2004;**201**:303–310.
18. Baldrick, P. The safety of chitosan as a pharmaceutical excipient. *Reg. Toxicol. Pharm.* 2009, to appear.

19. Chatelet, C., O. Damour, and A. Domard. Influence of the degree of acetylation on some biological properties of chitosan films. *Biomaterials* 2001;**22**:261–268.
20. Dodane, V. and V. D. Vilivalam. Pharmaceutical applications of chitosan. *PSTT* 1998;**1**:246–253.
21. Mi, F. L., Y. B. Wu, and S. S. Shyu. Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. *Biomed. Mater. Res.* 2002;**59**:438.
22. Illum, L., I. J. Gill, M. Hinchcliffe, A. N. Fisher, and S. S. Davis. Chitosan as a novel nasal delivery system for vaccines. *Adv. Drug Deliv. Rev.* 2001;**51**:81–96.
23. Madihally, S. V. and H. W. T. Matthew. Porous chitosan scaffolds for tissue engineering. *Biomaterials* 1999;**20**:1133–1142.
24. Sinha, V. R., A. K. Singla, S. Wadhawan, S. Kaushik, R. Kumria, K. Bansal, and S. Dhawan. Chitosan microspheres as a potential carrier for drugs. *Int. J. Pharm.* 2004;**247**:1–33.
25. Je, J. Y., Y. S. Cho, and S. K. Kim. Cytotoxic activities of water-soluble chitosan derivatives with different degree of deacetylation. *Bioorg. Med. Chem. Lett.* 2006;**16**:2122–2126.
26. Gupta, K. C. and M. N. V. Ravi Kumar. Drug release behaviour of beads and micro granules of chitosan. *Biomaterials* 2000;**21**:1115–1119.
27. Chae, S. Y., M. K. Jang, and J. W. Nah. Influence of molecular weight on oral absorption of water soluble chitosans. *J. Control. Release* 2005;**102**:383–394.
28. Van der Lubben, I. M., V. J. Coos, G. Borchard, and H. E. Junginger. Chitosan and its derivatives in mucosal drug and vaccine delivery. *Eur. J. Pharm. Sci.* 2001;**14**:201–207.
29. Nishimura, Y., H. S. Kim, N. Ikota, H. Arima, H. S. Bom, Y. H. Kim, Y. Watanabe, M. Yukawa, and T. Ozawa. Radioprotective effect of chitosan in sub-lethally X-ray irradiated mice. *J. Radiat. Res.* 2003;**44**:53–58.
30. Lee, K. Y., M. Shibutani, H. Takagi, T. Arimura, S. Takigami, C. Uneyama, N. Kato, and M. Hirose. Subchronic toxicity study of dietary N-acetylglucosamine in F344 rats. *Food Chem. Toxicol.* 2004;**42**(4):687–695.
31. Onishi, H. and Y. Machida. Biodegradation and distribution of water-soluble chitosan in mice. *Biomaterials* 1999;**20**:175–182.
32. Minami, S., M. Ohoka, Y. Okamoto, K. Miyatake, A. Matsuhashi, Y. Shigemasa, and Y. Fukumoto. Chitosan-inducing hemorrhagic pneumonia in dogs. *Carbohydr. Polym.* 1996;**29**:241–246.
33. Kean, T. and M. Thanou. Biodegradation, biodistribution and toxicity of chitosan. *Adv. Drug Deliv. Rev.* 2010;**62**:3–11.
34. Park, J. H., G. Saravanakumar, K. Kim, and I. C. Kwon. Targeted delivery of low molecular drugs using chitosan and its derivatives. *Adv. Drug Deliv. Rev.* 2010;**62**:28–41.
35. Sun, Y. and A. J. Wan. Preparation of nanoparticles composed of chitosan and its derivatives as delivery systems for macromolecules. *J. Appl. Polym. Sci.* 2007;**105**:552–561.
36. Kim, E. M., H. J. Jeong, I. K. Park, C. S. Cho, C. G. Kim, and H. S. Bom. Hepatocyte-targeted nuclear imaging using 99mTc-galactosylated chitosan: Conjugation, targeting, and biodistribution. *J. Nucl. Med.* 2005;**46**:141–145.

37. Park, J. S., T. S. Han, K. Y. Lee, S. S. Han, J. J. Hwang, D. M. Moon, S. Y. Kim, and Y. N. Cho. N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles forintracytoplasmic delivery of drugs: Endocytosis, exocytosis and drug release. *J. Control. Release* 2006;**115**:37–45.

38. Zhang, J., C. G. Xi, Y. Y. Li, and C. S. Liu. Self-assembled nanoparticles based on hydrophobically modified chitosan as carriers for doxorubicin. *Nanomed. Nanotech. Biol. Med.* 2007;**3**:258–265.

39. Lee, K. Y., J. H. Kim, I. C. Kwon, and S. Y. Jeong. Self aggregates of deoxycholic acid modiefied chitosan as a novel carrier of adriamycin. *Colloid Polym. Sci.* 2000;**278**:1216–1219.

40. Liu, C. G., K. G. H. Desai, X. G. Chen, and H. J. Park. Linolenic acid-modified chitosan for formation of self assembled nanoparticles. *J. Agric. Food Chem.* 2005;**53**:437–441.

41. Chen, X. G., C. M. Lee, and H. J. Park. O/W emulsification for the self aggregation and nanoparticle formation of linoleic acid modiefied chitosan in aqueous system. *J. Agric. Food Chem.* 2003;**51**:3135–3139.

42. Subramanian, A., U. M. Krishnan, and S. Sethuraman. Development of biomaterial scaffold for nerve tissue engineering: Biomaterial mediated neural regeneration. *J. Biomed. Sci.* 2009;**16**:108.

43. Amidi, M., E. Mastrobattista, W. Jiskoot, and W. E. Hennink. Chitosan-based delivery systems for protein therapeutics and antigens. *Adv. Drug Deliv. Rev.* 2010;**62**:59–82.

44. Urrusuno, F. R., P. Calvo, R. C. Lopez, J. L. Jato, and M. J. Lonso. Enhancement of nasal absorption of insulin using chitosan nanoparticles. *Pharm. Res.* 1999;**16**:1576–1581.

45. Kotze, A. F., M. M. Thanou, H. L. Lueben, A. G. De Boer, J. C. Verhoef, and H. E. Junginger. Enhancement of paracellular drug transport with highly quaternized N-trimethyl chitosan chloride in neutral environments: In vitro evaluation in intestinal epithelial cells (Caco-2). *J. Pharm. Sci.* 1999;**88**,253–257.

46. Thanou, M., J. C. Verhoef, and H. E. Junginger. Oral drug absorption enhancement by chitosan and its derivatives. *Adv. Drug Deliv. Rev.* 2001;**56**:117–126.

47. Thanou, M. M., A. F. Kotze, T. Scharringhausen, H. T. Lueben, A. G. De Boer, J. C. Verhoef, and H. E. Junginger. Effect of degree of quaternization of N-trimethyl chitosan chloride for enhanced transport of hydrophilic compounds across intestinal Caco-2 cell monolayers. *J. Control. Release* 2000;**64**:15–25.

48. Sadeghi, A. M., F. A. Dorkoosh, M. R. Avadi, M. Weinhold, A. Bayat, F. Delie, R. Gurny, B. Larijani, M. R. Tehrani, and H. E. Junginger. Permeation enhancer effect of chitosan and chitosan derivatives: Comparison of formulations as soluble polymers and nanoparticulate systems on insulin absorption in Caco-2 cells. *Eur. J. Pharm. Biopharm.* 2008;**70**:270–278.

49. Thanou, M. M., B. I. Florea, M. W. E. Langemeyer, J. C. Verhoef, and H. E. Junginger. N-trimethylated chitosan chloride (TMC) improves the intestinal permeation of the peptide drug buserelin in vitro (Caco-2 cell) and in vivo rats models. *Pharm. Res.* 2000;**17**:27–31.

50. Thanou, M., M. T. Nihot, M. Jansen, J. C. Verhoef, and H. E. Junginger. Mono-N-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the

intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. *J. Pharm. Sci.* 2001;**90**,38–46.

51. Schipper, N. G. M., S. Olsson, J. A. Hoogstraate, A. G. De Boer, K. M. Varum, and P. Artursson. Chitosans as absorption enhancers for poorly absorbable drugs. 2. Mechanism of absorption enhancement. *Pharm. Res.* 1997;**14**:923–929.
52. Kotze, A. F., H. L. Luessen, B. J. De Leeuw, A. G. de Boer, J. C. Verhoef, and H. E. Junginger. N-Trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: in vitro evaluation in intestinal epithelial cells(CaCo-2). *pharm. Rev.* 1997;**14**:1197–1202.
53. Kast, C. E., W. Frick, U. Losert, and A. B. Schnürch. Chitosan-thioglycolic acid conjugate: A new scaffold material for tissue engineering. *Int. J. Pharm.* 2003;**256**:183–189.
54. Jeong, B., Y. K. Choi, Y. H. Bae, G. Zentner, and S.W. Kim. New biodegradable polymers for injectable drug delivery systems. *J. Control. Release* 1999;**62**:109–114.
55. Kast, C. E. and A. B. Schnürch. Thiolated polymers—thiomers: Development and in vitro evaluation of chitosan-thioglycolic acid conjugates. *Biomaterials* 2001;**22**:2345–2352.
56. Kast, C. E., W. Frick, U. Losert, and A. B. Schnürch. Chitosan-thioglycolic acid conjugate: A new scaffold material for tissue engineering. *Int. J. Pharm.* 2003;**256**:183–189.
57. Chang, S. J., C. C. Niu, C. F. Huang, and S. M. Kuo. Evaluation of chitosan-g-PEG copolymer for cell anti-adhesion application. *J. Med. Biol. Eng.* 2007;**27** (1):41–46.
58. Jian Du, E. and Y. L. Hsieh. PEGylation of chitosan for improved solubility and ber formation via electrospinning. *Cellulose* 2007;**14**:543–552.
59. Brown, C. D., L. Kreilgaard, M. Nakakura, N. C. Lelham, P. Wayne, R. Gombotz, and A. S. Hoffman. Release of PEGylated granulocyte-macrophage colony-stimulating factor from chitosan/glycerol films. *J. Control. Release* 2001;**72**:35–46.
60. Romaskevic, T., S. Budriene, A. Liubertiene, I. Gerasimcik, A. Zubriene, and G. Dienys. Synthesis of chitosan-graf-poly(ethylene glycol) methyl ether methacrylate copolymer and its application for immobilization of maltogenase. *CHEMIJA* 2007;**18**:33–38.
61. Glerentes, P., L. Vachoud, J. Doury, and A. Domard. Study of a chitin-based gel as injectable material in periodontal surgery. *Biomaterials* 2002;**23**:1295–1302.
62. Schipper, N. G. M., K. M. Varum, and P. Artursson. Chitosans as absorption enhancers for poorly absorbable drugs. 1. Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial (Caco-2) cells. *Pharm. Res.* 1996;**13**:1686–1692.
63. Rekha, M. R. and C. P. Sharma. Synthesis and evaluation of lauryl succinyl chitosan particles towards oral insulin delivery and absorption. *J. Control. Release* 2009;**135**:144–151.
64. Janes, K. A., P. Calvo, and M. J. Alonso. Polysaccheride colloidal particles as delivery systems for macromolecules. *Adv. Drug Deliv. Rev.* 2001;**47**:83–97.
65. Agnihotri, S. A., N. N. Mallikarjuna, and T. M. Aminabhavi. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Control. Release* 2004;**100**:5–28.

66. Lubben, V. L. M., J. C. Verhoef, G. Borchard G, and H. E. Junginger. Chitosan for mucosal vaccination *Adv. Drug Deliv. Rev.* 2001;**52**:139–144.
67. Lubben, V. L. M., F. Konings, G. Borchard, J. C. Verhoef, and H. E. Jungier. In vivo uptake of chitosan microparticles by murine Peyer's patches: Visualization studies using confocal laser scanning microscopy and immunohistochemistry. *J. Drug Target.* 2001;**9**:39–47.
68. Kato, Y., H. Onishi, and Y. Machida. N-succinyl-chitosan as a drug carrier: Water insoluble and water soluble conjugates. *Biomaterials* 2004;**25**:907–915.
69. Senel, S., M. J. Kremer, S. Kas, P. W. Wertz, A. A. Hincal, and C. A. Aquier. Enhancing effect of chitosan on peptide drug delivery across buccal mucosa. *Biomaterials* 2000;**21**:2067–2071.
70. Lueben, H. L., B. J. LeeuwDe, M. W. Langemeyer, A. G. Boer, J. C. Verhoef, and H. E. Junginger. Mucoadhesive polymers in peroral peptide drug delivery. VI. Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin in vivo. *Pharm. Res.* 1996;**13**:1668–1672.
71. Keong, L. C. and A. S. Halim. *In vitro* models in biocompatibility assessment for biomedical-grade chitosan derivatives in wound management. *Int. J. Mol. Sci.* 2009;**10**,1300–1313.
72. Angela, M. Chitosan nanoparticles: A new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. *Int. J. Pharm.* 2001;**224**:159–168.
73. Felt, O., P. Furrer, J. M. Mayer, B. Plazonnet, P. Buri, and R. Gurny. Topical use of chitosan in ophthalmology: Tolerance assessment and evaluation of precorneal retention. *Int. J. Pharm.* 1999;**180**:185–193.
74. Genta, I., B. Conti, P. Perugini, F. Pavaneto, A. Spadaro, and G. Puglisi. Bioadhesive microspheres for ophthalmic administration of acyclovir. *J. Pharm. Pharmcol.* 1997;**49**:737–742.
75. Liu, W., S. Sun, Z. Cao, X. Zhang, K. Yao, W. W. Lu, and K. D. K. Luk. An investigation on the physiochemical properties of chitosan/DNA polyelectrolyte complexes. *Biomaterials* 2005;**26**:2705–2711.
76. Lavertu, M., S. Methot, N. T. Khanh, and M. D. Buschmann. High efficiency gene transfer using chitosan/DNA nanoparticles with specific combinations of melocular weight and degree of deacetylation. *Biomaterials* 2006;**27**: 4815–4824.
77. Gao, Y., Z. Zhang, L. Cen, W. Gu, and Y. Li. Chitosan N-betainates/DNA self-assembly nanoparticles for gene delivery: In vitro uptake and transfection efficiency. *Int. J. Pharm.* 2009;**371**:156–162.
78. Thanou, M., B. I Florea, M. Geldof, H. E. Junginger, and G. Borchard. Quaternized chitosan oligomers as novel gene delivery vectors in epithelial cell lines. *Biomaterials* 2002;**23**:153–159.
79. Corrigan, D. O., A. M. Healy, and O. I. Corrigan. Preparation and release of salbutamol from chitosan and chitosan co-spray dried compacts and multiparticulates. *Eur. J. Pharm. Biopharm.* 2006;**62**:295–305.
80. Cremer, B. K. and J. Kreuter. Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model for antiinflammatory drugs. *J. Control. Release* 1996;**39**:17–25.

81. Nascimento, A., M. C. Laranjeira, V. T. Favere, and A. Josue. Impregnation and release of aspirin from chitosan/poly(acrylic acid) graft copolymer microspheres. *J. Microencapsul* 2001;**18**:679–684.
82. Obara, K., M. Ishihaarab, Y. Ozekia, T. Ishizukaa, T. Hayashib, S. Nakamuraa, Y. Saitod, H. Yurad, T. Matsuib, H. Hattorib, B. Takaseb, M. Ishiharac, M. Kikuchib, and T. Maeharaa. Controlled release of paclitaxel from photocrosslinked chitosan hydrogels and its subsequent effect on subcutaneous tumor growth in mice. *J. Control. Release* 2005;**110**:79–89.
83. Shu, X. Z. and K. J. Zhu. The influence of multivalent phosphate structure on the properties of ionically cross linked chitosan films for controlled drug release. *Eur. J. Pharm. Biopharm.* 2002;**54**:235–243.
84. Boucarda, N., C. Viton, D. Agay, E. Mari, T. Roger, Y. Chancerelle, and A. Domard. The use of physical hydrogels of chitosan for skin regeneration following third-degree burns. *Biomaterials* 2007;**28**:3478–3488.
85. Kweon, D. K., S. B. Song, and Y. P. Park. Preparation of water-soluble chitosan/heparin complex and its application as wound healing accelerator. *Biomaterials* 2003;**24**:1595–1601.
86. Li, Z., H. R. Ramay, K. D. Hauch, D. Xiao, and M. Zhang. Chitosan/alginate hybrid scaffolds for bone tissue engineering. *Biomaterials* 2005;**26**:3919–3928.
87. Jiang, T., I. Wafa, I. Fattah, and C. T. Laurencin. *In vitro* evaluation of chitosan/poly (lactic acid–glycolic acid) sintered microsphere scaffolds for bone tissue engineering. *Biomaterials* 2006;**27**:4894–4903.
88. Jin, R., L. S. M. Teixeira, P. J. Dijkstra, M. Karperien, C. A. Blitterswijk, Z. Y. Zhong, and J. Feijen. Injectable chitosan-based hydrogels for cartilage tissue engineering. *Biomaterials* 2009;**30**:2544–2551.
89. Deng, C. M., L. Z. He, M. Zhao, D. Yang, and Y. Liu. Biological properties of the chitosan-gelatin sponge wound dressing. *Carbohyd. Polym.* 2007;**69**: 583–589.
90. Attila, C., C. Yasemin, B. Irpanli, E. Bilensoyb, K. Yorgancia, C. Sema, Z. Saribas, and V. Kaynaroglua. Antibacterial activity of triclosan chitosan coated graft on hernia graft infection model. *Int. J. Pharm.* 2009;**381**:214–219.
91. Mi, F. L., Y. B. Wu, and S. S. Shyu. Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. *Biomed. Mater. Res.* 2002;**59**:438.
92. Shi, C., Y. Zhu, X. Ran, M. Wang, Y. Su, and T. Cheng. Therapeutic potential of chitosan and its derivatives in regenerative medicine. *J. Surg. Res.* 2006;**133**:185–192.
93. Ta, H. T., C. R. Dass, and D. E. Dunstan. Injectable chitosan hydrogels for localised cancer therapy. *J. Control. Release* 2008;**126**:205–216.
94. Ruel-Gariepy, E., M. Shive, A. Bichara, M. Berrada, D. L. Garrec, A. Chenite, and J.-C. Leroux. A thermosensitive chitosan-based hydrogel for the local delivery of paclitaxel. *Eur. J. Pharm. Biopharm.* 2004;**57**:53–63.
95. Chiou, S. H., W. T. Wu, Y. Y. Huang, and T. W. Chung. Effects of the characteristics of chitosan on controlling drug release of chitosan coated PLLA microspheres. *J. Microencapsul.* 2001;**18**:613–625.

96. Kaushik, A., P. R. Solanki, A. Ansari, G. Sumana, A. S. Bansi, and D. Malhotra. Iron oxide-chitosan nanobiocomposite for urea sensor. *Sensors Actuators* 2009;**138**:572–580.

97. Wang, S. F. and Y. M. Tan. Novel amperometric immunosensor based on Fe_3O_4 magnetic nanoparticles/chitosan composite lm for determination of ferritin. *Anal. Bioanal. Chem*. 2007;**387**:703–708.

98. Solanki, P. R., A. Kaushik, A. A. Anees, G. Sumana, and B. D. Malhotra Zinc oxide-chitosan nanobiocomposite for urea sensor, *Appl. Phys. Lett*. 2008;**93**:163903. [online journal]

99. Khan, R., A. Kaushik, P. R. Solanki, A. A. Ansari, M. K. Pandey, and B. D. Malhotra. Zinc oxide nanoparticles-chitosan composite film for cholesterol. *Biosensor. Anal. Chim. Acta* 2008;**616**:207–213.

100. Kaushik, A., R. Khan, P. R. Solanki, P. Pandey, J. Alam, S. Ahmad, and B. D. Malhotra. Iron oxide nanoparticles–chitosan composite based glucose biosensor. *Biosens. Bioelectron*. 2008;**24**:676–683.

101. Kaushik, A., P. R. Solanki, A. A. Ansari, S. Ahmad, and B. D. Malhotra. Chitosan–ironoxide nanobiocomposite based immunosensor for ochratoxin-A. *Electrochem. Commun*. 2008;**10**:1364–1368.

102. Miao, Y. and S. N. Tan. Amperometric hydrogen peroxide biosensor based on immobilization of peroxidase in chitosan matrix crosslinked with glutaraldehyde. *Analyst* 2000;**125**:1591–1594.

CHAPTER 5

CLINICAL USES OF ALGINATE

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Biodegradable Polymers in Clinical Use and Clinical Development, First Edition. Edited by Abraham J. Domb, Neeraj Kumar, and Aviva Ezra
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5.1 INTRODUCTION

The manufacture of chemicals from brown seaweed is one of the oldest branches of the chemical industry, dating back to 1720 when soda was first produced from kelp in France, and soon after in Ireland, Scotland, and Norway [1]. Toward the beginning of the nineteenth century, this was the only source of this chemical, upon which the glass and soap industries were developed. The kelp industry reached sizable proportions, as evidenced by the fact that in 1820 more than 20,000 tons of kelp were produced, involving the collection of several million tons of weed. However, by ca. 1870, this industry was struggling for survival due to the discovery of less expensive sources of potash and iodine. Foreseeing the end of the kelp industry, E. C. C Stanford, chief chemist of the principal company operating in this field, investigated that part of the seaweed that had been burnt during the previous 150 years in the hopes that it might contain further chemicals of potential value to the industry. As a result, he discovered alginic acid in 1883, suggested a formula for it, and found it to be a colloid not unlike cellulose in composition and properties [1]. Stanford developed the procedure of alkali extraction of a thick substance, *algin*, from the algae and later precipitated it using mineral acid [2, 3]. In 1896, algin was isolated by Krefting, whose experimention on the seaweeds of Norway produced a *tang acid* similar to Stanford's alginic acid: However, Krefting claimed that his preparation was nitrogen free [4–6]. It took 48 additional years before commercial production of algin began from the giant brown seaweed *Macrocystis pyrifera*—known in the United States as kelp—by the Kelco Co. in California. In addition to xanthan gum, Kelco has pioneered a number of other biogums, chemical synthetics, and organic solvent-soluble colloids. The first uses of the extracted material were as a boiler compound and for can-sealing purposes. In 1934, alginate was first used in foods as an ice-cream stabilizer. In 1944, propylene glycol alginate (PGA) was developed and fabricated on a commercial basis. Alginate production facilities were then founded in the United States, Europe, and Japan [7].

5.2 SOURCES

Alginates comprise a group of naturally occurring polysaccharides extracted from brown seaweed. They differ from agar and carrageenan, which are extracted from red seaweed [8]. Numerous dissimilar species of brown seaweed exist; the most widely used are *Laminaria hyperborea, M. pyrifera,* and *Ascophyllum nodosum*. *M. pyrifera* comprises the main group of alginates in the United States. It is a very large kelp found in sea beds ranging from 50 ft to 1 mile wide, several miles long, and 25–80 ft deep (Fig. 5.1). *M. pyrifera* affixes itself to the rock-strewn bottom with a rootlike structure termed *holdfast*, so-named because of the physically powerful ocean currents it battles. The stipes arise from the holdfast and branch three or four times near the base. Blades develop at irregular intervals along the stipe. The stipes are unbranched and each blade has a gas bladder at its base [9–11].

The kelp is cut to ~0.9 m below the ocean surface by mechanical means to allow sunbeam penetration and to support development. The cropped kelp is processed a short while later. In North America, more than a few additional *Laminaria* species can be found and are used in domestic production including: *Laminaria digitata* (found mostly on exposed sites on shores in the lower littoral where it can be the dominant algae, with a growth rate of 5.5% per day and a carrying capacity of about 40 kg wet weight per square meter—it may reach lengths of about 4 m and its distribution is limited by salinity, wave exposure, temperature, desiccation, and general stress [12, 13]); *Laminaria cloustoni* (a rigid and erect light-brown seaweed, with a cylindrical stem that is 0.9–1.8 m long and 5 cm thick at the base. Below the stem it divides into

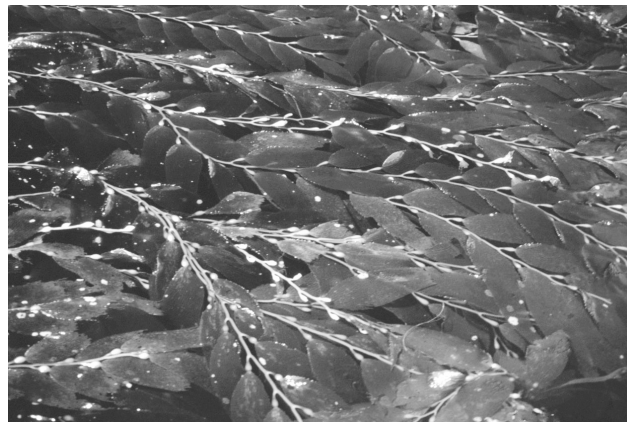

FIGURE 5.1 Giant kelp (*Macrocystis pyrifera*). (http://en.wikipedia.org/wiki/File:Giantkelp2_300.jpg; photograph, originally from the U.S. National Oceanic and Atmospheric Administration.)

rootlike branches, which spread and attach the plant to the submarine rocks. The plants grow on these rocks in the Atlantic, Pacific, and Arctic Oceans), and *Laminaria saccharina* (a brown alga in the Division Heterokontophyta, also known by the common name Sea Belt). In Europe, *Laminaria* species and *A. nodosum* are employed for manufacture, while the Japanese harvest *Eklonia cava* and the South Africans *Eklonia maxima*.

Alginate is found as a mixed calcium/sodium/potassium salt of alginic acid within the intercellular spaces and cell walls. Alginate molecules offer the strength and flexibility essential for algal growth in the ocean. The gum, which is generally sold as sodium alginate, is water soluble and used as a viscosity former, in addition to its gel-forming ability in the presence of calcium and/or other polyvalent metal ions. Trials to extract alginic acid from residual algae using the hot-water method were performed [14], but were unsuccessful since production is strongly influenced by the sulfated polysaccharide extraction method [14]. *Pseudomonas fluorescens* (an obligate aerobe that can be found in the soil and in water—certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration [15]) and *Pseudomonas putida* (a gram-negative rod-shaped saprotrophic soil bacterium [16]) grown in batch cultures on glucose and fructose (as carbon sources) can serve as a source for the synthesis of alginate-like polysaccharides [17]. Batch alginate production by immobilized *Azotobacter vinelandii* has also shown potential. The quantity of the produced alginate corresponds to ~60% of that recovered from a free-cell culture [18]. Another study dealt with the most favorable conditions for the production of bacterial alginate by *A. vinelandii* mutant NCIB 9068. The largest amount of bacterial alginate was obtained in ~110 h by a culture grown on optimal medium at 34°C and shaking at 170 rpm. The solution of bacterial alginate was more pseudoplastic than that of algal alginate, but no significant differences were observed among any of their other properties [19].

5.3 STRUCTURE

Stanford proposed that alginate is a nitrogenous substance, with the formula $C_{76}H_{76}O_{22}(NH_2)_2$ [8]. However, complementary contemporary isolation techniques have demonstrated that the pure product is nitrogen free. Throughout the 1950s, alginic acid was considered a polymer of anhydro-1,4-β-D-mannuronic acid [20], and it was only in the 1960s that L-guluronic acid was shown to be present as well. Consequently, we now know that alginic acid (Fig. 5.2) is a linear copolymer composed of D-mannuronic acid (M) and L-guluronic acid (G) [21, 22] units. Regions can be made up of either one unit or the other, or both in alternating sequence, that is, M blocks, G blocks, or heteropolymeric MG blocks, respectively. The monomers have a tendency to stay in their most energetically favorable structure in the polymer chain. For M-M, this is the $^4C_1$ chair form linked by a β-(1,4) glycosidic bond. For G-G, it is the $^1C_4$ chair

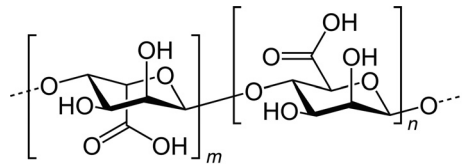

FIGURE 5.2 Structure of alginic acid. (http://en.wikipedia.org/wiki/File:Algins%C3%A4ure.svg; source: NEUROtiker.)

form linked by an α-(1,4) glycosidic bond. M blocks have an extended ribbon form, G blocks are buckled, and the MG-block regions are of intermediate rigidity [23]. The chemical composition of alginate has been determined by nuclear magnetic resonance (NMR) spectroscopy [23]. The behavior of a particular alginate depends on its molecular weight and on the proportion and arrangement of the M and G units. The composition of marketable brown seaweed has been investigated [24]: The percentage of M units ranged from 61% in *M. pyrifera* to 31% in *L. hyperborea*. The alternating-block region varied from 26.8% in *Laminaria* to 41.7% in *Macrocystis*. Diverse proportions of monomer have been found in "high M" (e.g., *Macrocystis* and *Ascophyllum*) and "high G" (*Laminaria*) alginates. Molecular weights of marketable alginates are in the range of 32,000−200,000, matching a degree of polymerization of 180−930 [25].

5.4 ALGINATE SOURCES AND PRODUCTION

5.4.1 Raw Materials

Only a small number of the abundant species of brown seaweed are suitable for commercial production. The three mentioned seaweeds, *L. hyperborea*, *M. pyrifera*, and *A. nodosum*, are harvested by coastal gathering, mechanical harvesting, or cutting by hand. The seaweed is then towed to the processing plant where it can be processed either wet or after drying [8].

5.4.2 Processing and Production of Propylene Glycol Alginate (PGA)

Today's methods of manufacturing PGA are modifications of processes invented by Green [26] and LeGloahec and Herter [27] in the 1930s, and information regarding these processes can be located elsewhere [8, 25]. Even though processing modifications and improvements can be noted in the numerous existing extraction plants, Stanford remains responsible for the fundamental process [25]. The extraction of calcium alginate includes size reduction of the raw material,

acid treatment, formaldehyde treatment, alkaline extraction, separation of insoluble seaweed residue including floatation, filtration, precipitation of calcium alginate, bleaching, and conversion of calcium alginate to alginic acid, dewatering of the alginic acid, and conversion of alginic acid to sodium alginate. A very comprehensive description of the process is given by McHugh [28].

Alginic acid can be treated with sodium carbonate to produce alginate salts or reacted with propylene oxide to produce PGA. PGA was first prepared ~63 years ago [29]. A better method was then published in a more informative patent [30], and an improvement upon that method was also reported [31]. These modifications decreased the reaction time to 2 or 3 h, mostly by removing inert gas from the reaction vessel. More recently, Noto and Pettitt [32] described a process using liquid propylene oxide mixed with partially neutralized alginic acid in a pressure vessel. Good esterification was achieved, even with very low neutralization of the alginic acid.

As already stated, PGA is an ester of alginic acid in which some of the carboxyl groups are esterified with propylene glycol, some are neutralized with an appropriate alkali, and some remain free. It is used as an emulsifier, stabilizer, and thickener in food products, with E number E405. Following the reaction, up to 90% of the carboxyl groups are esterified, and the remaining groups either remain free or are neutralized with sodium or calcium. Different PGAs can be produced on demand [8].

5.4.3 Alginate-like Polymers

In addition to the recognized traditional hydrocolloid extraction from brown seaweed, alginate-like polymers are manufactured by a number of bacteria as an exocellular secretion. *Pseudomonas aeruginosa* is a gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It is a partially acetylated secondary pathogen in patients with cystic fibrosis [33, 34], and the alginate that it produces includes variable amounts of M and G units. A similar polymer is produced by the soil bacterium *A. vinelandii*, which is a diazotroph that can fix nitrogen under aerobic growth conditions. Additional nonpathogenic species can be exploited as possible producers of alginate. Enzymatic modification with the enzyme mannuronan-C-5-epimerase from *Azotobacter* yields alginates with high G content, but this technique is not yet being used commercially. Another study described the optimal conditions of alginate production from glucose-based media by *A. vinelandii* [35]. Empirical models were used to estimate the C/N ratio (110 g-atom C per g-atom N) and concentrations of acetate and phosphate associated with optimum alginate yields, which ranged from 25 to 33% of the supplied glucose. More specifically, phosphate limitation (0.035 g/L) and no addition of acetate resulted in maximum alginate production as compared to excess phosphate (3.5 g/L) and acetate (3 g/L) [35].

5.5 PROCEDURES FOR PREPARATION OF ALGINATE SOLUTION

Alginate has to be dispersed before dissolution can take place. High-shear stirring and dry mixing with additional formulation components such as sugar or starch, or dispersion in oils or glycerol, promote the separation of alginate particles and subsequently their dissolution, provided these steps are taken before water is added. If dispersion is not performed properly, clumps form that are swollen on the outside, preventing contact between the water molecules and their center. In this case, very high-shear mixing must be performed to resolve the problem. Overheating during mixing must be avoided to eliminate degradation of the alginate, which results in reduced viscosity or gel strength. Soft water is suggested to avoid any undesirable crosslinking of the alginate by calcium. A long flow property of the smooth alginate solution is achieved after its dissolution in water. Solution viscosity can be tested by rotational viscometers designed for the study of non-Newtonian liquids.

A very weak gel may appear as a thick solution, making it difficult to differentiate between thickening and gelling, the former often resulting from limited calcium-alginate crosslinking. The flow characteristics of alginate solutions are affected by chemical and physical variables, including the presence of salts, sequestering agents, and polyvalent cations, polymer size, temperature, shear rate, concentration of the gum in solution, and the presence of other miscible solvents.

Solution viscosity relates to molecular weight, but it is also subject to the level of residual calcium from the manufacturing process; moreover, the higher the temperature, the lower the resulting viscosity of the hydrocolloid solution. Also, at high temperatures thermal depolymerization might occur. This is dependent on different variables, such as time, temperature, and pH. Depolymerization can be intentionally achieved via enzyme systems [36] for many industrial, medicinal, and other purposes. Cooling of the gum solution results in increased viscosity but does not result in gel formation. Frozen alginate solution sustains its viscosity after thawing. The addition of alcohols and glycols (water-miscible solvents) results in increased viscosity. pH influences alginate solutions differently, depending on the type of alginate used. Sodium-alginate solutions are not stable above pH 10, PGA is more stable at acidic pH, and sodium-alginate precipitates at pH < 3.5.

Sequestering agents prevent the calcium, which is inherent in the alginate, from reacting with it or prevent alginate's reaction with polyvalent ions in the solution. The addition of sequestering agents decreases viscosity relative to alginate without these agents. This holds true for both M- and G-type alginates. Furthermore, monovalent salts reduce the viscosity of dilute sodium alginate. The alginate polymer contracts as the ionic strength of the solution increases. Maximal viscosity is achieved at a salt level of 0.1 N. This effect can be reduced by both increasing the gum concentration and keeping the calcium level low. The effect of salt is prominent following an extended storage period and

depends upon the alginate source, the degree of polymerization, gum concentration in the solution, and the character of the monovalent salt used.

5.6 MECHANISM OF ALGINATE GELATION

A number of divalent cations react with alginate to form gels [37]. Calcium is particularly suitable for food applications due to its nontoxicity. Borax (Fig. 5.3), also known as sodium borate, sodium tetraborate, or disodium tetraborate, is an important boron compound, a mineral, and a salt of boric acid, that dissolves easily in water and has a wide variety of uses, such as the production of alginate gels for nonfood applications [8]. Until fairly recently, gels in general were thought to be produced by the ionic bridging of calcium ions of two carboxyl groups on adjacent polymer chains. However, even though these bonds are important for gelation, they are not regarded as energetically favorable enough to be solely responsible for it [38]. In poly-L-guluronate segments (with chain lengths of over 20 residues), enhanced binding of calcium ions takes place and a cooperative mechanism is involved in the gelation. Crosslinking occurs via carboxyl groups by primary valences and via hydroxyl groups by secondary valences. Coordinate bonds extend to two nearby hydroxyl groups of a third unit, which may be in the same molecular chain, to retain the macromolecule's coiled shape, or in another chain, resulting in the formation of a huge molecule with a three-dimensional (3D) netlike structure [25]. No such effects are observed for M blocks or alternating blocks.

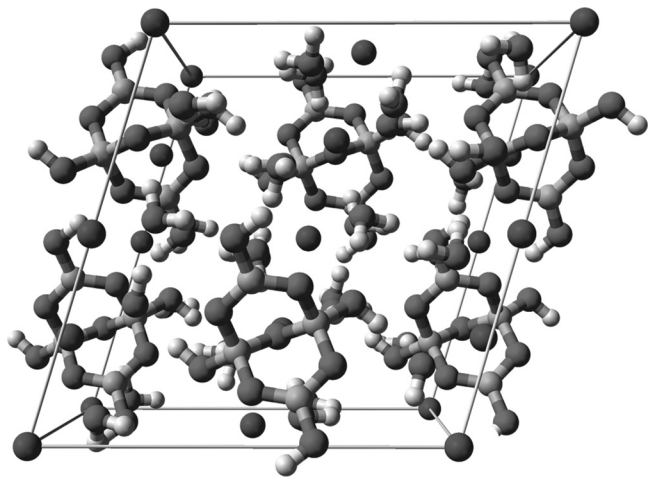

FIGURE 5.3 Ball-and-stick model of a unit cell of borax (sodium tetraborate decahydrate), $Na_2B_4O_7 \cdot 10H_2O$, better described as $Na_2[B_4O_5(OH)_4] \cdot 8H_2O$. (http://en.wikipedia.org/wiki/File:Borax-unit-cell-3D-balls.png; source: Ben Mills.)

Gel formation and the resulting gel strength obtained from an alginate are therefore very closely connected to the amount of G blocks and the average G-block length. High G content and long G blocks produce alginates with high calcium reactivity and the strongest gel-forming potential.

To obtain optimal gel strength, the degree of polymerization should be >200. A mechanism whereby a cavity acts as a binding site for calcium ions sandwiched between two diaxially linked G units is formed, producing a 3D structure called an "egg-box" (Fig. 5.4), within which the calcium interacts with the carboxyls and with the electronegative oxygen atoms of the hydroxyl groups. Gelation engages in chain dimerization, followed by aggregation of the dimers. Alginate gel is looked upon as a semisolid material, and the junction zones in which the alginate polymers are bound represent the solid state. Following gelation, the water molecules are actually entrapped by the alginate matrix or network but preserve their ability to migrate. This has significant outcomes for a range of applications. Gels with various consistencies can be formed by reacting soluble sodium alginate with calcium. A minute amount of calcium is sufficient to obtain a stiff texture. In fact, the stoichiometric reaction is 0.75 mg calcium per gram of algin (depending on the viscosity grade of the selected sodium alginate). The most favorable complexing of alginate and

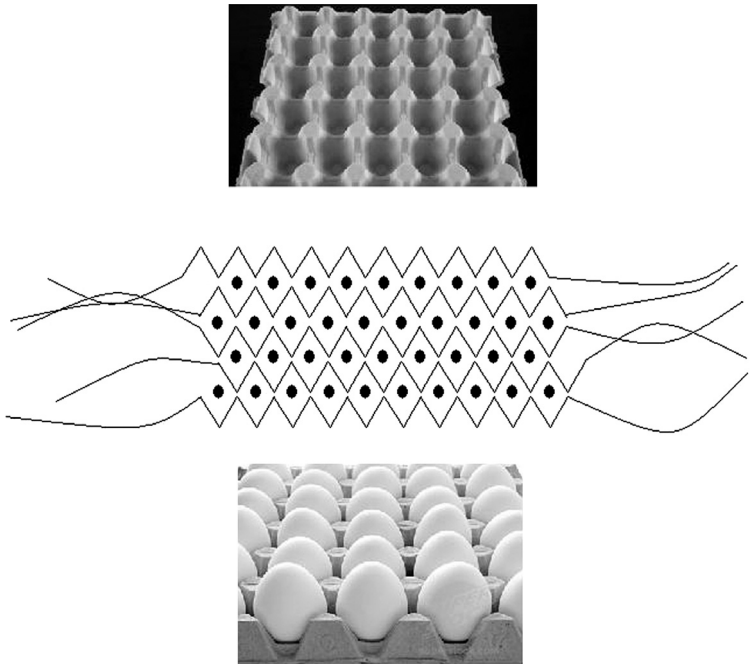

FIGURE 5.4 The "egg-box" model for alginate gel formation. (Adapted in part from A. Nussinovitch [8].)

calcium is obtained by using ~40% of the stoichiometric amount, which varies with calcium source, the presence of other soluble solids, and pH, among other things [39].

A mathematical model was developed to describe the kinetics of formation of calcium alginate gels in thermally set carrier gels [40]. The Sharp–Interface model was used to describe the crosslinking kinetics. The model presumes diffusion of calcium ions through a preformed gel of sodium alginate and a selected carrier, and an immediate reaction between the calcium and sodium alginate. The model can be employed to predict processing effects on food gels [40]. The structural changes in an alginate solution during the sol–gel transition induced by two divalent cations, Ca^{2+} and Cu^{2+}, were investigated by small-angle X-ray scattering. Purified alginates exhibited polyelectrolytic behavior [41]. Copper ions demonstrated a higher affinity for alginate than calcium ions. In the calcium-alginate system, the derived molecular parameters were correlated with the macroscopic properties of gelation. This correlation was not observed in the copper-alginate system, suggesting that different gelation mechanisms occur with calcium alginate and copper alginate [41]. Glucono-δ-lactone (GDL) E575 (Fig. 5.5) is a naturally occurring food additive used as a sequestrant, acidifier, and curing, pickling, or leavening agent. It is a lactone (cyclic ester) of D-gluconic acid. Pure GDL is a white odorless crystalline powder that is commonly found in honey, fruit juices, and wine. GDL is neutral but hydrolyzes in water to gluconic acid, which is acidic, adding a tangy taste to foods, with roughly one-third of the sourness of citric acid. The rate of GDL hydrolysis is increased by heat and high pH [42]. The effect of gum concentration on the strength and deformability modulus of a GDL alginate gel was characterized by a curve having a maximum strength for a particular concentration of hydrocolloid. Increasing the gum concentration beyond that point decreased the strength and the deformability modulus [43]. Sodium hexametaphosphate (SHMP) is a hexamer of composition $(NaPO_3)_6$ [44]. Commercial SHMP (Fig. 5.6) is typically a mixture of polymeric metaphosphates, of which the hexamer is one, and is usually the compound referred to by this name. SHMP hydrolyzes in aqueous solution, particularly under acidic conditions, to sodium trimetaphosphate and sodium orthophosphate [44]. Alginate gel set with SHMP exhibited similar mechanical behavior to

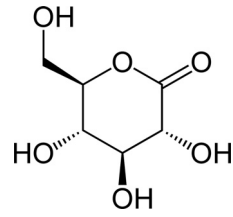

FIGURE 5.5 Glucono-δ-lactone: 2D skeletal diagram. (http://en.wikipedia.org/wiki/Glucono_delta-lactone; source: Benjah-bmm27.)

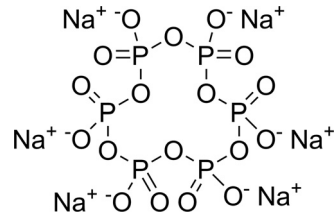

FIGURE 5.6 Chemical structure of sodium hexametaphosphate (SHMP). (http://en.wikipedia.org/wiki/File:Sodium_hexametaphosphate.png; source: Edgar181.)

GDL alginate gel, quite the opposite of an alginate gel set by calcium diffusion, which is characterized by a linear relationship between gel strength and gum concentration [43]. The stress–relaxation properties of alginate gels have been studied as well, in relation to their possible role in potential novel foods [45]. A strength–time study of alginate gels demonstrated that the compressive strength increases rapidly for ~15 h, then tends to stabilize asymptotically [46]. An empirical two-parameter mathematical model was used to estimate the asymptotic value on the basis of data obtained during the first 20 h. The common procedure of testing gels after 24 h was concluded to be satisfactory, and a similar two-parameter model was concluded to enable a reasonably accurate prediction of the gel's final weight [46].

5.7 ALGINATE GEL PREPARATION

5.7.1 Degree of Conversion and Thixotropy

Alginate gel properties depend on the type of alginate used, the degree of conversion to calcium alginate, the source of the calcium ions, and the method of preparation [8]. High-G alginates are required to form strong brittle gels. High-M alginates result in the formation of extra-elastic weaker gels that are less prone to syneresis. The degree of calcium conversion to calcium alginate (replacing sodium with calcium) has various effects. A small level of conversion results in increased viscosity. Higher conversion results in the development of gel structures. Additional conversion results in increased gel strength and thixotropic behavior whereby the produced gel converts into a fluid upon applying shear, and reforms when the shearing stops. Supplementary calcium leads to the production of irreversible gels: They do not reform with subsequent shearing [8]. Combinations of alginate and high-methoxy pectin (exhibiting synergism) can produce a gel at low solid contents and a wide range of pHs, in contrast to high-methoxy pectins that produce gels with high solid sugar contents [47, 48].

5.7.2 Diffusion Setting

Diffusion setting is another mechanism used for the manufacture of alginate gels. Gel beads (Fig. 5.7) can be produced by merely dropping alginate solution into a calcium chloride bath. The time needed for calcium to diffuse into the beads is not restrictive, predominantly if its concentration is deliberately increased. Concentrations on the order of 15% can be used for short times (on the order of seconds). For food purposes, less bitter calcium salts—such as calcium lactate or calcium acetate, are required. Acidic baths can be used when a calcium salt, which is only soluble under acidic conditions (e.g., calcium hydrogen orthophosphate), is solubilized within the alginate solution prior to extrusion [8]. When a product that contains low-molecular-weight ingredients (e.g., sucrose, acids) is prepared, diffusion occurs both in and out of the gel piece. Internal setting takes place when calcium is released under controlled conditions from within the system. The rate of calcium release is managed by pH and the solubility of the calcium salt. To avoid too rapid a reaction between the alginate and a crosslinking agent, a sequestering agent (such as SHMP) is added to the system and, depending upon pH, the system's aptitude to entrap or free the ions is changed [8]. Therefore, it is possible to make a preparation at a neutral pH and to change the pH suddenly to an acidic one, inducing the appropriate sequestering agent to release its entrapped ingredient, which then reacts with the alginate. Calcium release should be monitored using a special ion-selective calcium ion electrode. Full knowledge of the calcium concentration at any given stage of the process allows the creation of improved structured products [8].

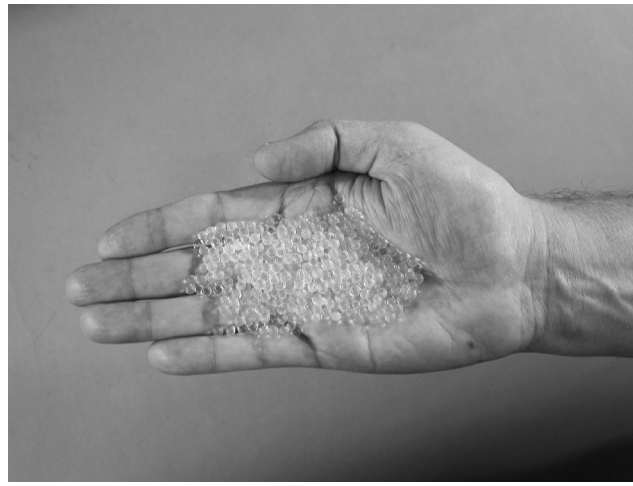

FIGURE 5.7 Alginate gel beads.

5.7.3 Establishing Dissimilarities Among Alginate Gels

Gel properties such as strength can be tested using a universal testing machine (UTM). Gel strength is regarded as the maximal stress (i.e., force divided by area) that breaks a gel (stress at failure). The key parameters influencing gel properties are temperature, pH, and the presence of proteins and neutral polymers [8]. Alginate gels are thermostable. In addition, once setting occurs, gels do not melt upon reheating. When the alginate is internally set and each of its constituents has been blended in, the system contains all the essentials for one-step gelation. Nevertheless, an increase in solution temperature confers additional thermal energy to the alginate chains, allowing their alignment, and consequently gelation is postponed. Upon cooling, associations occur. In such systems, due to the calcium's availability to all alginate molecules, a smaller degree of syneresis is detected. This is quite the opposite of systems in which gelation is induced by diffusion: There the molecules closest to the calcium source react first, resulting in instability, which induces greater gel shrinkage and syneresis [8]. An increase in alginate and calcium concentrations will require a higher temperature to prevent gelation. At 0.6% sodium alginate, and enough calcium to give 60% conversion (a molar ratio of 0.5 is theoretically sufficient for 100% replacement of the sodium), a temperature of 80°C is required to prevent gelation. Manufacture of a gel made of alginic acid (without calcium ions) is potentially possible by keeping the pH at below 3.5 (acid setting bath). Nevertheless the resultant gels are grainy and undergo syneresis. In a calcium setting bath, reducing pH lowers the amount of calcium required to obtain a gel. Upon inducing more acidic conditions, protein is also capable of interacting with sodium alginate [49] by electrostatic interaction. A decrease in pH increases this interaction's strength. At acidic pH, proteins are destabilized and denatured in the presence of alginate, and high-molecular-weight complexes are also produced [8].

5.8 CLINICAL APPLICATIONS, PRECLINICAL EXPERIMENTS AND POSSIBLE FUTURE OPTIONS FOR ALGINATE IMPLANTATION

5.8.1 Clinical Applications of Alginate in Urology

Use of Alginate for Treatment of Vesicoureteral Reflux. Normally, the distal ureter follows an oblique course through the bladder wall. When the urinary bladder distends, the distal ureter is compressed, thereby preventing urine reflux from the urinary bladder into the ureter and kidneys [50]. Vesicoureteral reflux (VUR) is caused by dysfunction of this mechanism, and the consequent retrograde flow of urine to the ureter and kidneys [51]. VUR increases the risk of recurrent ascending bacterial infections of the kidneys (pyelonephritis) and eventually, renal scarring and failure [50]. A grading system (I–V) has been established for the evaluation of VUR severity. Endoscopic injection of polytetrafluoroethylene (Fig. 5.8) into the ureterovesical junction for the treatment of VUR has been widely used since the 1980s

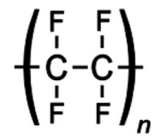

FIGURE 5.8 Polytetrafluoroethylene (PTFE), a synthetic fluoropolymer of tetrafluoroethylene. (http://en.wikipedia.org/wiki/Polytetrafluoroethylene.)

with a high rate of success (over 85%), although long-term effects remain unknown [52, 53]. Moreover, polytetrafluoroethylene has been found to migrate to distant organs, such as the lungs and brain [53], and its consequences there remain to be determined. The use of collagen for VUR treatment is of more limited efficacy, with an up to 70 or 80% success rate [53]. Moreover, over time, the use of collagen loses its efficacy [54]. It has been suggested that VUR be treated with endoscopic injection of autologous chondrocytes delivered in an alginate suspension [55]. Over time, the alginate biodegrades and is replaced with a cartilage matrix [55]. This concept was proven in four mini-pigs with induced VUR, in which chondrocytes were extracted from the auricle [52]. Injection of an alginate–chondrocyte–calcium sulfate solution resulted in reversal of VUR in all animals without ureter obstruction [52].

In a clinical trial conducted at two medical centers, 29 children aged 1–15 years, with grades II–IV VUR on at least one side, were included [53]. Cartilage was initially grafted from the ear concha, and chondrocytes were later extracted from the tissue. The chondrocytes were grown for 6 weeks, and then mixed with alginate. In the operating room, the mixture was further mixed with a solution of calcium chloride and calcium sulfate in order to obtain a thickened solution. The authors did not provide the concentration of calcium salts, but it is conceivable that they were related to a low level of conversion. The mixture was injected into the ureterovesical junction. The patients were followed for 3 months: 45% of the patients required a second injection of the alginate–chondrocyte mixture, resulting in an overall 83% success rate [53]. The adverse outcome rates were similar to those reported in other endoscopic therapies [53]. A 1-year follow-up of the study group found successful repair in 70% of the ureters, corresponding to 65% of the patients [54]. These percentages were comparable to the results following collagen injections. To improve the outcome, the authors reformulated the matrix for injection, increasing its viscosity and volume. However, no information is provided on the type of alginate used or the means used to increase viscosity [54]. Shifting of the injected material from the ureterovesical junction is the main cause of treatment failure. It is not yet known whether the use of chondrocytes or alginate or both is responsible for successful VUR treatment [54].

A phase III clinical trial is now being conducted to test prevention of surgery or antibiotic therapy after endoscopic implantation of a chondrocyte–alginate gel suspension (clinicaltrials.gov Identifier NCT00004487).

Alginate for Treatment of Intrinsic Urethral Sphincter Deficiency. Intrinsic urethral sphincter deficiency (ISD) may be congenital or acquired (e.g., following pelvic surgeries and treatment of prostate-related diseases) [56, 57]. It usually results in disabling stress urinary incontinence. Treatment may be surgical, although transplantation of periurethral bulking agents is an acceptable alternative [56]. Use of collagen as a bulking agent loses its efficacy in 3–19 months due to collagen biodegradation in some reports [56]. Nevertheless, it is an approved and widely used procedure in the United States [57]. Due to undesirable collagen degradation, an alternative was investigated: Chondrocytes (harvested from the ear pinna) were formulated into a hydrogel suspension consisting of an alginate–chondrocyte mixture. The alginate–chondrocyte suspension was added to a calcium solution to form an injectable gel, which was transplanted (transurethrally or periurethrally) in 32 patients with ISD. No information on the concentrations of either alginate or calcium was provided. One year following the procedure, it resulted in 50% full continence and an additional 31.3% improvement of incontinence [56]. The results were superior to those with commercial collagen implants (20–25% competency, and an overall 75% improvement) [56]. Adverse effects of injecting a chondrocyte–alginate matrix were minimal, except in patients that developed the inability to empty their bladder normally and required self-catheterization for 1 month [56]. In general, it can be concluded that alginate and autologous chondrocytes may be an efficient alternative to collagen for the treatment of VUR and ISD. Moreover, an alginate–chondrocyte mixture may have advantages over collagen in terms of providing longer-lasting relief. As it is inert, biodegradable, and does not spread systemically, alginate is suitable for these indications.

5.8.2 Clinical Applications of Alginate in Cardiology

Heart diseases are a common cause of morbidity and mortality. Coronary vascular diseases are estimated to cause 39% of deaths in the United Kingdom [58]. Myocardial infarction (MI) is caused by the sudden occlusion of a coronary artery or one of its branches. It is estimated that in the United States alone, 1 million patients develop MI annually [59]. Heart failure may result from various congenital or acquired causes [60], but MI remains the most common one, with a universal increase in prevalence in the last decade [60]. Heart failure is defined as the heart's inability to deliver blood supply to meet the body's demands [60]. Cases of heart failure may be divided into two types: systolic dysfunction due to contractile failure, and diastolic dysfunction caused by abnormal relaxation of the myocardium. The prognosis of symptomatically decompensated heart failure is very poor. It is frequently compared to the poor prognosis that accompanies malignant disorders [61]. The treatment usually includes inhibitors of angiotensin-converting enzyme (ACE), sympatholytic agents, diuretics, Na^+/K^+-ATPase pump inhibitors, aldosterone antagonists, and other pharmaceutical agents [60]. Nevertheless, treatment of advanced disease may require surgical implantation of a ventricular-assist device or heart

transplantation [58, 60]. Remodeling of the heart following initial damage is a complex process that involves mechanical, hormonal, and possibly genetic factors [61]. Remodeling may occur following MI and is characterized by cardiomyocyte hypertrophy, cellular loss, and increased fibrosis [61]. Over time, the infarcted area becomes thin, undergoes expansion, and loses its contractile abilities (being hypokinetic or dyskinetic) [59]. Eventually, the remodeling process causes a change in the normal myocardial geometry and myocardial failure [61]. Moreover, cardiac remodeling is associated with increased risk of early death. Reversal of the remodeling process may be induced in some patients following the use of ACE inhibitors or beta blockers [61]. It has been proposed that the process of remodeling and heart failure progression following MI is, in part, caused by damage to the extracellular matrix (ECM) during the early inflammatory phase [62, 63]. During that phase, monocytes and leukocytes infiltrate the affected tissue and secrete enzymes that damage the ECM [63]. For instance, matrix metalloproteases (MMPs) have been found to be secreted in the infarcted area and are believed to mediate some of the damage to ECM components [59]. The first 7 days following infarction are characterized by an acute-phase early inflammatory process, and early manipulations of the myocardium might be associated with increased risk for ventricular rupture. Therefore, treatments that attempt to prevent remodeling and improve contractile function following MI are usually initiated 7 days post infarction [59].

Alginate Injection for a Damaged Myocardium. Alginate, in the form of a crosslinked hydrogel, might present mechanical properties similar to those of the myocardial ECM [62]. Therefore, the use of alginate hydrogel as a substitute for the damaged ECM has been attempted in both recent and older MIs to reduce or reverse the remodeling. In one study, a calcium-crosslinked alginate solution was prepared from an aqueous solution of 30–50 kDa sodium alginate (1% w/v) by mixing it with a calcium gluconate solution (0.3% w/v) using homogenization to distribute the calcium ions throughout the solution. Calcium-crosslinked alginate (100–150 μL) of low viscosity (10–50 cP) or 150 μL of saline solution (as a control) was injected into the myocardium of 117 Sprague-Dawley rats: 67 of the rats had a recently induced MI (by intramural sewing up of the proximal left coronary artery) and 40 rats had a fully evolved MI, 2 months after induction [62]; 73 of the rats survived the MI induction and were evaluated. Results were compared among the alginate-injected group, the saline-injected control group, and a third group that was injected with 10^6 rat neonatal cardiomyocytes. Echocardiography was used for functional and dimensional evaluation. Eight weeks after the injections, the hearts were reevaluated. Biotin-labeled alginate was detected by using avidin-peroxidase immunostaining. It was demonstrated that 1 h post alginate injection, alginate was evident in 45 ± 4% of the infarcted scarred area. These levels decreased dramatically during the course of a few weeks, to 6 ± 1% at 6 weeks following injection. As time elapsed, the injected alginate was slowly replaced with

connective tissue and myofibroblasts [15]. The alginate injection was shown to significantly increase scar thickness and anterior wall thickness compared to saline or cardiomyocyte injection [62]. Both alginate and cardiomyocyte injections decreased the remodeling process in rats with recently induced MI [62]. Furthermore, the use of a low-viscosity alginate solution did not increase wall rigidity and did not cause diastolic dysfunction [62]. Interestingly, injection of alginate into the older infarcted area resulted in improvement of diastolic functions, via an unknown mechanism [62]. It is suspected that injection of alginate into the myocardial scar replaces the damaged ECM, provides mechanical support, and reduces wall stress [62]. The physical and functional similarities between alginate and ECM, and the potential use of alginate as a synthetic ECM have been reported [64]. Moreover, dyskinesis of the infarcted tissue decreases, along with a decrease in ventricular systolic dilatation [63]. This fact may be of clinical significance since the extent of systolic dilatation following MI is associated with bad prognoses [63]. The injected alginate was shown to undergo degradation over time, disappear from the myocardium, and be excreted by the kidneys [62]. Compared to injection of cardiomyocytes, alginate was superior in the prevention of remodeling [62].

In another study, a mixture of fibrinogen, fibronectin, factor XIII, and plasminogen was dissolved in an aprotinin solution to which gelatin-grafted alginate had been added. The mixture was injected intramyocardially with thrombin and 40 mM calcium chloride solution. After injection, a fibrinalginate composite formed within the myocardium, and this composite was tested for its possible effects on MI evolution in eight mature Yorkshire pigs, 7 days after MI induction [59]. Factor XIII is a coagulation factor that crosslinks fibrin molecules and is important for clot stabilization. Aprotinin is a protein that slows fibrinolysis. Thrombin is a coagulation protein that is important in the activation of several other coagulation factors and fibrin formation. Results were compared to those of nine pigs treated with saline. Injection of the fibrin–alginate mixture was associated with lower infarct expansion [59]. In the fibrin–alginate treatment group, the same infarction area was associated with a lower end-diastolic volume [59]. Moreover, injection of fibrin–alginate was found to be associated with a lesser degree of increase in MMP-2 in the infarcted area [59].

Other studies have supported the beneficial findings of alginate implantation in the aneurismal myocardial wall as a means of decreasing wall stress and myocardial dilatation [65]. Both fibrin and alginate induced an increase in ventricular function and restoration of myocardial geometry in rats with 5-week-old infarcts [65]. However, unlike in rats injected with fibrin glue, the alginate group showed persistent improvement in contractile function. Moreover, 5 weeks following transplantation, alginate was histologically evident, while fibrin was not [65]. Another study evaluated the effect of modified alginate, to which cell adhesion peptides containing the amino acid sequences arginine–glycine–asparagine (RGD) and tyrosine–isoleucine–glycine–serine–arginine (YIGSR) had been covalently attached, on an MI rat model [63]. The peptides

GGGGRGDY, GGGGRGEY, and YIGSRYIGSRY were covalently attached to alginate polysaccharides (30–50 kDa) via carbodiimide chemistry to yield a 0.2% rate of modification of uronic acid monomers by the peptide sequences. The peptide-modified alginate product was purified by dialysis and then lyophilized until dry. Solutions of calcium-crosslinked alginate (peptide-modified or unmodified) were prepared by thoroughly mixing an aqueous solution of 1% (w/v) polysaccharide with a solution (0.3% w/v) of D-gluconic acid/hemicalcium salt [63]. Both RGD and YIGSR peptides are frequently found in ECM proteins such as collagen fibronectin and laminin, respectively. They serve as cell recognition sites for some adhesive proteins found in the ECM. It was speculated that alginate modified with ECM proteins would improve myocardial healing and facilitate fibroblast recruitment: 29 rats survived the experiment and developed sufficiently large infarctions for testing. They were injected with different solutions 7 days after MI induction and were reevaluated 8 weeks later [63]. The following treatments were compared: unmodified alginate, RGD/YIGSR-modified alginate, RGE-modified alginate (a nonspecific peptide, arginine–glycine–glutamic acid, which has no role in the ECM), and saline solution. Injection of unmodified alginate was associated with preserved scar thickness on an echocardiograph, while both RGD/YIGSR- and RGE-modified alginates were associated with scar thinning, similar to saline controls [63]. Moreover, unmodified alginate injection was associated with an increase in contractile indexes and prevention of remodeling. In contrast, both RGD/YIGSR- and RGE-modified alginate injections were associated with remodeling and myocardial failure, similar to that observed in the saline-injected control group [63]. Compared to controls, microscopic observation of the tissue showed that the unmodified alginate group had thicker scars and decreased ventricular expansion [63]. Histologically, thicker scars and smaller expansion compared to controls was observed in the RGD/YIGSR-modified but not the RGE-modified alginate groups. The discrepancy between scar evolution in the RGD/YIGSR-modified alginate group as observed by echocardiograph and morphologically was explained to be a result of the different methods employed [63]. Moreover, histologically, in both modified and unmodified alginate groups there were cardiac cells in the infarcted area, compared to no cells in the saline-injected hearts. Overall, it was concluded that the use of unmodified alginate is superior to modified alginate in treating rats with induced MI and in preventing myocardial remodeling [63]. The disadvantages of the modified alginate may involve its enhanced viscosity (by four- to sevenfold) and decreased spread in the infarcted area [63]. Therefore, the effect of the modified alginate may be restricted to the small area of injection, and multiple injections might be required to enhance alginate distribution in the myocardial wall.

Use of Alginate as a Scaffold and Myocardial Implantation. Unmodified or RGD-modified alginate was used as a scaffold, in which human umbilical vein endothelial cells (HUVECs) were embedded [66]. The HUVECs were more

viable in RGD-modified alginate, suggesting that RGD supports cell proliferation better than unmodified alginate [66]. Integrin-mediated association to cell adhesion peptides, such as RGD, produces intercellular signal transduction, which may in turn produce, in some instances, cellular proliferation [67]. Both types of alginate and bovine serum albumin (BSA) in phosphate-buffered saline (PBS) as a control were compared for effectiveness following injection in rats with 5-week-old MIs. At 5 weeks following transplantation, both alginate preparations were associated with higher fractional shortening and other contractile indexes, a thicker ventricular wall, and less ventricular dilatation compared with controls [66]. It is probable that alginate injection alters mechanical stress on the myocardial wall and thereby reduces remodeling and prevents contractile dysfunction. Moreover, its mechanical properties prevent scar thinning and mechanical weakness of the scar tissue and thereby prevent paradoxical outward bulging of the scar during ventricular contraction (i.e., dyskinesis), which is associated with ineffective contraction. The finding of thicker scars following injection of unmodified alginate into infarcted areas is in line with previous publications [62, 63].

The myocardium has limited regeneration ability since cardiomyocytes are unable to divide. Moreover, there are limited pharmacological treatment options for advanced heart failure. In addition, the limited availability of heart donors is making heart transplantation a less feasible option for patients with established heart failure [68]. Therefore, the engineering of contractile tissue designed for implantation was attempted, based on an alginate scaffold [68, 69]. In one demonstrative study [68], scaffolds based on sodium alginate and calcium gluconate were freeze-dried. Then (after freeze-dehydration), neonatal rat cardiomyocytes were implanted at a concentration of 3×10^5 cells/scaffold (6 mm diameter $\times$ 1.0 mm height). The grafts were transplanted 7 days after MI induction in six rats, and compared to six sham-transplanted rats (in which the scar was stitched once, without any graft implantation) serving as controls. Alginate can be used to make hydrophilic porous 3D scaffold structures with relatively large pores (50–150 μm in diameter), enabling large-scale transplantation of cardiomyocytes [68, 69]. Cells were found to remain viable and preserve their contractile abilities in the alginate scaffold [68]. Moreover, the graft integrated into the scar tissue and neovascularization was demonstrated in the implanted scaffold [68]. Nevertheless, it has yet to be determined whether it is the alginate alone or a combination of the neonatal rat cardiomyocytes and alginate that encourages neovascularization [58], although the implantation of an unseeded scaffold was associated with significantly less neovascularization than with the seeded graft [68]. It is also possible that the designed 3D porous matrix facilitates neovascularization. A few weeks after implantation, the alginate scaffold was absorbed and replaced by cell-driven ECM [68, 69]. Furthermore, the alginate scaffold prevented remodeling following MI and preserved contractile function [68]. In another study, implantation of human embryonic stem cells (hESCs) on an alginate scaffold made no significant contribution to myocardial regeneration [58]. The use of centrifical force

during cell implantation on the alginate scaffold resulted in a 3D structure with high cell density [69].

Alginate and Restoration of Myocardial Blood Supply. Ischemia is defined as restricted blood supply to an organ or its parts. Although ischemia resides in the pathogenesis of MI evolution, and most cases of heart failure are induced by ischemia, not all patients with cardiovascular heart disease are candidates for revascularization, by either percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG) [70]. Moreover, up to 37% of the patients that undergo CABG will not obtain complete revascularization [71]. Therefore, novel techniques to improve myocardial blood supply have been sought. As mentioned above, an implanted alginate scaffold provides new vascular distribution [68]. Promising approaches, including gene therapy [70] and transmyocardial revascularization with laser [72], are being investigated. Other studies have found that alginate may promote angiogenesis, possibly via a proinflammatory response [65]. RGD-modified alginate (see section on alginate injection for a damaged myocardium) promotes angiogenesis more strongly than unmodified alginate (via integrin–ligand interactions and an effect on intercellular signaling pathways). Both regimens promote neovascularization more strongly than control PBS injection [66]. In contrast, it has also been reported that unmodified alginate injection into MI borders is not superior to saline injection in the induction of angiogenesis [73]. Therefore, the role of alginate in angiogenesis remains controversial. Bearing in mind that MI is the consequence of vascular insufficiency and ischemia, many strategies have attempted to induce angiogenesis by administration of growth factors, proteins, and genes to the myocardium [73]. Nevertheless, both systemic absorption and technical issues have limited the use of these approaches. Several growth factors are potentially capable of inducing vascular formation in ischemic tissue [70]. The use of alginate as an injectable carrier for vascular endothelial growth factor (VEGF) and platelet-derived growth factor BB (PDGF-BB) was successful [73]. VEGF may induce angiogenesis, while PDGF-BB assists in recruiting smooth muscle cells to the forming vessel [73]. A solution (100 μL) containing alginate, 3 μL VEGF or 3 μL PDGF-BB, or both, was injected into the border of the infarcted area 7 days after MI induction in rats. Alginate with VEGF or both VEGF and PDGF-BB induced more angiogenesis than alginate alone [73]. In that study, injection of alginate alone did not alter vascular density compared with saline injection [73]. Furthermore, it was found that proangiogenic therapy improves the systolic velocity–time integral but not ejection function [73]. The conflicting results were explained by the different measurement methodologies used for these indexes [73].

Basic fibroblast growth factor (bFGF) is another promising peptide that was evaluated for its ability to induce angiogenesis [70]. bFGF enables mesenchymal cell proliferation, angiogenesis, and growth of fibrous connective tissue [74]. Sterilized heparin sepharose beads were mixed with filter-sterilized sodium alginate. The mixture was dropped into a solution of $CaCl_2$

(1.5% w/v) and heparin–alginate beads formed instantaneously [71]. First, it was demonstrated that implantation of bFGF carried in heparin–alginate beads in a swine model of MI results in significant angiogenesis [71]. Prompted by these results, a phase I randomized double-blind placebo-controlled trial was conducted in 16 CABG patients to examine the proangiogenic effect of epicardial transplantation of heparin–alginate beads containing bFGF (10 or 100 μg). Technically, the beads were transplanted in the epicardial fat adjacent to an ischemic area that could not undergo revascularization with a bypass graft. Results were compared to a control group consisting of eight patients in which heparin–alginate beads with no bFGF were transplanted. Implantation of high-dose bFGF–alginate beads during CABG was associated with symptomatic improvement and improved perfusion in thallium scans. On the other hand, a low dose of bFGF showed no major change in the perfusion scan results, while 50% of the control group had deteriorated perfusion results [71]. Polypyrrole (PPy), a conductive polymer, was mixed with alginate and injected into 1-week-old infarctions in a rat model. The alginate–PPy mixture was found to be superior to alginate alone and to PBS in the induction of angiogenesis [75]. The role of alginate alone in the induction of angiogenesis remains to be established. Further research is also required to evaluate the connection between alginate-associated angiogenesis and systolic function.

Conclusions. Alginate may have several future clinical applications in cardiology in the prevention of myocardial remodeling, treatment of recent or old MIs, use as a cellular vehicle in the engineering of 3D myocardial tissues, as a means of delivering drugs or growth factors to the myocardium, and in the induction of angiogenesis. Moreover, there is at least one encouraging phase I study in humans that may promote the use of alginate in conjugation with other materials for the treatment of myocardial ischemia. Today, cardiac catheterization is performed on a routine basis and the myocardium is easily accessible. Therefore, transplantation of cells and biologically compatible compounds such as alginate directly into the myocardium is feasible [76], and may become clinically routine based on accumulated experimental evidence.

5.8.3 Clinical Applications of Alginate in Treating Diabetes Mellitus

Diabetes mellitus (DM) is a disease associated with major morbidity that is becoming increasingly common worldwide. Type 1 DM (DM1), also known as insulin-dependent DM, is caused by autoimmune destruction of the insulin-secreting ß cells in the pancreas. DM type 2 (DM2) is a metabolic disease that is far more common than DM1 [77]. It was formerly known as non-insulin-dependent DM and is caused by increased insulin resistance and a decreased effect of circulating insulin. In DM1, insulin is the preferred initial treatment. In contrast, the initial therapies in DM2 include lifestyle modifications, drugs that decrease the liver's production of glucose, and drugs that increase the

secretion of insulin from the pancreas due to hyperglycemia. Nevertheless, insulin treatment can eventually be required in DM2. Several long-term complications may be encountered in patients with DM: retinopathy that may progress to visual loss, nephropathy that may be complicated by end-stage renal disease, and neuropathy that may cause neuropathic pain [77, 78]. In addition, diabetic foot may lead to amputation. Moreover, DM accelerates atherosclerosis and is associated with increased risk of MI and stroke: 70% of patients with DM2 die from cardiovascular complications [77]. Other morbid conditions associated with DM2 include increased risk for fractures, depression, and cognitive deterioration [78].

Since the 1970s, treatment of diabetes with pancreatic islet cell transplantation has been attempted in animal models [79]. Some of these attempts have been successful. Islet transplantation has an advantage over pancreas transplantation in that it requires a less extensive operation and less immunosuppression [80]. Lim and Sun [81] were the first investigators to propose the use of encapsulated cells for transplantation. They demonstrated that microencapsulated cells are functional for almost 3 weeks in a rat model of diabetes, compared to 6–8 days for nonencapsulated cells [81]. During the 1990s, human trials of islet transplantation resulted in a roughly 6% success rate [82]. Not surprisingly, it was suggested that implantation success depends on cell quality and immunosuppression [82]. Nevertheless, the need for chronic immunosuppression poses a major disadvantage for transplantation [83]. A lack of sufficient organ donors [80, 82, 83] is another problem that is not easily solved. Moreover, transplantation of a partial pancreas segment from a living donor may be complicated by a 25% chance of the donor developing diabetes or by pancreatitis and pancreatic pseudocysts, which may be life-threatening [80]. The use of alginate for encapsulation of xenograft islet cells has the potential of overcoming both obstacles since it may prevent immune reaction against the graft [83, 84], and it enables the use of nonhuman tissue, which is far more available.

The use of alginate with a high-M content for islet encapsulation resulted in unstable beads. Therefore high-G alginate was used for islet encapsulation by Soon-Shiong et al. [84]. Encapsulated islets were transplanted on two different occasions in a 38-year-old patient with DM1. This patient received low-dose immunosuppressants (i.e., cyclosporin and azathiopine) following kidney transplantation that was required due to diabetes-associated end-stage renal disease. During the 9 months of reported follow-up, the patient developed no side effects and required lower doses of insulin supplements until he could withdraw from the use of exogenous insulin altogether [84]. The functionality of the transplanted islets was confirmed by an elevation in C-peptide (a by-product of insulin synthesis) [84]. Moreover, during the follow-up, the patient reported less neuropathic pain and showed improved axonal nerve function by electromyography, more rapid healing of diabetic ulcers, and stable renal function. Furthermore, the patient reported an improvement in quality of life [84].

Elliott et al. [85] reported the case of a 41-year-old male with DM1 who had undergone chronic insulin treatment for 9 years and who underwent transplantation of fetal pig pancreatic islets embedded in alginate-poly-L-lysine (PLL)–alginate beads. This involves a polyanion-polycation reaction. The patient was not treated with immunosuppressant. In total, he was implanted with 1.3 million islet equivalents. The beads were laparoscopically injected into the peritoneal cavity. Following transplantation, the insulin required to maintain normal glucose levels in the serum was reduced by up to 30% for 14 months [85]. At 4 months following transplantation, urine levels of porcine C-peptide reached a maximum of 9.5 ng/mL in a 24-h collection. Nevertheless, 14 months after transplantation, the patient required the same insulin doses used prior to transplantation; 9.5 years following transplantation, the patient underwent a second laparotomy, during which nonfibrotic nodules were apparent on the mesenterium. Histological examination revealed undamaged capsules that contained viable porcine cells, most of which were glucagon-secreting cells and a few were insulin-secreting cells (Fig. 5.9) [85]. This report demonstrated that alginate may be a safe and efficient carrier for animal tissue transplantation, and may provide long-term survival for some of the implanted cells. It is possible that a different capsule design might enable long-term survival of more transplanted cells, enabling the implant to provide insulin support for longer periods of time.

A standardized procedure for the encapsulation of pancreatic islets for clinical use was suggested by Calafiore et al. [83]: The islets (at least 400,000 islet equivalents with at least 80% purity) are placed in 1.6% alginate solution and are dripped into 1.2% $CaCl_2$ solution. The ratio of islet to alginate should result in one islet per bead and up to 5% empty alginate beads [83]. A subsequent covering with poly-L-ornithine (PLO) and additional alginate coating eventually produces alginate–PLO–alginate beads. The beads should be incubated for 24 h and then diluted in 100 mL saline solution, and injected into the peritoneal cavity [83].

Subsequently, Calafiore et al. [86] published some encouraging results of encapsulated-islet transplantation in two DM1 patients who were followed up for 6–12 months and showed a decreased need for external insulin. A phase I clinical study is now underway (www.ClinicalTrials.gov identifier NCT00790257) to test the immune reaction toward, and the insulin secretion of, subcutaneously implanted allogeneic human pancreatic islets embedded in an alginate-based matrix.

Immunoprotective Properties of Alginate Beads. Alginate beads form a semipermeable matrix that protects grafted cells from the recipient's immune system. The matrix prevents antibodies, as well as cytotoxic cells, from reaching the entrapped cells [87], especially those in alginate–PLL–alginate beads [88]. Alginate has a negative charge and therefore cells that have negatively charged membranes are repelled by it [87]. Use of a positively charged PLL coating has the opposite effect, accelerating macrophage and fibroblast adhesion to the

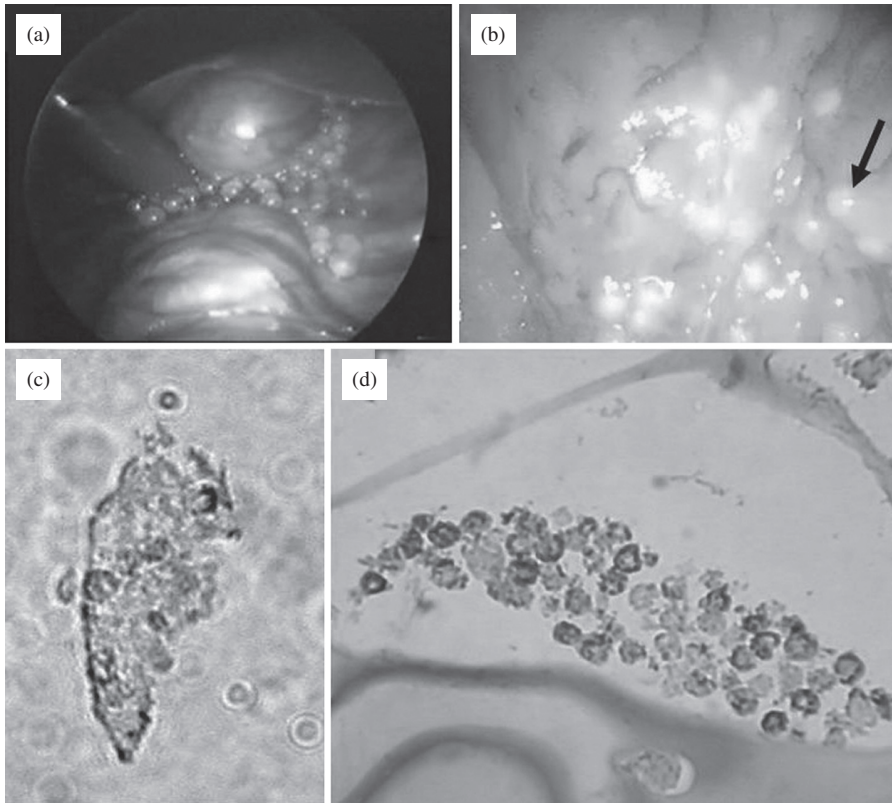

FIGURE 5.9 (a) Laparoscopic view of encapsulated neonatal porcine islets at time of implantation. (b) Laparoscopic view at time of biopsy showing the omentum embedded with nodules of previously implanted capsules. Arrow points to a nodule. Positive immunohistochemical staining for (c) insulin or (d) glucagon in porcine islets retrieved from the peritoneum 9.5 years after implantation. (Adapted from Elliott et al. [85].)

beads [88]. Moreover, it is suggested that PLL can cause toxic damage to encapsulated islets (the strongest effect encountered in alginate with high-G content and PLL coating) [88]. In addition to the immune response that may be mediated by macrophages, the attached cells are competing for nutrients and thus may hamper the function of the transplanted cells [88].

High-M content of alginate may cause stimulation of monocytes via CD14, and toll-like receptor (TLR) 4 and 2 [87]. TLRs are membranous receptors which play an important role in the functions of the innate immune system. High-M content has been shown to stimulate the secretion of interleukin-1 (IL-1), IL-6, and TNFα [89], all of which are proinflammatory cytokines [90]. Moreover, cytokine excretion may cause dysfunction of the insulin-secreting cells [88]. In addition, hypoxia should be avoided since it may cause excretion of

stress proteins, facilitating the host immune response [88]. Finally, antibodies against the M component have been detected in mice [87]. Both immune reactions can be avoided by washing the beads before transplantation, thereby removing some of the non-crosslinked M component [87]. Nonpure alginate contains endotoxins that cause an immune reaction. Therefore, the use of ultrapure alginate ($<$100 EU/g endotoxin) is recommended for clinical applications [87].

Capsule design poses a challenge since the encapsulated cells should not be in contact with the beads' external surface, and one needs to allow for the free diffusion of glucose, oxygen, and nutrients into and out of the beads and proteins. It has been suggested that xenotransplantation be performed in a less porous alginate matrix in order to prevent diffusion of foreign antigens out of the beads because they may cause an exaggerated immune reaction [88].

Conclusions. The use of alginate encapsulation for transplanted pancreas islets was first suggested about 30 years ago. Nevertheless, to date, experiments have included only a small number of patients and have yielded inconsistent results. Different types of alginate, different microcapsule sizes, and different purification levels have been suggested as the cause of these inconsistencies [91]. Moreover, some studies used barium and not calcium for crosslinking (since barium–alginate gels provide higher mechanical strength than calcium–alginate gels) and others used PLL coatings [91]. Although transplantation has resulted in prolonged improvement of quality of life, decreased dependence on insulin, and lower occurrence of hypoglycemia, no complete lifetime cure has yet been achieved. This obstacle may be resolved by improvements in alginate matrix design or by the use of repeat transplantations. Further research is also required to support the long-term survival of functional beads.

5.8.4 Clinical Applications in Neurology

The human central nervous system (CNS) is a very complex structure. It is vulnerable to various insults, such as trauma, infections, degenerative diseases, and autoimmune mechanisms, to name a few. All may result in major chronic disability and decreased quality of life. Moreover, with age, axonal growth potential decreases and consequently there is a decrease in the CNS's capacity for self-rehabilitation [92]. There are several explanations for the CNS's limited ability to correct neural damage. First, secretion of neurotrophic factors (which facilitate neural growth and survival of neurons) decreases. Second, there is increased expression of inhibitory factors that limit axonal growth [92]. Therefore, the medical world is striving for new therapeutic approaches to CNS-directed treatments.

Treatment of Spinal Cord Dissection. Spinal injury may be associated with severe disability, such as paralysis [93, 94]. Annually, 10,000 individuals are

affected in the United States alone, most of whom are young adults [93]. Overall, there are about 250,000 people in the United States with spinal injuries [95]. Following spinal injury, the transected axon undergoes Wallerian degeneration [95]. Despite encouraging results in rats, restoration of dissected spinal cord function is currently impossible in humans [94]. In rats and mice, implantation of intercostal nerves, mouse embryonic stem cells (ESCs), or embryonic or fetal spinal cord segments have resulted in restitution of neural functions [94]. Different approaches to neural regeneration have been attempted, most of which have focused on cell therapy [93]. Several cells have been investigated for implantation: peripheral nerve cells, ependymal cells derived from the choroid plexus, olfactory ensheathing cells, hESCs, and Schwann cells [96 ,97]. Nevertheless, to date, these methods have shown inadequate efficacy and are also limited by a lack of available cell sources and a possible immunological reaction against the implant [96]. Moreover, transfer of disease remains a great concern [98]. In some of these methods, axonal elongation was found to be insufficient [97]. In addition, glial scar tissue formation and associated proteoglycans, oligodendrocytes, and myelin-derived growth-inhibitory proteins all decrease axonal regeneration [95, 97, 98]. Other therapeutic strategies for the induction of neural regeneration have been attempted with the use of neurotrophic factors, degradation of chondroitin sulfate proteoglycan (which is inhibitory to neural growth, using MMP-2), or the use of genetically modified fibroblasts [99, 100]. Implantation of genetically modified Schwann cells with enhanced release of neurotrophic factors has also been considered as a possible therapy for neural damage [100]. Both synthetic and natural polymers were evaluated with variable results [101]. The use of collagen for spinal cord regeneration yielded disappointing results [101]. Therefore, alginate, as a natural extracted polymer, was evaluated. In one study, freeze-dried alginate gel was used in 30 rats as a neural-gap filler following resection of two 2-mm gaps in the spinal cord [102]. After 45 days, the sensorimotor brain cortex was stimulated and motor-evoked potentials (MEPs) were recorded. In addition, somatosensory-evoked potentials (SEPs) were recorded following distal stimulation. The presence of both MEPs and SEPs indicated bidirectional neural bridging of the spinal gaps [102]. Histological examination of the transplanted area revealed residual alginate, replaced by connective tissue and a large number of myelinated axons, Schwann-like cells, some perineural-like structures, and blood vessels [102]. Moreover, another study reported that 6 weeks following alginate implantation in transected spinal cord, recorded SEPs were similar to those prior to spinal transaction [98] (Fig. 5.10), suggesting that alginate enabled the formation of a synaptic interface [98]. Restoration of motor function was poor in experiments following complete spinal cord resection, but gain of coordination was reported following treatment of partial resection [50].

In another experiment following a similar methodology, a 3-mm gap was induced in the T9–10 spinal cord segment of 50 rats and was filled with alginate sponges [96]. After 21 weeks, the gap was replaced with areas of myelinated and nonmyelinated axons, as well as mast cells [96]. Labeling of the corticospinal

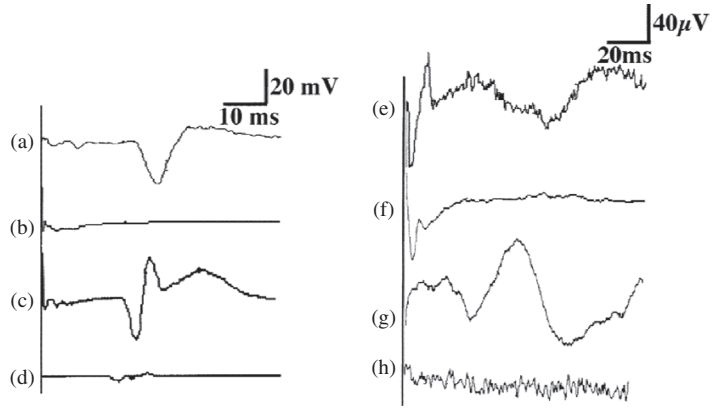

FIGURE 5.10 Recorded SEPs 6 weeks following alginate implantation in transected spinal cord were similar to those prior to spinal transaction. Evoked electromyogram (a) before surgery, (b) 24 h after surgery (the same waveform in rats in both groups), (c) 6 weeks after surgery in a rat with implanted alginate, and (d) 6 weeks after surgery in a rat in the control group. SEP (e) before surgery, (f) 24 h after surgery (the same waveform in rats in both groups), (g) 6 weeks after surgery, and (h) 6 weeks after surgery in a rat in the control group. (Adapted from Kataoka et al. [98].)

tract neurons with B-subunit-conjugated horseradish peroxidase demonstrated extension of axons through the alginate-filled gap and up to 200–300 μm caudal to the gap [96]. Moreover, the neurons grew 1–1.5 cm rostral to the lesion [96]. Nevertheless, it was apparent that the ascending neurons possessed a more significant proportion of regenerative neural tissue [96]. Electrophysiological evaluation confirmed that the outgrown tissue was functional and hence had developed synapses with distant nerves [96]. Dissection of a spinal cord section without implantation of alginate or other biomaterial was associated with the formation of a scar composed of connective tissue [97]. Therefore, alginate transplantation prevents scar formation and thus enables axonal regeneration and elongation of astrocytic processes [97]. In addition, alginate offers an alternative to other attempted methods of controlling scar formation, including irradiation and enzymatic digestion of chondroitin sulfate [97]. How elongation of the astrocyte processes, enhanced astrocye migration, and reduced gliosis occur in alginate implants, is unknown [98]. Nevertheless, all of these processes promote axonal regeneration. Alginate was superior to collagen in terms of number of regenerated axons and axonal penetration into the implant [97]. Moreover, the collagen implant facilitated undesirable connective tissue invasion [97]. It was suggested this connective tissue invasion (which does not occur with alginate implantation) impedes the extension of regenerating axons from the spinal cord [97].

Alginate was observed to enable axonal growth, which was limited and disorganized [93, 96]. In an attempt to support organized axonal growth, a

structured alginate anisotropic parallel capillary gel with approximately 27-μm-wide capillaries was constructed [93]. The rationale for this ultra-3D structure was the need to guide the transected axon growth caudally toward its original continuation in the distal stump. Organized neural structures were demonstrated both in vitro (using rat dentate gyrus of the hippocampus) and in vivo (spinal cord implantation following dorsal column transection in rats) [45]. Moreover, the engineered alginate scaffold survived in the rat's CNS for more than 6 weeks and, therefore, potentially supports slow neural growth [93]. Nevertheless, further research is needed to evaluate the consequent restoration of neural function. Such an alginate-based scaffold could also be used to deliver regenerative cells to the neural system with 3D orientation.

Treatment for Peripheral Nerve Regeneration. Peripheral nerve injuries are far more common than spinal cord transactions [103]. In cases of traumatically transected peripheral nerve, the use of autologous nerve implantation has a better clinical outcome than suturing the two stumps with applied tension [103]. Nevertheless, the implantation method has several disadvantages, namely, limited sources of nerves for grafting and the limited length of those nerves. Moreover, harvesting a healthy nerve can result in iatrogenically induced sensory loss and skin scarring [74, 103]. It is generally accepted that the use of an implant is required in cases of peripheral nerve defects of more than 20 mm [104].

The use of several synthetic materials as neural bridges has been attempted (i.e., silicone, polyglactin, polyglycolic acid, collagen), but these were found to support up to only 30 mm of neural growth [104, 105]. Implantation of ECM-driven gap filler may encourage fibroblast growth as well [105], and ECM substances are produced from animals that may carry a risk of disease transfer (e.g., prion infections) when transplanted into humans [105].

Therefore, an implantable biomaterial may produce an alternative method for the treatment of peripheral nerve damage. Alginate preparations with ethylenediamine and water-soluble carbodiimide were freeze-dried, and then later transplanted in rats with a surgically induced 1-cm gap in the right sciatic nerve [106]. Four weeks after transplantation, the distal parts of the alginate implant underwent degradation and were replaced by straight axonal regeneration [106]. Moreover, both fibroblast and Schwann cells were found in the lesion, as well as a few macrophages. This is an important observation as Schwann cells enhance axonal regeneration [100], and mast cells may also contribute to nerve regeneration [106]. Electrophysiologically, stimulation proximal to the stump resulted in action potentials that were of smaller amplitude and longer latency compared with normal action potentials [106]. It was suggested that alginate degradation supports the axonal elongation process [106]. Another study described a similar composite of alginate and both ethylenediamine and carbodiimide, which was freeze-dried and coated with polyglycolic acid mesh. This was then implanted in 10 cats with a 50-mm gap in the right sciatic nerve [105]. Compound muscle action potentials (CMAPs) and

SEPs were recoded 3 and 13 weeks after implantation. The conduction's latency of peak amplitude in the implanted cats was 89 ± 11% that of the contralateral nerve, which served as a control [105]. A near-normal SEP suggested near-normal conduction of sensory nerves following transplantation [105]. Seven months after transplantation, the alginate had been entirely absorbed. Regenerated axons, some of which were myelinated, were arranged in a fascicular structure and were smaller than normal axons [105].

In another study, two implantation methods were applied in 12 cats with a 5-mm gap of the right sciatic nerve: a tubular method in which the coated alginate was implanted and connected to the nerves by sutures, and a nontubular method in which alginate was merely positioned in the gap. In both groups, 3 months after implantation, there was electrophysiological activity with near-normal amplitude and latency [104]. Eight months after surgery, the alginate had been absorbed and replaced with myelinated nerves, although the regenerated nerves appeared smaller and with less myelin tissue. The study showed that nontubular implantation of alginate may be an important development in the treatment of nerve dissection [104]. Traumatic injury to the 7th cranial nerve (i.e., the facial nerve) may be caused by blunt or penetrating trauma or may be secondary to surgery. Among other functions, the facial nerve is responsible for facial expressions. Any injury to this nerve is associated with an asymmetrical face, hemifacial paralysis, eating difficulties, and inability to close the eye on the affected side. Direct suturing is applied when possible. Otherwise, the use of autologous or synthetic implants is usually advised [107]. In one experiment, five cats underwent facial nerve dissection, which was treated with alginate implants [107]. In that study, ethylenediamine and carbodiimide were also dissolved in alginate to form a gel and later freeze-dried. The alginate sponge was transplanted into the 5-mm gap of the facial nerve without any suturing. Twelve weeks later, eyelid blinking had been recovered and there was normalization of electrophysiological parameters [107]. Histologically, there were no traces of alginate, and it had been replaced by a high density of small axons [107]. Use of alginate for facial nerve injury provides a sutureless alternative, especially in cases of distorted anatomy and difficulties in reconnecting fine neural branches.

ECM proteins such as fibronectin can influence neural regeneration and can induce Schwann cell proliferation [52]. A mixture of Schwann cells or 0.05% fibronectin supplement or both was added to a formed alginate matrix. Gelatinization was achieved by immersion in 0.1 M $CaCl_2$ solution. The matrix was tested for its neural regeneration abilities in sciatic nerve gaps [100]. In vitro, the alginate–fibronectin mixture enhanced Schwann cell proliferation. Moreover, implantation of Schwann cells was associated with axonal regeneration, especially when fibronectin was added to the alginate matrix [100]. It has been suggested that it is the porous structure of alginate that makes it suitable for axonal growth [104]. Moreover, alginate may enable the diffusion of neurotrophic factors from the distal stump to the proximal regenerating nerve and thereby both enhance growth, and guide the direction of growth in

regenerating neurons [107]. Nevertheless, the mechanism by which alginate enables neural regeneration remains to be determined [104, 105].

Surgical Uses of Alginate. Peripheral nerve disorders are a common medical condition. Carpal tunnel syndrome (CTS) is caused by pressure on the median nerve of the wrist. Severe CTS has an estimated prevalence of 4% in the general population [108]. Surgical treatment of CTS may be unsuccessful in 3% of surgeries, mainly due to fibrosis and adhesions [108]. These complications are not easily treated and may require additional surgical decompression due to the formation of scar tissue [108]. Therefore, it was speculated that injection of alginate into the surgical bed may efficiently reduce postoperative scarring and adhesions. Forty rats were experimentally operated on: The right sciatic nerve was exposed and dissected, followed by local injection of 500 μL of 1–5% alginate solution [108]. The left sciatic nerve served as a control. Six weeks later, alginate treatment was associated with the presence of loose connective tissue and recovery of the perineurium, compared to thick granulation tissue surrounding the sciatic nerve in the untreated group [108].

Use for the Delivery of Neurotrophic Factors. Neurotrophic factors can facilitate neural regeneration and growth, even in mature neural tissue [92]. There are several known neurotrophic factors that may play a role in enhancing neural rehabilitation: brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), ciliary-derived neurotrophic factor (CNTF), and others [92]. The use of an alginate vehicle for bFGF transfer was attempted for the purpose of neural regeneration. bFGF enhances growth of neural extensions and recruitment of astrocytes, oligodendrocytes, and Schwann cells [74]. Because bFGF is vulnerable to photolytic degradation, alginate may provide a stable slow-release matrix [74]. Ethylenediamine was used for crosslinking of alginate and heparin in order to produce a matrix compatible with the slow release of a total 0.05 μg bFGF. Later it was freeze-dried and implanted in a 1-cm gap induced in the right sciatic nerve of rats [74]. Results of bFGF release were compared with a group that was implanted with bFGF-free matrix. Axonal regeneration was found to be accelerated in the alginate-containing bFGF group, although angiogenesis was found to be decelerated [74].

Alginate as a Scaffold for Cell Implantation. Various cells can be used for implantation into the CNS: Stem cells are potentially capable of replacing damaged neural tissue, while genetically modified cells may be capable of secreting neurotrophins and thereby encourage neural regeneration. Alginate has several advantages under these circumstances because it can provide a structural support for the implanted cells, as well as dampen the immune response against the implanted cells [109]. BDNF is a neurotrophin capable of regenerating axons and preventing neural atrophy [99]. However, implantation of BDNF-producing fibroblasts (by using retroviral vector for cell transfection)

requires the use of chronic immunosuppression. The use of an alginate coating as a substitute for immunosuppression therapy was tested. BDNF-producing fibroblast cells (Fb/BDNF) were added to low-viscosity alginate solution, which was dropped into a $CaCl_2$ solution in order to form beads. Thirty-six rats underwent subtotal hemisection of the spinal cord and received beads containing Fb/BDNF cells, alginate beads without cells, or Fb/BDNF without alginate encapsulation (with and without immunosuppression with cyclosporine). Alginate-encapsulated Fb/BDNF cells remained viable and functional for 2 months in vitro [99]. Moreover, transplantation of the encapsulated cells into the damaged spinal cord eliminated the need for immunosuppressants, induced axon growth, and showed a marked improvement in motor functions compared to other groups [99]. In addition, cells that were not encapsulated and without administration of immunosuppressants were lost and consequently there was no effect on neural regrowth [99]. In that particular experiment, alginate beads that contained no cells did not induce axonal growth [99]. However, in that experiment, and in contrast to earlier studies [98, 102], alginate was not freeze-dried and was delivered in the form of beads and not scaffolding.

Alginate was also used as a delivery system for recombinant human colony-stimulating factor 1 (hCSF-1) delivery. CSF-1 is hematopoietic growth factor that is also produced by astrocytes in the CNS. Proliferation and survival of microglial cells (immune cells in the CNS with phagocytic ability) are mediated by CSF-1. CSF-1 has also been found to promote the survival of ischemic neural tissue [110]. LM-10 is a fibroblast-like cell that produces CSF-1. LM-10 cells were incorporated into chitosan–alginate beads. These beads were transplanted into the exposed brain tissue of osteopetrotic mice following induction of cortical ischemia [110]. Ultimately, implantation of LM-10-containing beads resulted in a 2.2-fold increase in neural survival relative to nontransplanted mice [110]. Such a delivery system for long-term release holds a great advantage because CSF-1 is easily degraded.

An alginate scaffold enables survival of neural progenitor cells (NPCs) [93]. NPCs may replace nonfunctional neural tissue and therefore may be used to treat stroke, Parkinson's disease, spinal cord injuries, amyotrophic lateral sclerosis, and other diseases [109]. NPC implantation has also been proposed to cause the recipient's own neural regeneration (via the secretion of neurotrophins and enzymes that degrade proteins that are inhibitory to neural regeneration) [109]. The neurotrophins found to be secreted by NPCs include nerve growth factor (NGF), glial-derived neurotrophic factor (GDNF), BDNF, and MMP-2. Integration of NPC in beads of several compositions—alginate with high-G content (68%) and alginate with high-M content (54%)—was tested. Both a PLL coating layer and noncoated matrices were evaluated [109]. PLL provided mechanical stability to the alginate beads, as well as additional immunoprotection by blocking IgG penetration [88, 109]. Moreover, PLL altered the porosity of the matrix [88]. Release of NGF, BDNF, and GDNF from the beads was evaluated. High-G content alginate beads with no PLL covering were the only beads that allowed release of all

three factors. The PLL-coated high-M alginate beads were mechanically unstable [109]. Both high-G and high-M alginate beads with no PLL covering allowed NPC proliferation [109].

Alginate was also used as a scaffold for spinal implantation of fetal neural cells, constructed into neurospheres [111]. First, alginate was implanted into a 1-mm-thick dissected spinal cord section. Later, the neurospheres were slowly injected into the alginate-filled lesion. Four weeks after neural cell transplantation, a proportion of the fetal neural cells had migrated to the animal's neural tissue [111]. Without the use of alginate as a carrier, almost no transplanted neural cells survived beyond 2 weeks of transplantation [111].

Treatment of Pain. Neuropathic pain may be resistant to available analgesic therapies [112]. Adrenal chromaffin cells are capable of releasing catecholamines and met-enkephalin, which may have an analgesic effect [112]. Moreover, adrenal chromaffin cells release neurotrophic factors that may reduce neural degeneration [112]. Xenogenic cell transplantation may provide a source for chromaffin cells. Nevertheless, the immune response limits such an approach and immunosuppressants are required to mask the transplant from the immune system. Therefore, bovine adrenal medullary tissue was added to a 1.4% sodium alginate solution and later dropped into $CaCl_2$ to form beads with dimensions of 100–300 μm, which were then covered with PLL [112]. The sciatic nerve was tightened slightly to cause neural edema and chronic pain. Fifteen rats were transplanted with beads containing chromaffin cells (into the lumbar subarachnoid space). This group was compared to 15 other rats transplanted with cell-free alginate beads [112]. With time, implantation of chromaffin cells was associated with higher levels of catecholamines and met-enkephalin in the cerebrospinal fluid, and rats suffered less from neuropathic pain [112].

Due to a concern of prion-associated diseases (e.g., Jacob–Creutzfeldt disease) that may be caused by the use of bovine chromaffin cells, other studies tested porcine adrenal chromaffin cells [113]. In vitro experiments demonstrated that porcine chromaffin cells produce larger amounts of analgesic substances than bovine chromaffin cells [113]. Alginate beads consisting of porcine or bovine chromaffin cells were produced [113] in a technique that was similar to that in an earlier described study [112]. Beads were implanted in the subarachnoid space as well. Pain reduction (evaluated by a mechanical allodynia test) was superior in the porcine-driven cell group [113].

Although an alginate scaffold was not used in human subjects, the use of pain relief in two cancer patients by injecting donor medullary cells into the lumbar cerebrospinal fluid was reported [114]. The injected medullary cells induced 1 year of sustained pain control and increased cerebrospinal levels of met-enkephalin [114]. Experiments with chromaffin cells implanted into the subarachnoid space were performed at two medical centers, with further promising results [115].

Treatment of Parkinson's Disease. Parkinson's disease (PD) is a degenerative neural disorder characterized by loss of dopamine-producing cells in the substantia nigra and concomitant reduction of dopamine release in the corpus striatum [116]. Clinically, patients with PD exhibit uncontrolled tremors, rigidity, slowness of movement, and a shuffling gait [117, 118]. The disease is progressive by nature and is associated with substantial disability and decreased quality of life [118]. Current treatment of PD focuses on exogenous supplementation of dopamine or its precursor with systemic absorption, along with systemic side effects. Moreover, the desirable effects of levodopa (L-dopa) decrease with time [117]. In addition, current treatment modalities do not prevent further loss of dopaminergic cells: They merely provide symptomatic treatment. Other therapeutic approaches have focused on restoration of dopamine release through the use of cell therapy. Retinal pigment epithelial (RPE) cells are capable of dopamine release as well as secretion of neurotrophic factors such as BDNF and GDNF [116]. In one particular experiment, porcine RPE cells were suspended in a 2.4% alginate solution and then entrapped in beads following dripping of the mixture into a $CaCl_2$ solution. The beads were covered with PLL and were then transferred to a 0.24% alginate solution for a few hours. Subsequently, the beads were treated with a sodium citrate solution (producing alginate–PLL–alginate beads). It is important to note that embedding the RPE cells in alginate beads did not affect dopamine secretion [116]. The 200- to 300-μm beads were implanted in the right corpus striatum of nine rats with PD [116]. Results were compared to a PD group implanted with empty alginate beads and another PD group treated with saline injection. Response to treatment was evaluated by apomorphine-induced rotation (AIR). The group treated with saline showed no alteration in AIR [68]. The cell-free alginate beads caused a brief decrease, of less than 2 weeks, in AIR. Of the nine rats treated with encapsulated RPE cells, three sustained a lasting reduction in AIR, while six showed no beneficial effect [116]. Survival of the beads was found to be consistent with improved symptoms, while mechanical disruption of the beads was found in some nonresponsive rats [116].

Glial-derived neurotropic factor has been shown to improve survival of dopamine-producing cells [117]. Nevertheless, there is some controversy regarding its potential use for the treatment of PD in light of both encouraging and discouraging results in clinical trials that included human subjects [117]. It was speculated that the lack of beneficial effect in some trials was due to the short life of GDNF in the CNS in the absence of a slow-release sustained matrix. Therefore, continuous release of GDNF from transfected rat fibroblasts embedded in alginate–PLL–alginate beads was investigated for the treatment of PD in a rat model following implantation into the striatum [117]. Transplantation resulted in a continuous decrease in AIR over 24 weeks [117].

The successful use of autologous medullary chromaffin cells in the caudate nucleus has been reported [119]. Nevertheless, autotransplantation of chromaffin cells may carry high surgical risk despite its effectiveness in

some patients [119]. Therefore, alternative sources for chromaffin cells were evaluated. Alginate was proposed for decreasing rejection risks in the implantation of animal tissue.

Implantation of bovine chromaffin cells was tested in a methylphenyltetrahydropyridine (MPTP)-induced PD model in monkeys. The embedding of chromaffin cells in alginate–PLL–alginate beads was compared to cell-free beads and implantation of chromaffin cells without encapsulation [120]. The monkeys that were treated with empty beads showed no improvement in AIR. In contrast, unencapsulated chromaffin cells induced a 1-month-long decrease in AIR, while encapsulated chromaffin cells were associated with a 9-month decrease in AIR [72]. Therefore, alginate encapsulation of chromaffin cells may produce sustained improvement of PD in primates and possibly, in the future, in humans.

The tobacco alkaloid nicotine may have a beneficial effect in PD [118]. To prolong nicotine release from the alginate beads beyond 2 weeks, a bead composition of alginate–hydroxyethylcellulose was investigated in vitro [118]. However, review of the medical literature revealed no in vivo attempts to investigate a CNS-implanted slow-release nicotine system, most probably due to the long experience with skin patches and nicotine gums, which do not require complicated surgical procedures. Nevertheless, currently used methods of nicotine supplementation involve systemic distribution of nicotine, which may cause nausea and gastrointestinal side effects in some patients, potentially resulting in low compliance [121].

Conclusions. Alginate's bioabsorptive properties make it a good candidate for neural regeneration [104]. It may have different potential applications in neurological disorders, including use as a scaffold and as a vehicle for the release of bioactive substances. The use of alginate for the treatment of neurological diseases is currently in the preclinical stages. The use of alginate as a carrier for chromaffin cells for pain management in cancer patients and for the treatment of PD seems especially promising. We believe that the concomitant use of alginate as a scaffold with 3D orientation, which favors neural regeneration, and as a matrix for the slow release of neurotrophins, holds promise for the treatment of various heretofore permanent neural injuries.

5.8.5 Uses of Alginate in Dental Care

Emdogain is a commercial product composed of an enamel matrix protein derivative (EMD) and PGA [122]. It is used as an adjunct to periodontal surgery and is applied to the exposed surface of the tooth's root. Inflammatory periodontal disease may cause instability of the tooth [123]. The EMD component in Emdogain enhances periodontal regeneration and has been found efficient for the treatment of intrabony defects due to moderate or severe periodontitis [124]. The EMD is the biologically active component, while PGA is used as a carrier matrix. A randomized controlled trial

(clinicaltrials.gov Identifier NCT00757159) is ongoing to evaluate the treatment of periodontal defects with Emdogain or synthetic hydroxyapatite.

5.8.6 Uses of Alginate in Oncology

Cancer is a leading cause of morbidity and mortality in the western world. Major advances have been made in antineoplastic therapy in recent years. Targeted therapy is now available, as are potent drugs that prevent tumor spread. These drugs are generally delivered systemically. However, systemic treatment is commonly complicated by adverse effects and toxicity. An alginate-based local chemotherapeutics-release technique has been proposed [125, 126], as has the use of alginate for chemoembolization with mitomycin-C [127]. Local administration of chemotherapeutics is advantageous due to high systemic toxicity. In addition, alginate may be used for the local delivery of peptides that induce apoptosis, thereby providing additional local antineoplastic therapy [128].

Moreover, alginate gels can contain radiation-emitting substances, thereby enabling localized radiotherapy [129]. Tumors are usually very vascular due to neovascularization induced by malignant cells. Inhibition of angiogenesis is a different therapeutic approach to cancer therapy. Angiostatin, an antiangiogenic material, can be produced by genetically engineered cells. Alginate was found useful, with successful proof of concept, in a rat model for prolonged cell survival following transplantation [130]. Endostatin is another antiangiogenic agent whose release from an alginate matrix has shown promising results in vitro [131].

In addition, alginate can be used for entrapment and support of retroviral vector-producing cells, which may be used for the local delivery of suicide genes into malignant cells [132]. In vivo experimentation on mice has demonstrated improved survival in the treatment group [132].

In conclusion, alginate may be used for the localized delivery of different antitumor treatments with enhanced local effect. This has especially high therapeutic potential in cases of confined tumors without metastasis.

5.8.7 Uses of Alginate in Artificial Liver Techniques

The liver is a vital organ that supports metabolism. It produces bioactive proteins and peptides and plays an important role in glucose homeostasis as well as bile production, and in the detoxification of toxins and drugs. Acute hepatic failure is a life-threatening condition that is associated with encephalopathy [133]. End-stage liver disease or acute hepatic failure may require liver transplantation. In one experiment, a model of acute liver failure was introduced in pigs. Plasmapheresis was then conducted with an extracorporeal bioartificial liver composed of alginate beads containing hepatocytes. The treatment reduced encephalopathy and intracranial pressure [133] and prolonged survival [134]. These results were further supported by other studies

[135]. Moreover, transplantation of alginate-encapsulated hepatocytes from various sources may be useful in the restoration of hepatic functions [136]. In addition, alginate may be used as a scaffold for de novo engineering of liver tissue [137, 138].

Liver regeneration following partial hepatectomy was found to be accelerated following intraperitoneal transplantation of bone marrow stem cells encapsulated in alginate beads in a rat model [139]. Moreover, survival was increased in 90% of hepatectomized rats following transplantation. This beneficial effect was presumably caused by the secretion of factors that stimulate liver regeneration by the bone marrow stem cells [139]. In conclusion, due to a dearth of organ donors, alginate may be used for liver replacement therapy, either in the transplantation of foreign encapsulated cells or as part of an extracorporeal bioartificial liver.

5.8.8 Alginate Applications for Cartilage Substitution Therapy

The cartilage is a dense connective tissue that contains chondrocytes. It is found throughout the body, either on articular surfaces or as a structural support for the airways, nose, and external ear. An alginate–chondrocyte mixture was investigated for its possible use as injectable cartilage, which can maintain different shapes created by external molding. This technique has been suggested for use in facial plastic surgeries [140, 141]. Moreover, alginate may be used for 3D tissue engineering and reconstruction of neocartilage prior to transplantation [142–144]. Chondrocytes embedded in alginate survive for long periods (Fig. 5.11) and preserve their ability to secrete connective tissue products (proteoglycan, glycosaminoglycan, and collagen type II).

5.8.9 Alginate Applications for Local Drug Release

Localized drug administration has important advantages: It enables a higher concentration of the drug where its effect is required, prevents undesirable rapid drug degradation, enables sustained release, and decreases systemic side effects. Alginate has been suggested as a vehicle for the delivery of different types of antibiotics in the treatment of local infections [145–149]. VEGF enhances neovascularization, and therefore local administration of VEGF to ischemic tissue has been proposed. Alginate is a suggested carrier for the local release of VEGF to treat peripheral vascular disease [150]. Moreover, as already noted, in an animal trial, alginate-based local intramyocardial delivery of VEGF following MI had beneficial effects [73]. In addition, alginate may be used for the delivery of proangiogenic drugs such as bFGF [151] or other proangiogenic approaches [152, 153]. Embolization is a common treatment for vascular lesions (i.e., arteriovenous malformations, vascular aneurysms, highly vascularized tumors, and vascular hemorrhages). Alginate has been suggested for use in this approach [154, 155]. Moreover, renal artery embolization with

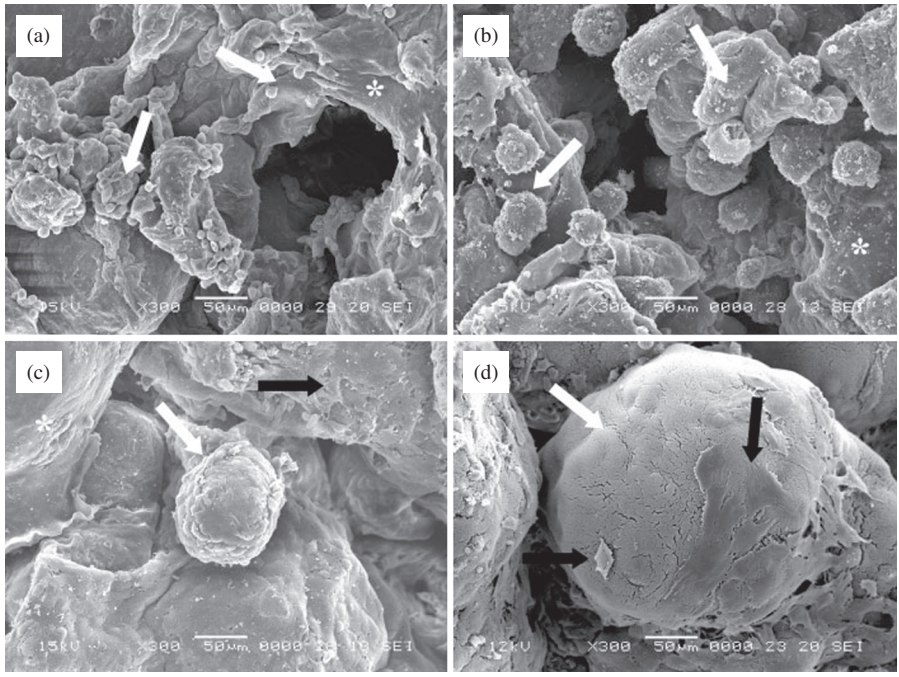

FIGURE 5.11 Scanning electron micrograph of chondrocyte cells grown on alginate scaffolds for 1 week (a), 2 weeks (b), 3 weeks (c) and 4 weeks (d). White asterisks indicate alginate; white arrows indicate chondrocyte cells and clusters; black arrows indicate fibroblast-like chondrocyte cells. (Adapted from Lin et al. [143].)

alginate beads for the treatment of renal malignancy is possible, with or without embedded chemotherapeutics [156, 157]. Alginate may be used as a matrix for enzyme delivery [158–160]. Anticoagulants such as heparin and low-molecular-weight heparin have a short half-life following administration. The use of alginate–chitosan–polyethylene glycol capsules has been suggested for their slow release [161]. In conclusion, alginate matrix can be used safely and effectively for local delivery of various drugs, proteins, and enzymes, although experience in human subjects is currently limited.

5.8.10 Alginate Applications for Local Gene Therapy

Gene therapy may provide future treatment options for various diseases in which expression of a particular protein(s) is lacking. In addition, local delivery of gene therapy may be required in cases of disease limited to an organ or tissue. Moreover, the use of local gene therapy via viral vectors in alginate carriers can potentially be applied [162]. As described above, encapsulated

retroviral vector-producing cells may be locally implanted to deliver suicide genes into malignant cells [132]. The use of alginate for virus delivery decreases the host immune response against the virus itself and enables viral protein expression [163].

REFERENCES

1. Woodward, F. N. The Scottish seaweed research association. *J. Marine Biol. Assoc. UK* 1951;**29**:719–725.
2. Stanford, E. C. C. On algin: A new substance obtained from some of the commoner species of marine algae. *Chem. News* 1883;**46**:254.
3. Stanford E. C. C. On the economic applications of seaweeds. *J. Soc. Arts* 1884; **32**:717.
4. Krefting, A. An improved method of treating seaweed to obtain valuable products therefrom. British Patent 1896, 11, 538.
5. Krefting, A. An improved method of treating seaweed to obtain valuable products (alginic acid, "tang acid") therefrom. English Patent, 11538, 1896.
6. Krefting, A. An improved system or apparatus for treating seaweed (alginic acid) for the manufacture of rroducts therefrom. English Patent 12416, 1898.
7. McNeely, W. H. Algin. In *Industrial Gums*. R. L. Whistler (Ed.). Academic: New York, 1959, pp. 55–82.
8. Nussinovitch, A. *Gum technology in the food and other industries*. Blackie Academic & Professional: London, 1997.
9. Mondragon, J. and J. Mondragon. *Seaweeds of the Pacific Coast*. Sea Challengers: Monterey, CA, 2003.
10. Abbott, I. A. and G. J. Hollenberg. *Marine Algae of California*. Stanford University Press: Stanford, 1976.
11. Kain, J. M. Culivation of attached seaweeds. In *Seaweed Resources in Europe: Uses and Potential*. M. D. Guiry and G. Blunden (Ed.). Wiley: New York; 1991.
12. Lewis, J. R. *The Ecology of Rocky Shores*. English Universities Press: London; 1964.
13. Seip, K. L. A mathematical model of competition and colonization in a community of marine benthic algae. *Ecol. Model.*1980;**10**:77–104.
14. Nishide, E., A., Mishima, and H. Anzai. Properties of alginic acid from sulfated polysaccharides extracted from residual algae by the hot water method. *Bull. Coll. Agricul. Vet. Med.,* Nihon University, 1992;**49**:140–142.
15. Jay, J. M. *Taxonomy, role, and significance of microorganisms in food*. In *Modern Food Microbiology*. Aspen: Gaithersburg, MD, 2000, p. 13.
16. Anzai, Y., H. Kim, J. Y. Park, H. Wakabayashi, and H. Oyaizu. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol.* 2000;**50**:1563–1589.
17. Conti, E., A. Flaibani, M. Oregan, and I. W. Sutherland. Alginate from *Pseudomonas fluorescens* and *P. putida*—production and properties. *Microbiology-UK* 1994;**140**:1125–1132.

18. Lebrun, L., G. A. Junter, T. Jouenne, and L. Mignot. Exopolysaccharide production by free and immobilized microbial cultures. *Enz. Microb. Technol.* 1994;**16**:1048–1054.
19. Chen, W. P., J. Y. Chen, S. C. Chang, and C. L. Su. Bacterial alginate produced by a mutant of Azotobacter vinelandii. *Appl. Environ. Microbiol.* 1985;**49**:543–546.
20. Steiner, A. B. and W. H. McNeely. Algin in review. *Adv. Chem.* 1954;**11**:68–82.
21. Whistler, R. L. and K. W. Kirby. Composition of alginic acid of *Macrocystis pyrifera*. *Hoppe-Seyler's Z. Physiol. Chem.* 1959;**314**:46.
22. Hirst, E. and D. A. Rees. Structure of alginic acid. 5. Isolation and unambiguous characterization of some hydrolysis products of methylated polysaccharide. *J. Chem. Soc.* 1965;1182–1187.
23. Grasdalen, H. High-field, H-1-NMR spectroscopy of alginate—sequential structure and linkage conformations. *Carbohyd. Res.* 1983;**118**:255–260.
24. Haug, A. *Composition and properties of alginates. Report no. 30. Trondheim*, Norway: Norwegian Institute of Seaweed Research. 1964.
25. Glicksman, M. *Gum Technology in the Food Industry*. Academic: New York; 1969.
26. Green, H. C. Fibrous alginic acid. U.S. Patent 2,036,934, 1936.
27. LeGloahec VCE, Herter JR. Treating seaweed. U.S. Patent, 2,138,551, 1938.
28. McHugh, D. J. Production and utilization of products from commercial seaweeds. FAO Fisheries technical paper. Food and Agriculture Organization of the United Nations: Rome, 1987, p. 288.
29. Steiner, A. B. and Manufacture of glycol alginates. U.S. Patent 2,426,215, 1947.
30. Steiner, A. B. and W. H. McNeely. High-stability glycol alginates and their manufacture. *U.S. Patent* 2,494,911, 1950.
31. Pettitt, D. J. and V. H. Noto. Process for the preparation of propylene glycol alginate from partially neutralized alginic acid. U.S. Patent 3,772,266, 1973.
32. Noto, V. H. and D. J. Pettitt. Production of propylene glycol alginic acid esters. British Patent 1,563,019, 1980.
33. Doggett, R. G. and G. M. Harrison. Significance of the pulmonary flow associated with chronic pulmonary disease in cystic fibrosis. In *Proc. 5th Int. Cystic Fibrosis Conf. London*: D. Lawson (Ed.). Cambridge University Press: Cambridge, 1969, pp. 175–188.
34. Hassett, D. J. Anaerobic production of alginate by *Pseudomonas aeruginosa*: Alginate restricts diffusion of oxygen. *J. Bacteriol.* 1996;**178**:7322–7325.
35. Clementi, F., P. Fantozzi, F. Mancini, and M. Moresi. Optimal conditions for alginate production by *Azotobacter vinelandii*. *Enz. Microb. Technol.* 1995;**17**: 983–988.
36. Takeuchi, T., K. Murata, and I. Kusakabe. A method for depolymerization of alginate using the enzyme system of *Flavobacterium multivolum*. *J. Jpn. Soc. Food Sci. Technol-Nippon Shokuhin Kagaku Kogaku Kaishi* 1994;**41**:505–511.
37. McDowell, R. H. Applications of alginates. *Rev. Pure Appl. Chem.* 1960;**10**:1–15.
38. Rees, D. A. Structure, conformation and mechanism in formation of polysaccharide gels and networks. In. *Advances in Carbohydrate Chemistry and Biochemistry*, **Vol. 24**. M. L. Wolform and R. S. Tipson (Eds.). New Academic: New York, 1969, pp. 267–332.

39. Messina, B. T. and D. Pape. Ingredient cuts heat-process time. *Food Eng*. 1966;**8**:48–51.
40. Chavez, M. S., J. A. Luna, and R. L. Garrote. Cross-linking kinetics of thermally present alginate gels. *J. Food Sci*. 1994;**59**:1108–1110.
41. Wang, Z.Y., J. W. White, M. Konno, S. Saito, and T. Nozawa. A small angle X-ray scattering study of alginate solution and its sol-gel transition by adding of divalent cations. *Biopolymers* 1995;**35**:227–238.
42. Pocker, Y. and E. Green. Hydrolysis of D-glucono-delta-lactone. I. General acid–base catalysis, solvent deuterium isotope effects, and transition state characterization. *J. Am. Chem. Soc*. 1973;**95**:113–119.
43. Nussinovitch, A., I. J. Kopelman, and S. Mizrahi. Effect of hydrocolloids and minerals content on the mechanical properties of gels. *Food Hydrocolloids* 1990;**4**:257–265.
44. Van Wazer, J. R. *Phosphorus and Its Compounds*. Wiley — Interscience: New York, 1958.
45. Nussinovitch, A., M. Peleg, and M. D. Normand. A modified Maxwell and a nonexponential model for characterization of the stress relaxation of agar and alginate gels. *J. Food Sci*. 1989;**54**:1013–1016.
46. Nussinovitch, A. and M. Peleg. Strength-time relationships of agar and alginate gels. *J. Texture Studies* 1990;**21**:51–60.
47. Toft, K. Interactions between pectins and alginates. *Progr. Food Nutr. Sci* 1982;**6**:89–96.
48. Toft, K., H. Grasdalen, and O. Smidsrod. *Synergistic gelation of alginates and pectins*. In Fishman ML, Jen JJ, editors. *Chemistry and Function of Pectins*, ACS Symposium Series No. 310. M. L. Fishman and J. J. Jen (Eds.). Washington D.C.: American Chemical Society: 1986.
49. Imeson, A. P. In *Gums and Stabilizers for the Food Industry*, Vol. 2. G. O. Phillips, D. J. Wedlock, P. A. Williams, (Eds.). Pergamon: Oxford, 1984, p. 189.
50. Lim, R. Vesicoureteral reflux and urinary tract infection: Evolving practices and current controversies in pediatric imaging. *Am. J. Roentgenol*. 2009;**192**:1197–1208.
51. Hensle, T. W. and A. L. Grogg. Part 1: Vesicoureteral reflux treatment: The past, present, and future. *Curr. Med. Res. Opin*. 2007;**23**(Suppl 4):S1–5.
52. Atala, A., W. Kim, K. T. Paige, C. A. Vacanti, and A. B. Retik. Endoscopic treatment of vesicoureteral reflux with a chondrocyte-alginate suspension. *J. Urol*. 1994;**152**:641–643.
53. Diamond, D. A. and A. A. Caldamone. Endoscopic correction of vesicoureteral reflux in children using autologous chondrocytes: Preliminary results. *J. Urol*. 1999;**162**:1185–1188.
54. Caldamone, A. A. and D. A. Diamond. Long-term results of the endoscopic correction of vesicoureteral reflux in children using autologous chondrocytes. *J. Urol*. 2001;**165**:2224–2227.
55. Atala, A. Engineering tissues, organs and cells. *J. Tissue Eng. Regen. Med*. 2007;**1**:83–96.
56. Bent, A. E. R. T. Tutrone, M. T. McLennan, L. K. Lloyd, M. J. Kennelly, and G. Badlani. Treatment of intrinsic sphincter deficiency using autologous ear chondrocytes as a bulking agent. *Neurourol. Urodyn*. 2001;**20**:157–165.

57. Westney, O. L. R. Bevan-Thomas, J. L. Palmer, R. D. Cespedes, and E. J. McGuire. Transurethral collagen injections for male intrinsic sphincter deficiency: The University of Texas–Houston experience. *J. Urol.* 2005;**174**:994–997.

58. Jawad, H., N. N. Ali, A. R. Lyon, Q. Z. Chen, S. E. Harding, and A. R. Boccaccini. Myocardial tissue engineering: A review. *J. Tissue Eng. Regen. Med.* 2007;**1**:327–342.

59. Mukherjee, R., J. A. Zavadzkas, S. M. Saunders, J. E. McLean, L. B. Jeffords, C. Beck, R. E. Stroud, A. M. Leone, C. N. Koval, W. T. Rivers, S. Basu, A. Sheehy, G. Michal, and F. G. Spinale. Targeted myocardial microinjections of a biocomposite material reduces infarct expansion in pigs. *Ann. Thorac. Surg.* 2008;**86**:1268–1276.

60. Nussinovitch, U. and Y. Shoenfeld. Autoimmunity and heart diseases: Pathogenesis and diagnostic criteria. *Arch. Immunol. Ther. Exp. (Warsz)* 2009;**57**:95–104.

61. Jessup, M. and S. Brozena. Heart failure. *N. Engl. J. Med.* 2003;**348**:2007–2018.

62. Landa, N., L. Miller, M. S. Feinberg, R. Holbova, M. Shachar, I. Freeman, S. Cohen, and J. Leor. Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat. *Circulation* 2008;**117**: 1388–1396.

63. Tsur-Gang, O., E. Ruvinov, N. Landa, R. Holbova, M. S. Feinberg, J. Leor, and S. Cohen. The effects of peptide-based modification of alginate on left ventricular remodeling and function after myocardial infarction. *Biomaterials* 2009;**30**: 189–195.

64. Rowley, J. A., G. Madlambayan, and D. J. Mooney. Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials* 1999;**20**:45–53.

65. Yu, J., K. L. Christman, E. Chin, R. E. Sievers, M. Saeed, and R. J. Lee. Restoration of left ventricular geometry and improvement of left ventricular function in a rodent model of chronic ischemic cardiomyopathy. *J. Thorac. Cardiovasc. Surg.* 2009;**137**:180–187.

66. Yu, J., Y. Gu, K. T. Du, S. Mihardja, R. E. Sievers, and R. J. Lee. The effect of injected RGD modified alginate on angiogenesis and left ventricular function in a chronic rat infarct model. *Biomaterials* 2009;**30**:751–756.

67. Kantlehner, M., P. Schaffner, D. Finsinger, J. Meyer, A. Jonczyk, B. Diefenbach, B. Nies, G. Holzemann, S. L. Goodman, and H. Kessler. Surface coating with cyclic RGD peptides stimulates osteoblast adhesion and proliferation as well as bone formation. *Chem. Bio. Chem.* 2000;**1**:107–114.

68. Leor, J., S. Aboulafia-Etzion, A. Dar, L. Shapiro, I. M. Barbash, A. Battler, Y. Granot, and S. Cohen. Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium?*Circulation* 2000;**102**:III56–61.

69. Dar, A., M. Shachar, J. Leor, and S. Cohen. Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. *Biotechnol. Bioeng.* 2002;**80**:305–312.

70. Mukherjee, D. Current clinical perspectives on myocardial angiogenesis. *Mol. Cell Biochem.* 2004;**264**:157–167.

71. Laham, R. J., F. W. Sellke, E. R. Edelman, J. D. Pearlman, J. A. Ware, D. L. Brown, J. P. Gold, and M. Simons. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: Results of a phase I randomized, double-blind, placebo-controlled trial. *Circulation* 1999;**100**:1865–1871.

72. Horvath, K. A. Transmyocardial laser revascularization. *J. Card. Surg.* 2008;**23**:266–276.
73. Hao, X., E. A. Silva, A. Mansson-Broberg, K. H. Grinnemo, A. J. Siddiqui, G. Dellgren, E. Wardell, L. A. Brodin, D. J. Mooney, and C. Sylven. Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. *Cardiovasc. Res.* 2007;**75**:178–185.
74. Ohta, M., Y. Suzuki, H. Chou, N. Ishikawa, S. Suzuki, M. Tanihara, Y. Mizushima, M. Dezawa, and C. Ide. Novel heparin/alginate gel combined with basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve. *J. Biomed. Mater. Res. A* 2004;**71**:661–668.
75. Mihardja, S. S., R. E. Sievers, and R. J. Lee. The effect of polypyrrole on arteriogenesis in an acute rat infarct model. *Biomaterials* 2008;**29**:4205–4210.
76. Oron, U., O. Halevy, T. Yaakobi, G. Hayam, L. Gepstein, T. Wolf, and S. Ben-Haim. Technical delivery of myogenic cells through an endocardial injection catheter for myocardial cell implantation. *Int. J. Cardiovasc. Intervent.* 2000;**3**:227–230.
77. Nathan, D. M. Clinical practice. Initial management of glycemia in type 2 diabetes mellitus. *N. Engl. J. Med.* 2002;**347**:1342–1349.
78. Gerstein, H. C., M. E. Miller, R. P. Byington, D. C. Goff, Jr., J. T. Bigger, J. B. Buse, W. C. Cushman, S. Genuth, F. Ismail-Beigi, R. H. Grimm, Jr., J. L. Probstfield, D. G. Simons-Morton, and W. T. Friedewald. Effects of intensive glucose lowering in type 2 diabetes. *N. Engl. J. Med.* 2008;**358**:2545–2559.
79. Ballinger, W. F. and P. E. Lacy. Transplantation of intact pancreatic islets in rats. *Surgery* 1972;**72**:175–186.
80. Robertson, R. P. Islet transplantation as a treatment for diabetes—a work in progress. *N. Engl. J. Med.* 2004;**350**:694–705.
81. Lim, F. and A. M. Sun. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980;**210**:908–910.
82. Robertson, R. P. Successful islet transplantation for patients with diabetes—fact or fantasy? *N. Engl. J. Med.*2000;**343**:289–290.
83. Calafiore, R., G. Basta, G. Luca, A. Lemmi, L. Racanicchi, F. Mancuso, M. P. Montanucci, and P. Brunetti. Standard technical procedures for microencapsulation of human islets for graft into nonimmunosuppressed patients with type 1 diabetes mellitus. *Transplant Proc.* 2006;**38**:1156–1157.
84. Soon-Shiong, P., R. E. Heintz, N. Merideth, Q. X. Yao, Z. Yao, T. Zheng, M. Murphy, M. K. Moloney, M. Schmehl, and M. Harris, R. Mendez, and P. A. Sandford. Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. *Lancet* 1994;**343**:950–951.
85. Elliott, R. B., L. Escobar, P. L. Tan, M. Muzina, S. Zwain, and C. Buchanan. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation* 2007;**14**:157–161.
86. Calafiore, R., G. Basta, G. Luca, A. Lemmi, M. P. Montanucci, G. Calabrese, L. Racanicchi, F. Mancuso, and P. Brunetti. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: First two cases. *Diabetes Care* 2006;**29**:137–138.
87. de Vos, P., M. M. Faas, B. Strand, and R. Calafiore. Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials* 2006;**27**:5603–5617.

88. King, A., A. Andersson, B. L. Strand, J. Lau, G. Skjak-Braek, and S. Sandler. The role of capsule composition and biologic responses in the function of transplanted microencapsulated islets of Langerhans. *Transplantation* 2003;**76**:275–279.

89. Son, E. H., E. Y. Moon, D. K. Rhee, and S. Pyo. Stimulation of various functions in murine peritoneal macrophages by high mannuronic acid-containing alginate (HMA) exposure in vivo. *Int. Immunopharmacol.* 2001;**1**:147–154.

90. Nussinovitch, U. and Y. Shoenfeld. Intravenous immunoglobulin—indications and mechanisms in cardiovascular diseases. *Autoimmun. Rev.* 2008;**7**:445–452.

91. Mallett, A. G. and G. S. Korbutt. Alginate modification improves long-term survival and function of transplanted encapsulated islets. *Tissue Eng. Part A* 2008;**15**:1301–1309.

92. Bregman, B. S., M. McAtee, H. N. Dai, and P. L. Kuhn. Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. *Exp. Neurol.* 1997;**148**:475–494.

93. Prang, P., R. Muller, A. Eljaouhari, K. Heckmann, W. Kunz, T. Weber, C. Faber, M. Vroemen, U. Bogdahn, and N. Weidner. The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. *Biomaterials* 2006;**27**:3560–3569.

94. Yoshii, S., S. Ito, M. Shima, A. Taniguchi, and M. Akagi. Functional restoration of rabbit spinal cord using collagen-filament scaffold. *J. Tissue Eng. Regen. Med.* 2009;**3**:19–25.

95. Ruff, R. L., L. McKerracher, and M. E. Selzer. Repair and neurorehabilitation strategies for spinal cord injury. *Ann. N.Y. Acad. Sci.* 2008;**1142**:1–20.

96. Suzuki, Y., M. Kitaura, S. Wu, K. Kataoka, K. Suzuki, K. Endo, Y. Nishimura, and C. Ide. Electrophysiological and horseradish peroxidase-tracing studies of nerve regeneration through alginate-filled gap in adult rat spinal cord. *Neurosci. Lett.* 2002;**318**:121–124.

97. Kataoka, K., Y. Suzuki, M. Kitada, T. Hashimoto, H. Chou, H. Bai, M. Ohta, S. Wu, K. Suzuki, and C. Ide. Alginate enhances elongation of early regenerating axons in spinal cord of young rats. *Tissue Eng.* 2004;**10**:493–504.

98. Kataoka, K., Y. Suzuki, M. Kitada, K. Ohnishi, K. Suzuki, M. Tanihara, C. Ide, K. Endo, and Y. Nishimura. Alginate, a bioresorbable material derived from brown seaweed, enhances elongation of amputated axons of spinal cord in infant rats. *J. Biomed. Mater. Res.* 2001;**54**:373–384.

99. Tobias, C. A., S. S. Han, J. S. Shumsky, D. Kim, M. Tumolo, N. O. Dhoot, M. A. Wheatley, I. Fischer, A. Tessler, and M. Murray. Alginate encapsulated BDNF-producing fibroblast grafts permit recovery of function after spinal cord injury in the absence of immune suppression. *J. Neurotrauma* 2005;**22**:138–156.

100. Mosahebi, A., M. Wiberg, and G. Terenghi. Addition of fibronectin to alginate matrix improves peripheral nerve regeneration in tissue-engineered conduits. *Tissue Eng.* 2003;**9**:209–218.

101. Novikova, L. N., L. N. Novikov, and J. O. Kellerth. Biopolymers and biodegradable smart implants for tissue regeneration after spinal cord injury. *Curr. Opin. Neurol.* 2003;**16**:711–715.

102. Suzuki, K., Y. Suzuki, K. Ohnishi, K. Endo, M. Tanihara, and Y. Nishimura. Regeneration of transected spinal cord in young adult rats using freeze-dried alginate gel. *Neuroreport* 1999;**10**:2891–2894.

103. Battiston, B., S. Geuna, M. Ferrero, and P. Tos. Nerve repair by means of tubulization: Literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. *Microsurgery* 2005;**25**:258–267.

104. Sufan, W., Y. Suzuki, M. Tanihara, K. Ohnishi, K. Suzuki, K. Endo, and Y. Nishimura. Sciatic nerve regeneration through alginate with tubulation or nontubulation repair in cat. *J. Neurotrauma* 2001;**18**:329–338.

105. Suzuki, Y., M. Tanihara, K. Ohnishi, K. Suzuki, K. Endo, and Y. Nishimura. Cat peripheral nerve regeneration across 50 mm gap repaired with a novel nerve guide composed of freeze-dried alginate gel. *Neurosci. Lett*. 1999;**259**:75–78.

106. Hashimoto, T., Y. Suzuki, M. Kitada, K. Kataoka, S. Wu, K. Suzuki, K. Endo, Y. Nishimura, and C. Ide. Peripheral nerve regeneration through alginate gel: Analysis of early outgrowth and late increase in diameter of regenerating axons. *Exp. Brain Res*. 2002;**146**:356–368.

107. Wu, S., Y. Suzuki, M. Tanihara, K. Ohnishi, K. Endo, and Y. Nishimura. Repair of facial nerve with alginate sponge without suturing: An experimental study in cats. *Scand. J. Plast. Reconstr. Surg. Hand Surg*. 2002;**36**:135–140.

108. Ohsumi, H., H. Hirata, T. Nagakura, M. Tsujii, T. Sugimoto, K. Miyamoto, T. Horiuchi, M. Nagao, T. Nakashima, and A. Uchida. Enhancement of perineurial repair and inhibition of nerve adhesion by viscous injectable pure alginate sol. *Plast. Reconstr. Surg*. 2005;**116**:823–830.

109. Purcell, E. K., A. Singh, and D. R. Kipke. Alginate composition effects on a neural stem cell-seeded scaffold. *Tissue Eng. Part C Methods* 2009;**15**:541–550.

110. Maysinger, D., O. Berezovskaya, and S. Fedoroff. The hematopoietic cytokine colony stimulating factor 1 is also a growth factor in the CNS. (II) Microencapsulated CSF-1 and LM-10 cells as delivery systems. *Exp. Neurol*. 1996;**141**:47–56.

111. Wu, S., Y. Suzuki, M. Kitada, M. Kitaura, K. Kataoka, J. Takahashi, C. Ide, and Y. Nishimura. Migration, integration, and differentiation of hippocampus-derived neurosphere cells after transplantation into injured rat spinal cord. *Neurosci. Lett*. 2001;**312**:173–176.

112. Jeon, Y., K. Kwak, S. Kim, Y. Kim, J. Lim, and W. Baek. Intrathecal implants of microencapsulated xenogenic chromaffin cells provide a long-term source of analgesic substances. *Transplant Proc*. 2006;**38**:3061–3065.

113. Kim, Y. M., K. H. Kwak, J. O. Lim, and W. Y. Baek. Reduction of allodynia by intrathecal transplantation of microencapsulated porcine chromaffin cells. *Artif. Organs* 2009;**33**:240–249.

114. Bes, J. C., J. Tkaczuk, K. A. Czech, M. Tafani, R. Bastide, C. Caratero, G. D. Pappas, and Y. Lazorthes. One-year chromaffin cell allograft survival in cancer patients with chronic pain: Morphological and functional evidence. *Cell Transplant* 1998;**7**:227–238.

115. Pappas, G. D., Y. Lazorthes, J. C. Bes, M. Tafani, and A. P. Winnie. Relief of intractable cancer pain by human chromaffin cell transplants: Experience at two medical centers. *Neurol. Res*. 1997;**19**:71–77.

116. Zhang, H. L., J. J. Wu, H. M. Ren, J. Wang, Y. R. Su, and Y. P. Jiang. Therapeutic effect of microencapsulated porcine retinal pigmented epithelial cells transplantation on rat model of Parkinson's disease. *Neurosci. Bull*. 2007;**23**:137–144.

117. Grandoso, L., S. Ponce, I. Manuel, A. Arrue, J. A. Ruiz-Ortega, I. Ulibarri, G. Orive, R. M. Hernandez, A. Rodriguez, R. Rodriguez-Puertas, M. Zumarraga, G. Linazasoro, J. L. Pedraz, and L. Ugedo. Long-term survival of encapsulated GDNF secreting cells implanted within the striatum of parkinsonized rats. *Int. J. Pharm.* 2007;**343**:69–78.

118. Choonara, Y. E., V. Pillay, R. A. Khan, N. Singh, and L. C. du Toit. Mechanistic evaluation of alginate-HEC gelisphere compacts for controlled intrastriatal nicotine release in Parkinson's disease. *J. Pharm. Sci.* 2009;**98**:2059–2072.

119. Drucker-Colin, R., L. Verdugo-Diaz, C. Morgado-Valle, G. Solis-Maldonado, R. Ondarza, C. Boll, G. Miranda, G. J. Wang, and N. Volkow. Transplant of cultured neuron-like differentiated chromaffin cells in a Parkinson's disease patient. A preliminary report. *Arch. Med. Res.* 1999;**30**:33–39.

120. Xue, Y. L., Z. F. Wang, D. G. Zhong, X. Cui, X. J. Li, X. J. Ma, L. N. Wang, K. Zhu, and A. M. Sun. Xenotransplantation of microencapsulated bovine chromaffin cells into hemiparkinsonian monkeys. *Artif. Cell Blood Substit. Immobil. Biotechnol.* 2000;**28**:337–345.

121. Singh, N., V. Pillay, and Y. E. Choonara. Advances in the treatment of Parkinson's disease. *Prog. Neurobiol.* 2007;**81**:29–44.

122. Koike, Y., S. Murakami, K. Matsuzaka, and T. Inoue. The effect of Emdogain® on ectopic bone formation in tubes of rat demineralized dentin matrix. *J. Periodont. Res.* 2005;**40**:385–394.

123. Sculean, A., A. Kiss, A. Miliauskaite, F. Schwarz, N. B. Arweiler, and M. Hannig. Ten-year results following treatment of intra-bony defects with enamel matrix proteins and guided tissue regeneration. *J. Clin. Periodontol.* 2008;**35**:817–824.

124. Casarin, R. C. V., E. D. P. Ribeiro, F. H. Nociti, A. W. Sallum, G. M. B. Ambrosano, and M. Z. Casati. A double-blind randomized clinical evaluation of enamel matrix derivative proteins for the treatment of proximal class-II furcation involvements. *J. Clin. Periodontol.* 2008;**35**:429–437.

125. Bouhadir, K. H., E. Alsberg, and D. J. Mooney. Hydrogels for combination delivery of antineoplastic agents. *Biomaterials* 2001;**22**:2625–2633.

126. Vechasilp, J., B. Tangtrakulwanich, K. Oungbho, and S. Yuenyongsawad. The efficacy of methotrexate-impregnated hydroxyapatite composites on human mammary carcinoma cells. *J. Orthop. Surg. (Hong Kong)* 2007;**15**:56–61.

127. Misirli, Y., E. Ozturk, H. Kursaklioglu, and E. B. Denkbas. Preparation and characterization of mitomycin-C loaded chitosan-coated alginate microspheres for chemoembolization. *J. Microencapsul.* 2005;**22**:167–178.

128. Kuijlen, J. M., B. J. de Haan, W. Helfrich, J. F. de Boer, D. Samplonius, J. J. Mooij, and P. de Vos. The efficacy of alginate encapsulated CHO-K1 single chain-TRAIL producer cells in the treatment of brain tumors. *J. Neurooncol.* 2006;**78**:31–39.

129. Holte, O., A. Skretting, T. Bach-Gansmo, P. K. Hol, K. Johnsrud, H. H. Tonnesen, and J. Karlsen. Localized internal radiotherapy with 90Y particles embedded in a new thermosetting alginate gel: a feasibility study in pigs. *Nucl. Med. Commun.* 2006;**27**:185–190.

130. Cirone, P., J. M. Bourgeois, and P. L. Chang. Antiangiogenic cancer therapy with microencapsulated cells. *Hum. Gene Ther.* 2003;**14**:1065–1077.

131. Joki, T., M. Machluf, A. Atala, J. Zhu, N. T. Seyfried, I. F. Dunn, T. Abe, R. S. Carroll, and P. M. Black. Continuous release of endostatin from microencapsulated engineered cells for tumor therapy. *Nat. Biotechnol.* 2001;**19**:35–39.

132. Dvir-Ginzberg, M., A. Konson, S. Cohen, and R. Agbaria. Entrapment of retroviral vector producer cells in three-dimensional alginate scaffolds for potential use in cancer gene therapy. *J. Biomed. Mater. Res. B Appl. Biomater.* 2007;**80**:59–66.

133. Desille, M., B. Fremond, S. Mahler, Y. Malledant, P. Seguin, A. Bouix, Y. Lebreton, J. Desbois, J. P. Campion, and B. Clement. Improvement of the neurological status of pigs with acute liver failure by hepatocytes immobilized in alginate gel beads inoculated in an extracorporeal bioartificial liver. *Transplant Proc.* 2001;**33**:1932–1934.

134. Desille, M., S. Mahler, P. Seguin, Y. Malledant, B. Fremond, V. Sebille, A. Bouix, J. F. Desjardins, A. Joly, J. Desbois, Y. Lebreton, J. P. Campion, and B. Clement. Reduced encephalopathy in pigs with ischemia-induced acute hepatic failure treated with a bioartificial liver containing alginate-entrapped hepatocytes. *Crit. Care Med.* 2002;**30**:658–663.

135. Mai, G., T. H. Nguyen, P. Morel, J. Mei, A. Andres, D. Bosco, R. Baertschiger, C. Toso, T. Berney, P. Majno, G. Mentha, D. Trono, and L. H. Buhler. Treatment of fulminant liver failure by transplantation of microencapsulated primary or immortalized xenogeneic hepatocytes. *Xenotransplantation* 2005;**12**:457–464.

136. Cai, J., M. Ito, H. Nagata, K. A. Westerman, D. Lafleur, J. R. Chowdhury, P. Leboulch, and I. J. Fox. Treatment of liver failure in rats with end-stage cirrhosis by transplantation of immortalized hepatocytes. *Hepatology* 2002;**36**:386–394.

137. Yang, J., M. Goto, H. Ise, C. S. Cho, and T. Akaike. Galactosylated alginate as a scaffold for hepatocytes entrapment. *Biomaterials* 2002;**23**:471–479.

138. Chung, T. W., J. Yang, T. Akaike, K. Y. Cho, J. W. Nah, S. I. Kim, and C. S. Cho. Preparation of alginate/galactosylated chitosan scaffold for hepatocyte attachment. *Biomaterials* 2002;**23**:2827–2834.

139. Liu, Z. C., and T. M. Chang. Transplantation of bioencapsulated bone marrow stem cells improves hepatic regeneration and survival of 90% hepatectomized rats: A preliminary report. *Artif. Cell Blood Substit. Immobil. Biotechnol.* 2005;**33**:405–410.

140. Dobratz, E. J., S. W. Kim, A. Voglewede, and S. S. Park. Injectable cartilage: Using alginate and human chondrocytes. *Arch. Facial Plast. Surg.* 2009;**11**:40–47.

141. Chang, S. C., G. Tobias, A. K. Roy, C. A. Vacanti, and L. J. Bonassar. Tissue engineering of autologous cartilage for craniofacial reconstruction by injection molding. *Plast. Reconstr. Surg.* 2003;**112**:793–799.

142. Caterson, E. J., L. J. Nesti, W. J. Li, K. G. Danielson, T. J. Albert, A. R. Vaccaro, and R. S. Tuan. Three-dimensional cartilage formation by bone marrow-derived cells seeded in polylactide/alginate amalgam. *J. Biomed. Mater. Res.* 2001;**57**:394–403.

143. Lin, Y. J., C. N. Yen, Y. C. Hu, Y. C. Wu, C. J. Liao, and I. M. Chu. Chondrocytes culture in three-dimensional porous alginate scaffolds enhanced cell proliferation, matrix synthesis and gene expression. *J. Biomed. Mater. Res.A* 2009;**88**:23–33.

144. Chang, S. C., J. A. Rowley, G. Tobias, N. G. Genes, A. K. Roy, D. J. Mooney, C. A. Vacanti, and L. J. Bonassar. Injection molding of chondrocyte/alginate constructs in the shape of facial implants. *J. Biomed. Mater. Res.* 2001;**55**:503–511.

145. Ueng, S. W., M. S. Lee, S. S. Lin, E. C. Chan, and S. J. Liu. Development of a biodegradable alginate carrier system for antibiotics and bone cells. *J. Orthop. Res.* 2007;**25**:62–72.

146. Ueng, S. W., L. J. Yuan, N. Lee, S. S. Lin, E. C. Chan, and J. H. Weng. In vivo study of biodegradable alginate antibiotic beads in rabbits. *J. Orthop. Res.* 2004;**22**:592–599.

147. Park, Y. J., J. Y. Lee, H. R. Yeom, K. H. Kim, S. C. Lee, I. K. Shim, C. P. Chung, and S. J. Lee. Injectable polysaccharide microcapsules for prolonged release of minocycline for the treatment of periodontitis. *Biotechnol. Lett.* 2005;**27**: 1761–1766.

148. Liu, D. Z., W. P. Chen, C. P. Lee, S. L. Wu, Y. C. Wang, and T. W. Chung. Effects of alginate coated on PLGA microspheres for delivery tetracycline hydrochloride to periodontal pockets. *J. Microencapsul.* 2004;**21**:643–652.

149. Ueng, S. W., S. S. Lee, S. S. Lin, E. C. Chan, B. R. Hsu, and K. T. Chen. Biodegradable alginate antibiotic beads. *Clin. Orthop. Relat. Res.* 2000: 250–259.

150. Jay, S. M. and W. M. Saltzman. Controlled delivery of VEGF via modulation of alginate microparticle ionic crosslinking. *J. Control. Release* 2009;**134**:26–34.

151. Chinen, N., M. Tanihara, M. Nakagawa, K. Shinozaki, E. Yamamoto, Y. Mizushima, and Y. Suzuki. Action of microparticles of heparin and alginate crosslinked gel when used as injectable artificial matrices to stabilize basic fibroblast growth factor and induce angiogenesis by controlling its release. *J. Biomed. Mater. Res. A* 2003;**67**:61–68.

152. Freeman, I. and S. Cohen. The influence of the sequential delivery of angiogenic factors from affinity-binding alginate scaffolds on vascularization. *Biomaterials* 2009;**30**:2122–2131.

153. Keshaw, H., A. Forbes, and R. M. Day. Release of angiogenic growth factors from cells encapsulated in alginate beads with bioactive glass. *Biomaterials* 2005;**26**:4171–4179.

154. Chretien, C., V. Boudy, P. Allain, and J. C. Chaumeil. Indomethacin release from ion-exchange microspheres: Impregnation with alginate reduces release rate. *J. Control. Release* 2004;**96**:369–378.

155. Becker, T. A., and D. R. Kipke. Flow properties of liquid calcium alginate polymer injected through medical microcatheters for endovascular embolization. *J. Biomed. Mater. Res.* 2002;**61**:533–540.

156. Li, S., X. T. Wang, X. B. Zhang, R. J. Yang, H. Z. Zhang, L. Z. Zhu, and X. P. Hou. Studies on alginate-chitosan microcapsules and renal arterial embolization in rabbits. *J. Control. Release* 2002;**84**:87–98.

157. Eroglu, M., H. Kursaklioglu, Y. Misirli, A. Iyisoy, A. Acar, A. Isin Dogan, and E. B. Denkbas. Chitosan-coated alginate microspheres for embolization and/or chemoembolization: in vivo studies. *J. Microencapsul.* 2006;**23**:367–376.

158. Ribeiro, C. C., C. C. Barrias, and M. A. Barbosa. Calcium phosphate-alginate microspheres as enzyme delivery matrices. *Biomaterials* 2004;**25**:4363–4373.

159. Taqieddin, E., and M. Amiji. Enzyme immobilization in novel alginate-chitosan core-shell microcapsules. *Biomaterials* 2004;**25**:1937–1945.

160. Srivastava, R., J. Q. Brown, H. Zhu, and M. J. McShane. Stabilization of glucose oxidase in alginate microspheres with photoreactive diazoresin nanofilm coatings. *Biotechnol. Bioeng*. 2005;**91**:124–131.

161. Chandy, T., G. H. Rao, R. F. Wilson, and G. S. Das. Delivery of LMW heparin via surface coated chitosan/peg-alginate microspheres prevents thrombosis. *Drug Deliv*. 2002;**9**:87–96.

162. Wang, Y., J. K. Hu, A. Krol, Y. P. Li, C. Y. Li, and F. Yuan. Systemic dissemination of viral vectors during intratumoral injection. *Mol. Cancer Ther*. 2003;**2**:1233–1242.

163. Sailaja, G., H. HogenEsch, A. North, J. Hays, and S. K. Mittal. Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector-specific immune response. *Gene Ther*. 2002;**9**:1722–1729.

CHAPTER 6

DEXTRAN AND PENTOSAN SULFATE – CLINICAL APPLICATIONS

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Biodegradable Polymers in Clinical Use and Clinical Development, First Edition. Edited by Abraham J. Domb, Neeraj Kumar, and Aviva Ezra
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6.1 DEXTRAN

6.1.1 Introduction

Dextran is a polysaccharide of microbial origin made up of glucose units. The most common source of dextran is *Leuconostoc mesenteroides*. There are many clones of *L. mesenteroides* to produce various grades of dextran differing in their molecular weight distribution and degree of branching. Dextran used for clinical applications should be free from pathogens and other contaminations.

6.1.2 Chemistry of Dextran

Dextran is chemically a homopolymer of α-D-glucose. The backbone linear chain contains the glucose units attached with each other by an α-D-1,6-glycosidic linkage, while the branches begin with an α-D-1,3 linkage at the attachment to the backbone, as opposed to an α-D-1,4 linkage of backbone and α-(1,6) linkage at

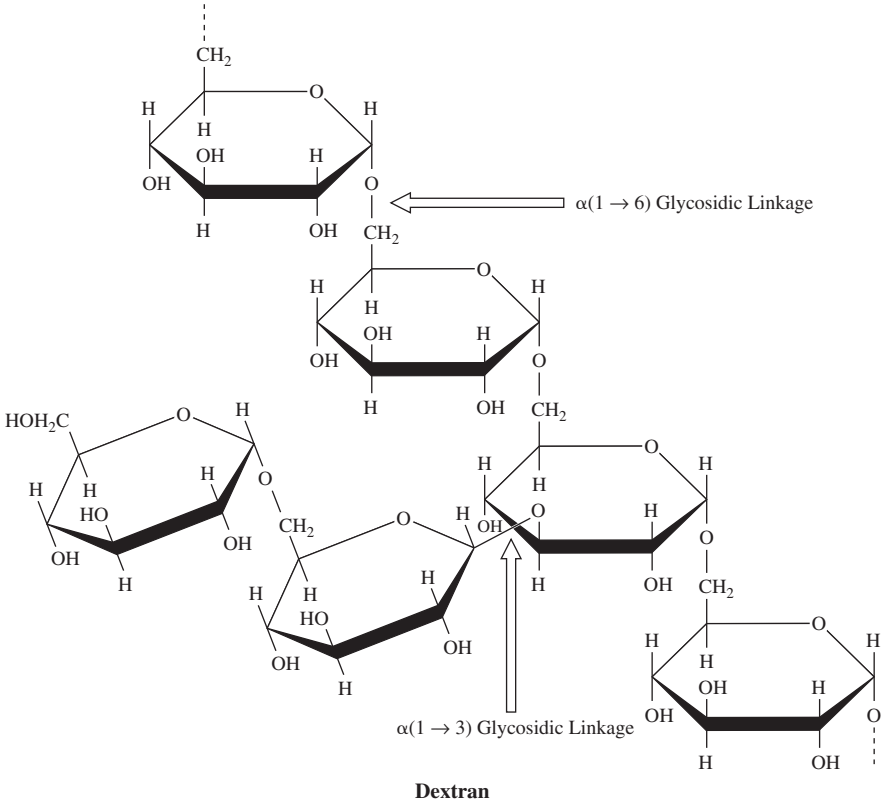


FIGURE 6.1 Structure of dextran.

the branching as in starch (Fig. 6.1). Branches are mostly one to two glucose units long. The branches in dextran can also be attached in an α-1,4 and α-1,2 fashion. The structure, molecular weight distribution, and degree of branching of an individual dextran vary with the strain of microorganism and the environmental growth conditions. Dextran is soluble in water because of its abundant hydroxyl groups but insoluble in ethanol. The CAS (Chemical Abstract Service) registry number of dextran is 9004-54-0. The molecular weight of dextran ranges from 10 to 150 kDa. Generally, the average molecular weight is suffixed to the name, say dextran 1, dextran 40, dextran 70, and so forth where the number denotes the average molecular weight distribution in kilodaltons [1]. Dextran is also produced by certain strains of *Streptococcus, Acetobacter* species, and *Lactobacillus brevis*. It is a major component of dental plaques.

6.1.3 Dextran Derivatives

Natural dextran is modified chemically to give special properties to the modified polymer for specific applications.

Dextran Sulfate. It is a polysulfated product of dextran. The free hydroxyl groups at 2, 3, and 4 positions are sulfated and generally exist as sodium salt. The sulfate groups confer a polyanionic property to the otherwise non-charged dextran. On average 2.3 sulfate groups present per glucose residue [Fig. 6.2(a)]. Dextran sulfate is used to artificially induce colitis in experimental animals.

Diethylaminoethyl–Dextran (DEAE–Dextran). It is produced by reacting dextran with diethylaminoethyl chloride, and the degree of substitution corresponds to approximately one DEAE group per three glucose units. The DEAE group confers a polycationic property to the molecule [Fig. 6.2(b)]. DEAE dextran is used as an adjuvant in vaccine production and as a transfection agent to deliver nucleic acids and proteins.

Iron Dextran. Iron is complexed with dextran and used as an intravenous iron supplement in treating iron deficiency anemia. Some popular brands of iron–dextran are Dexferrum (American Regent Labs, NY) and InFed (Watson Pharma, AZ).

6.1.4 Synthesis of Dextran

Dextran is a polysaccharide from a natural source and is biosynthesized by *Leuconostoc* and *Streptococcus* species from sucrose by the enzyme dextransucrase, which utilizes the glucose part of sucrose to build the dextran polymer and liberates free fructose as a by-product. *Acetobacter* species convert dextrin to dextran by converting the α-1,4 linkages to α-1,6 linkages.

6.1.5 Production of Dextran

Dextran is produced by a fermentation process from various bacterial strains of *L. mesenteroides* (B512, FT 045 B, Lcc4, etc.). Many strains of this organism, including artificially mutated strains, have been studied to optimize the substrate, yield, molecular weight distribution, and degree of branching, and numerous patents have been granted for different manufacturing processes. A typical bioprocess of clinical dextran comprises a two-stage fermentation process. In the first stage, dextransucrase is produced from *L. mesenteroides* at a pH of 6.7 for 24 h. In the second stage the cell-free broth containing enzyme and sucrose are input into a continuous fermentation process at a pH of 5.2 for dextran synthesis [2]. Sugarcane molasses and corn steep liquor can be used as carbon and nitrogen sources, respectively, on an industrial scale [3]. The molecular weight distribution of native dextran is so wide and not suitable for clinical use. In order to get a specific molecular weight range 40–70 kDa, which is most frequently used clinically, a two-stage fermentation process is used. The native high-molecular-weight dextran product from the first stage is fermented with *Lipomyces starkeyi,* which produces dextranase, an enzyme that cleaves dextran into smaller fragments [4, 5]. Finally, the desired molecular weight fraction of dextran is obtained

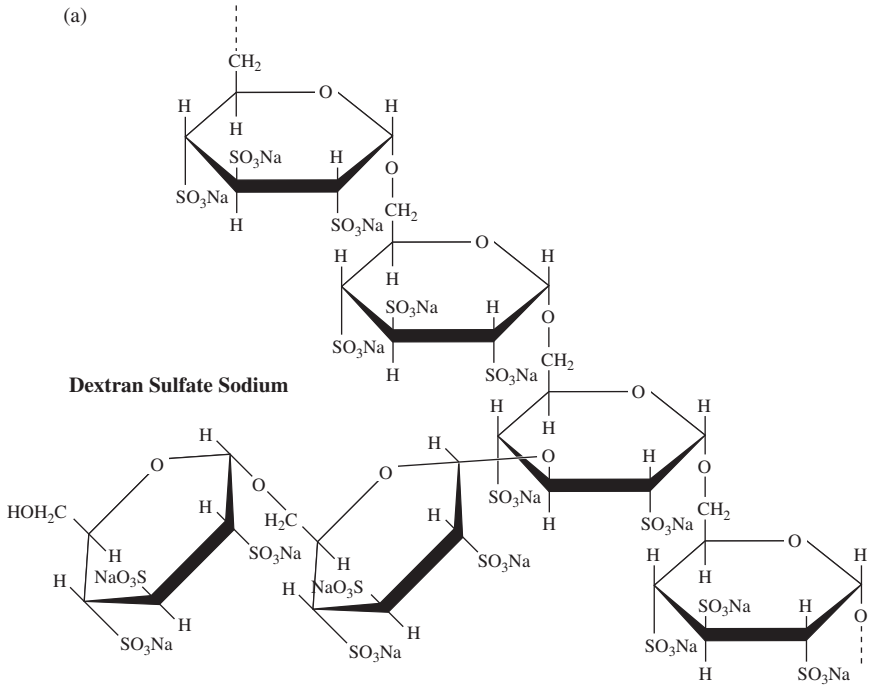


FIGURE 6.2 Structure of (a) dextran sulfate sodium and (b) diethylaminoethyl dextran.

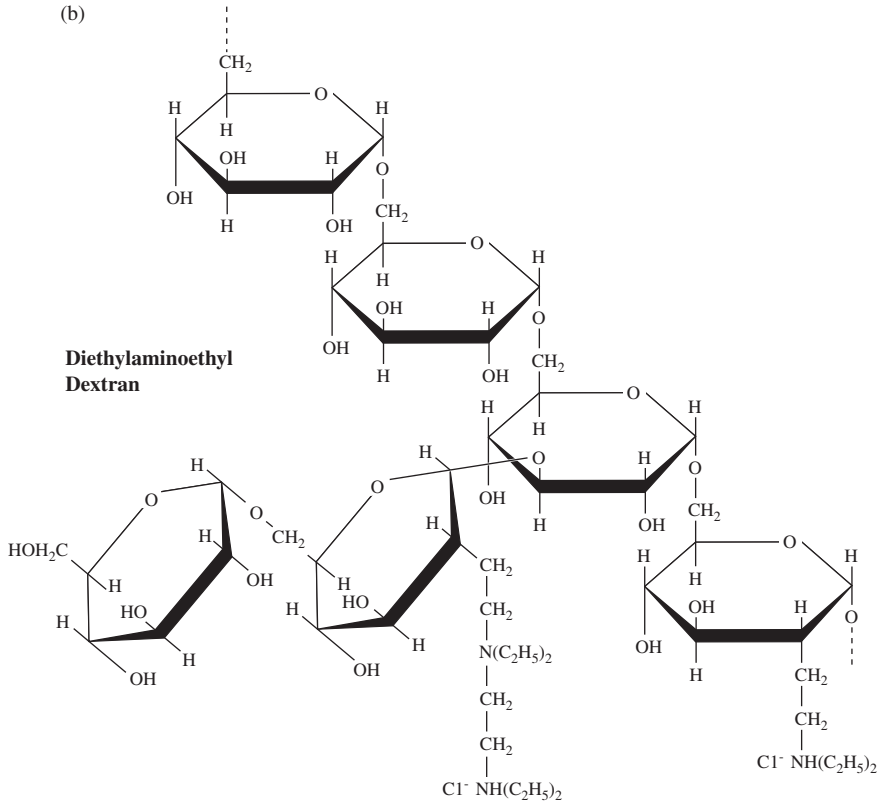


FIGURE 6.2 (Continued).

by removing the larger and smaller fragments by membrane filtration or size-exclusion continuous chromatography [6] and precipitated with organic solvents such as ethanol. A mixed-culture fermentation method employing *L. starkeyi* and *L. mesenteroides,* using sucrose and starch to produce dextrans of selected size was, also reported [7]. A new separation method to control the molecular weight distribution and improved quality of dextran using a direct-current (dc) potential during membrane ultrafiltration coupled with solvent crystallization was reported by Chen et al. [8]. Sucrose content of media affects the molecular weight and degree of branching of the dextran produced. Kim et al. has reported that high sucrose content decreases the yield of high-molecular-weight dextran and increases the yield of low-molecular-weight dextran and increases the degree of branching [9]. Temperature has very little effect on the size of dextran but has a significant effect on the degree of branching as high temperature favors more branching. Neither molecular weight nor degree of branching is affected by pH between 4.5 and 6.0 (Table 6.1). Emergence of recombinant deoxyribonucleic acid (rDNA) technology has enabled the production of dextran using recombinant dextransucrase enzyme expressed in non-*Leuconostoc* hosts [10].

TABLE 6.1 Effect of Sucrose Concentrations, pH, and Temperature on Molecular Weight Distribution of Dextrans Synthesized by *L. mesenteroides* B-12FMCM Dextransucrase

Percent dextran				
Sucrose[a] (M)	10^3-10^4	10^4-10^5	10^5-10^6	$>10^6$ MW[b]
	I	II	III	IV
0.1	0.12	0	0.8	99.8
0.3	1.27	0.73	0	98.0
1.0	5.8	18.6	17.9	57.7
1.5	8.7	30.9	3.8	56.6
2.0	61.5	0	0	38.5
3.0	68.4	0	0	31.6
4.0	69.9	0	0	30.1
pH[c]				
4.5	13.2	1.2	26.8	58.8
5.0	1.6	1.1	10.4	86.9
5.2	1.3	0	10.1	88.6
5.5	0.5	0	4.5	95.0
6.0	0.3	0	0	99.7
°C[d]				
4	0	0.4	16.4	83.2
15	4.2	0	20.6	75.2
23	6.1	0	9.1	84.8
28	2.3	7.8	15.4	74.5
37	1.3	0	33.4	65.3
45	0	0	15.9	84.1

[a] Reactions conducted at pH 5.5 and 28°C and different sucrose concentrations.
[b] Molecular weights divided into four divisions.
[c] Reactions conducted at 1.5 M sucrose and 28°C and different pH values.
[d] Reactions conducted at 1.5 M sucrose and pH 5.5 and different temperatures.

Source: Kim et al., *Carbohydr. Res.* 2003;**338**:1183–1189.

Besides natural sources of dextran, a few chemical syntheses have been reported. A cationic ring-opening polymerization of 1,6-anhydro-2,3,4-tri-*O*-allyl-α-D-glucopyranose was carried out using BF_3-OEt_2 at 0.5°C for 140 h followed by isomerization using tris(triphenylphosphine)-chlororhodium catalyst and then deprotection by hydrochloric acid in acetone to give dextran, a (1→6)-α-D-glucopyranan [11, 12] (Fig. 6.3).

6.1.6 Clinical Uses of Dextran

Dextran has been used in clinical practice for more than a half century. It is biocompatible [13] and biodegradable [14]. This makes it useful in in vivo

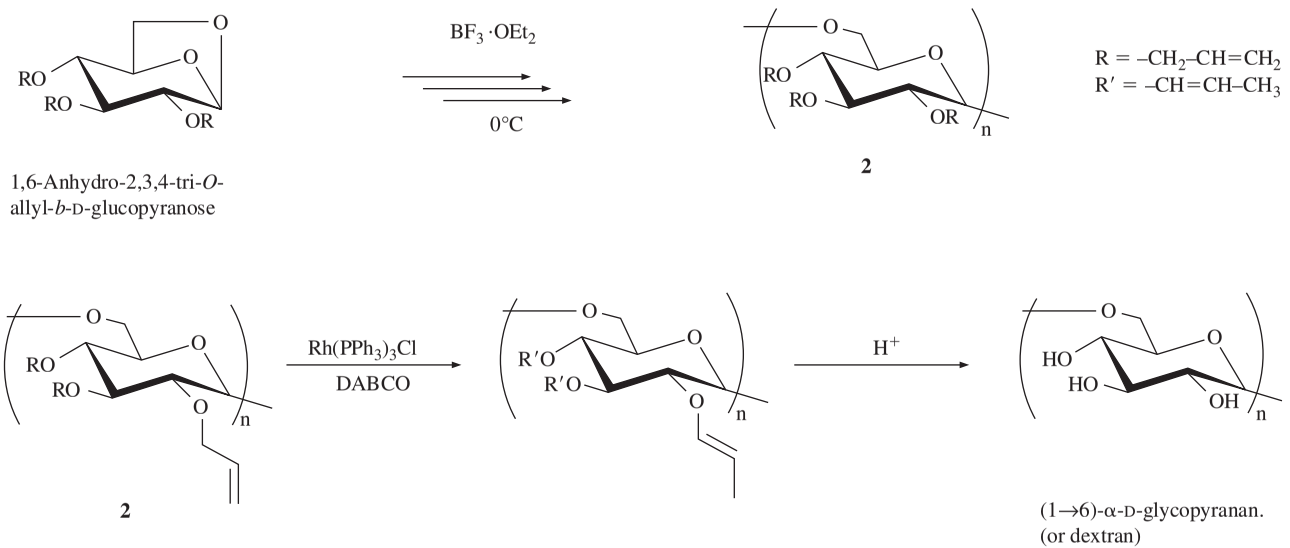


FIGURE 6.3 Scheme of dextran synthesis by cationic ring-opening polymerization reaction. (*Source*: Kakuchi et al., *Macromol. Rapid Commun*. 2000;**21**:1003–1006.)

applications. A molecular weight (MW) range of 40,000–70,000 is most frequently used for clinical applications. A few commercial brands of dextran for clinical uses are Macrodex (MW 70,000) and Rheomacrodex (MW 40,000). A brief account on the past and present investigations and applications of dextran is highlighted below.

Dextran as Lymph Node Imaging Agent. Dextran can be used as an imaging agent in diagnostic procedures after labeling with a radionuclide. In an animal experiment using rabbits, dextran labeled with technetium (^{99m}Tc) when given intradermally preferentially accumulated at the lymph nodes while the blood radioactivity levels were reported as too low [15]. Thus, the lymphatic system can be visualized by scintigraphy using ^{99m}Tc–dextran as a radioimaging agent (Fig. 6.4). Identification of sentinel lymph node (SLN), a hypothetical lymph node where a metastatic cancer can possibly reach first before migrating to other lymph nodes, can improve the assessment of the pathological condition of tumor cancer spread and to plan the treatment strategy. A preclinical study using pigs had analyzed the SLN mapping of the prostate using [^{99m}Tc]diethylenetetramine pentaacetic acid–mannosyl-dextran (Lymphoseek, Neoprobe Corporation) to determine the SLN [16]. Lymphoseek is now in a phase III clinical trial. Other imaging agents, such as superparamagnetic iron oxide nanoparticles or gadolinium-based nanoparticles are coated with dextran sulfate to confer biocompatibility and monodispersity in water [17] (Fig. 6.5).

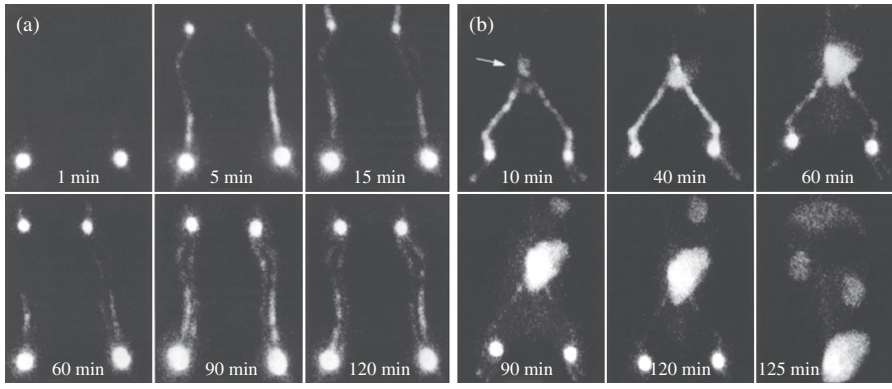


FIGURE 6.4 Scintigraphic imaging of lymph nodes by ^{99m}Tc–dextran. (a) Scintigraphic anterior images at times indicated after ID injection of 400 μCi (0.05 mL) ^{99m}Tc–dextran in both hind legs of a rabbit: The injection sites, the bilateral lymph channels extending up to popliteal lymph nodes are all visible. (b) The upper region showing the popliteal lymph nodes and the channels extending up to external iliac lymph nodes (*arrow*) later obscured by the urinary bladder. The image at 125 min taken from the abdomen shows the accumulation in liver, kidneys and urinary bladder. (*Source:* Ercan et al., *Eur. J. Nucl. Med.* 1985;**11**:80–84.)

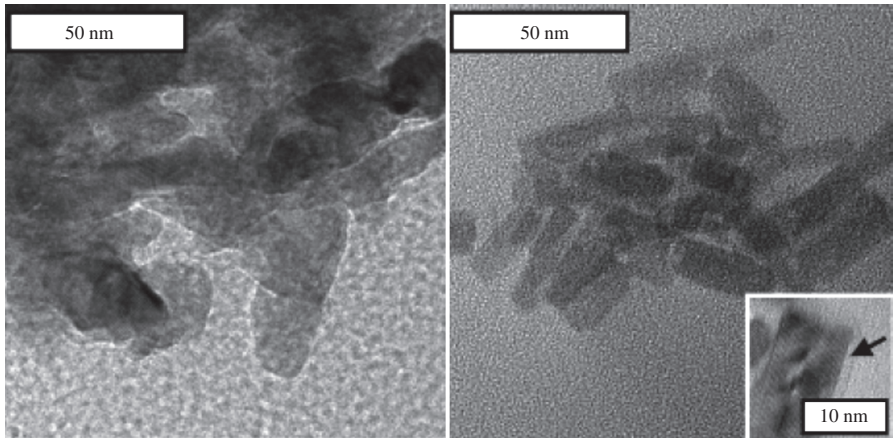


FIGURE 6.5 TEM images of $GdPO_4$ particles without (*left*) and with (*right*, PGP/dextran-K01) the dextran coating. The arrow in the right picture indicates the dextran coating material (*Source*: Hifumi et al., *J. Am. Chem. Soc.* 2006;**128**:15090–15091.)

Dextran in Surgical Procedures. Spinal anaesthesia is achieved by injecting a local anaesthetic agent by an epidural puncture. But sometimes this causes a headache after the surgical procedure. Hence a safe material is needed for epidural patches. In an animal experiment dextran injected in rats intrathecally was reported to cause no neurotoxicity and hence may be suitable for epidural patches [18].

Blood-Related Applications of Dextran. Dextran sulfate was reported to inhibit coagulation and complement activation. In clinical islet transplantation, contact of human pancreatic islets with blood triggers an instant blood-mediated inflammatory reaction (IBMIR) and leads to tissue loss. Administration of a low-molecular-weight dextran sulfate (MW 5000) shows a dose-dependent inhibition of IBMIR with inhibition of coagulation and complement activation [19]. It is reported that dextran 70 shows a dose-dependent C3 activation and terminal complement complex formation when incubated with fresh serum in vitro [20]. Dextran has been used as a plasma volume expander. When blood is lost in an accident or surgery, the primary measure is to stop the bleeding and secondly to replace the lost volume with a suitable blood substitute such that the remaining red blood cells (RBCs) can still oxygenate the body tissue until a blood transfusion can restore the lost RBCs. Plasma volume expanders do expand and maintain the volume of blood and facilitate the heart to keep pumping without any shortage of volume. Dextran is a plasma expander, and, because of its high molecular weight, it does not pass out of the vessels and is a potent osmotic agent, biocompatible, and biodegradable. But it is also a potential anticoagulant, which raises the question of its usage in surgical

procedures as a volume expander. In a study conducted to establish the clinical significance of any increased intraoperative blood loss associated with the use of dextran 70 during transurethral resection of the prostate (TURP) 500–1000 mL of 6% dextran 70 was administered. The regression analysis found no indication that dextran 70 given within the recommended dose range increases the blood loss during TURP [21]. Hemodilution facilitates free flow of blood through vessels and has beneficiary effect in ischemic stroke. A combination of venesection, a surgical procedure to remove excess of blood from the circulation, and hemodilution by dextran 40 administration was reported as a clinically safe therapeutic regimen in the treatment of acute cerebral infarction that could improve long-term clinical outcome [22]. In treating congenital heart disease by open-heart surgery especially in children, the extracorporeal blood circulation induces lung injury. Dextran sulfate was reported to prevent this injury by blocking leukocyte-endothelial cell adhesion. Dextran sulfate was given 60 mg/kg by intravenous (IV) infusion just before the cardiopulmonary bypass. Respiratory index was preserved and granulocyte elastase was significantly lower in the dextran sulfate group, indicating amelioration of postperfusion lung damage by dextran sulfate [23]. Incidences of surgical adhesion of organs after surgery are of major concern in clinical practice. Intraperitoneally administered 32% high-molecular-weight dextran 70 was reported to reduce the incidence of surgical adhesions [24]. In reconstructive hip surgery dextran 40 was given to patients pre- and postoperatively and it effectively prevented thrombus formation and was a safe and efficacious means of deep venous thromboprophylaxis [25].

Dextran in Anemic Treatment. Iron–dextran is a complex of ferric oxyhydroxide with dextrans of molecular weights from 5000–7000 Da containing about 50 mg/mL of iron as a viscous solution. It is administered as a parenteral IV infusion and is used as a hematinic in the treatment of anemia. Iron–dextran is given to patients who are intolerant to oral iron supplementation treatment. Typically, the calculated dose is given in divided doses. Total dose infusion (TDI) of iron–dextran is commonly not used because of possible side effects such as anaphylactic reactions. The most frequent side effects of TDI are nausea, headache, vomiting, chills, and backache. However, recently Reddy et al. have reported that anaphylactic reactions were not observed with TDI of iron–dextran, and so it is reported as a safe and efficacious treatment in iron deficiency anemia [26]. Arthralgia-myalgia syndrome observed in some patients can be treated effectively with methylprednisolone [27]. (For a review on iron–dextran toxicity see Burns and Pomposelli [28]). Iron–sucrose was proposed as a safer iron supplement than iron–dextran; but a recent report found no difference between these two [29].

Dextran as an Antimucus Agent. Cystic fibrosis (CF) is a hereditary disease characterized by a thick mucus production in the lung and other complications. The thick mucus affects the rheology and ciliary transportability of sputum and

facilitates adhesion and infection by airway pathogens such as *Pseudomonas aeruginosa*. An in vitro experiment in which the sputum was treated with dextran 4000 (4–40 mg/mL) and the viscoelastic properties of sputum was studied and compared with control. Dextran 4000 treatment is reported to reduce viscoelasticity and increase mucociliary and cough clearability, suggesting potential application of dextran 4000 to reduce crosslink density and cohesiveness of mucus in CF treatment [30].

Dextran in Organ Preservation. In organ transplantations, the grafts are preserved in preservation solutions such as the University of Wisconsin (UW) solution from the time of organ collection until surgery to preserve the function. UW solution contains hydroxyethylstarch colloid as a key component. A dextran-40-based preservation solution was compared with UW solution and reported to be comparable with that of UW solution in preserving kidneys [31]. Similarly graft preservation using a low-potassium dextran preserving solution leads to immediate and intermediate graft function and long-term survival was reported for lung transplantation [32].

Dextran Conjugates as Drug Delivery Systems. Dextran is a biocompatible polymer and biodegradable under physiological conditions. These properties make it a potential candidate as a carrier for many drugs, proteins, nucleic acids, and the like. Many drug conjugates of dextran have been prepared and reported as drug delivery systems. Nystatin was conjugated with oxidized dextran through a Schiff linkage, and the resultant conjugate was highly water soluble and more effective than the parent drug [33]. Another report shows a 12-fold increase in the solubility of acyclovir, an antiviral drug that is active against the hepatitis B virus, after conjugating to dextran. Free drug was released from the conjugate in vitro at pH 7.4, and the pharmacokinetic studies in mice after IV administration showed higher distribution of the drug in liver with acyclovir–dextran conjugate compared to acyclovir alone [34].

A preclinical study of a conjugate of the camptothecin analog with carboxymethyldextran polyalcohol linked by a peptide spacer showed a long retention of high blood levels of conjugate, preferential accumulation in the tumor, and sustained release of the drug and the drug linker in the tumor tissue, leading to enhanced antitumor efficacy with a high therapeutic index by a single dose [35]. Choosing the right linker that can be cleaved only by certain conditions will enhance the drug release specific to a particular tissue. A dextran–peptide–methotrexate conjugate was reported for tumor-targeted delivery of the drug mediated via matrix metalloproteinase II (MMP-2) and metalloproteinase IX (MMP-9), the tumor-associated enzymes that are overexpressed in many tumors such as prostate, colon, ovary, bladder, and gastric carcinoma. The anticancer drug was linked to the polymer via a short peptide of Pro–Val–Gly–Leu–Ile–Gly, the sequence that can be cleaved by the target MMP enzymes (Fig. 6.6). In the presence of the target enzymes the linker was cleaved and methotrexate–peptide is released in the cancer cells alone, while the

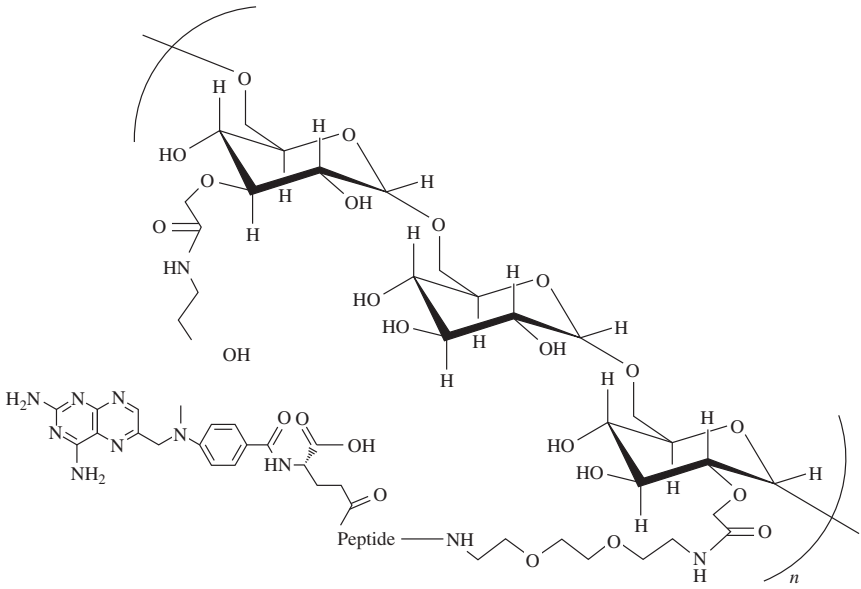
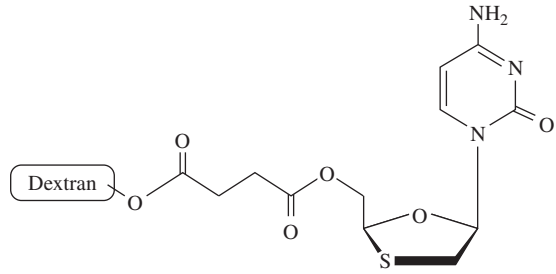


FIGURE 6.6 Dextran–peptide–methotrexate conjugate. Anticancer drug methotrexate is conjugated with dextran via a short peptide Pro–Val–Gly–Leu–Ile–Gly, the sequence that can be cleaved by the target matrix metalloproteinase enzymes. (*Source:* Chau et al., *Bioconjug. Chem.* 2004;**15**:931–941.)

drug in the conjugate remains unreleased in the blood, making the tumor-targeting capability of drug conjugates possible [36].

Drug targeting to a particular tissue can be done by conjugating a ligand for the targeted tissue. A norfloxacin–dextran–mannose conjugate was reported that used mannose as a homing device to direct the drug into macrophages, where the tuberculosis bacteria live, to achieve antimycobacterial activity in vivo in mice [37]. Similarly, a liver-selective prodrug of lamivudine, an antiviral drug used in the treatment of hepatitis B and HIV (human immunodeficiency virus) infection, was reported. Lamivudine was conjugated with dextran through a succinate linker, and the in vitro and in vivo studies of conjugate were shown to decrease the clearance and volume of distribution of the drug, with a 50-fold increase in the accumulation of the drug conjugate in the liver and the gradual release of free drug [38] (Fig. 6.7). Another dextran conjugate of ketorolac was shown to have reduced the ulcerogenic nature of the parent drug [39].

Dextran Hydrogels as Drug Delivery Systems. Hydrogels are crosslinked three-dimensional networks of polymer chains that are not soluble in water but are capable of swelling by absorbing water into the network. If the hydrogel is



Lamivudine–Succinate–Dextran Conjugate (3TCSD)

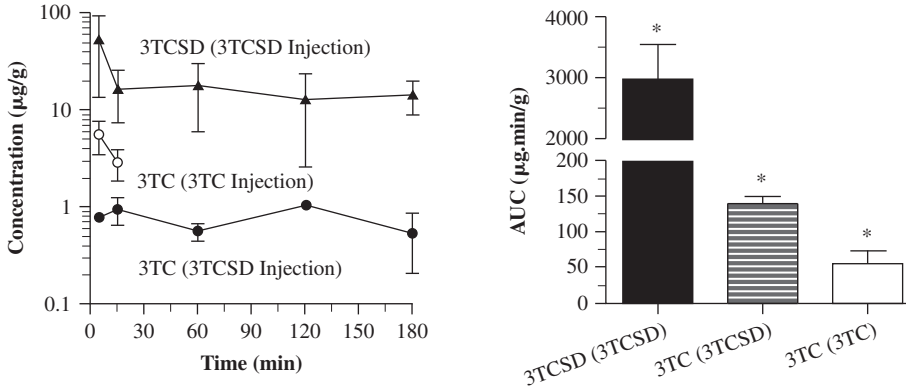


FIGURE 6.7 Higher hepatic accumulation of lamivudine–succinate–dextran conjugate (3TCSD) and slow release of lamivudine (3TC). Structure of 3TCSD (*top*) and liver concentration–time courses (*bottom left*) and AUC values (*bottom right*) of parent (3TC) and/or conjugated (3TCSD) lamivudine after IV administration of single 5-mg/kg doses (3TC equivalent) of 3TC or 3TCSD to rats. Standard deviation values are shown as error bars ($n = 3$ rats for each time point). Asterisks indicate significant differences from the other two groups. (*Source:* Chimalakonda et al., *Bioconjug. Chem.* 2007; **18**:2097–2108.)

formed in the presence of the drug, the latter is entrapped in the network and will be released upon swelling by absorbing water, providing a means of delivering the drug in a controlled fashion. Doxorubicin was incorporated into dextran methacrylate hydrogel by photopolymerization, and the loaded hydrogel released the drug following a simple Fickian diffusion at an early stage of release [40]. A pH-sensitive drug delivery matrix was reported based on dextran–methacrylate hydrogel, which was further functionalized with acidic residue by phthalic anhydride to confer a pH-sensitive swelling behavior. The incorporated ibuprofen was retained in the stomach pH and released in the intestinal pH in a sustained manner dependent on the degree of methacrylic

group derivatization. Also in vivo studies in rats were reported to protect them from the ulcerogenic effect of ibuprofen [41]. A hydrogel based on oxidized dextran and gelatin loaded with dopamine was reported to release the drug when implanted unilaterally in the lesioned striatum of a hemiparkinsonian rat model and abolished the apomorphine-induced contralateral rotational behavior. This technology can be an alternative method for treating Parkinson's disease and also reducing the oral dose of drugs that show severe adverse effects and develop tolerance [42].

Dextran Protein Conjugates. The advantages of dextran–drug conjugates can be extended to proteins as well. Aprotinin is a protein that is used to reduce bleeding during complex surgical procedures. In order to prolong the in vivo lifetime of aprotinin and to target the liver, it was conjugated with clinical dextran and used galactose residues for specific cellular uptake by the liver. The conjugate showed a decreased elimination rate and effective liver targeting [43]. Similarly, a conjugate of a mouse epidermal growth factor with dextran by a reductive amination of free amino end group of proteins with an aldehyde group on the reductive end of dextran was reported for specific binding to the epidermal growth factor receptor [44].

Dextran in Gene Delivery. Diethylaminoethyl–dextran (DEAE–dextran) is a polycationic derivative of dextran. The polycationic nature confers its ability to bind with DNA and facilitates its transfer into the cell, thus making it a transfection agent useful in gene delivery. An enhancement of adenovirus-mediated gene transfer to the airways by sodium caprate pretreatment when the adenovirus is complexed with DEAE–dextran by 45-fold over the virus alone was reported [45]. Stabilizing the nonviral vectors during lyophilization is important to maintain the transfection rate and physical characters upon reconstitution. Sucrose can protect nonviral vectors during freeze-drying despite the osmotic incompatibility with subcutaneous or intramuscular injection. Dextran 3000 was shown to be offering similar protection as that of sucrose, with 40% reduced osmolality compared to sucrose [46].

Anti-HIV Activity of Dextran Sulfate. Dextran was one of the candidates tested for HIV treatment in the late 1980s. Attachment of a viral particle to the target T lymphocyte receptor, the CD4 antigen is the first step in HIV-1 infection. Dextran sulfate, molecular weight 8000, was found to block the binding of virus to T lymphocytes and showed inhibitory effect against HIV-1 in vitro at concentrations clinically attainable in humans. Also dextran sulfate was reported to have suppressed replication of HIV-2 in vitro [47]. A clinical trial for tolerance and safety of oral dextran sulfate in treating HIV patients with the acquired immunodeficiency syndrome (AIDS) reported that the drug was well tolerated, but there was no appreciable change in CD4 lymphocyte numbers and no decline in beta-2 microglobulin level [48]. Pharmacokinetic analysis of dextran sulfate in rats was studied with radiolabeled dextran sulfate,

molecular weight 8000, given IV and the initial plasma half-life was found to be 30 min, a minor breakdown of the molecules. On oral administration the apparent bioavailability was found to be 6.8%, but it is absorbed after breakdown into smaller fragments of MW < 200, while in vitro testing revealed that dextran sulfate MW < 2300 had no anti-HIV effect, suggesting that oral administration of dextran sulfate was unlikely to produce anti-HIV effect in vivo [49]. Also it was reported that the anti-HIV effect of dextran sulfate is strain dependent such that two clinical isolates of HIV-1 (TM and SP) were less susceptible to dextran sulfate compared to two cloned isolate (HIV-1 WMF and HIV-2 ROD) and a prototype lab strain (HIV-1 IIIB). Also high-molecular-weight dextran sulfate (500,000) was antagonistic (in HIV-1 TM and SP isolates) while low-molecular-weight dextran (8000) was synergistic (in all five HIV isolates) when dextran sulfate is given in combination with dideoxynucleosides [50]. Sulfated polysaccharides including dextran sulfate and pentosan polysulfate were reported to inhibit transmission of HIV-1 from lymphocyte to epithelial cells in vitro, suggesting a possible vaginal formulation that could inhibit HIV-1 transmission [51] (Fig. 6.8).

Antitumor Activity of Dextran Sulfate. In a preclinical study, dextran sulfate at a dose of 300 mg/kg intraperitoneally (IP) given to rats prior to the IV injection of radiolabeled lung carcinoma cells significantly decreased the pulmonary metastasis compared to a control group, suggesting dextran sulfate

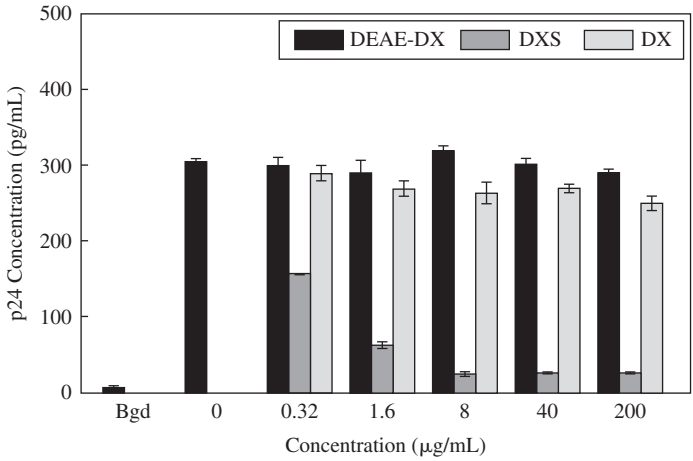


FIGURE 6.8 Effect of dextrans on cell-mediated HIV-1 infection. Neutrally charged dextran (DX) and positively charged dextran (DEAE-DX) did not inhibit HIV-1 infection. Dextran sulfate (DXS) inhibited HIV-1 infection by 50% at 0.3 μg/mL. p24 values represent mean of 2 wells ± SD; experiment was repeated 3 times. Bgd, background p24 value due to nonspecific antibody binding in the absence of test supernatant. (*Source:* Pearce-Pratt et al., *Biol. Reprod.* 1996;**54**:173–182.)

can be used for inhibiting the metastasis of carcinoma [52]. Bone marrow transplantation is one of the surgical procedures to treat tumor patients. The bone marrow should be processed to remove the RBCs and to enrich the hematopoietic progenitor cells (HPC) in vitro. A method that uses dextran 110 for bone marrow processing was reported. Blood was treated with dextran in transfer bags for 30 min to allow sedimentation followed by flow cytometry to assay the recovery of $CD34^+$ cells. There was no difference in mean RBC count in postdextran sedimentation bone marrow (BM) allografts and ficoll-treated BM allografts, suggesting dextran sedimentation as an efficient method of depleting red cells in major ABO-incompatible bone marrow allografts without significant loss of HPC [53]. Intraperitoneal dextran sulfate 500,000 showed antiadherent therapy against metastasis of carcinoma, and toxicity studies in mice and rabbits have shown that the IP dextran sulfate is safe as an antiadherent treatment against peritoneal carcinomatosis [54]. Magnetic nanoparticles could heat in an alternating magnetic field, causing magnetic fluid hyperthermia that can inhibit tumor growth in vivo. A toxicity study of dextran-coated magnetic fluid given subcutaneously (SC) was reported in mice. At a dose of 30 mg/0.3 mL dextran–magnetic-fluid-activated glutamicoxalacetictransaminase and glutamicpyruvictransaminase no change in cell number of blood was noted, suggesting that dextran–magnetic fluid was safe, biocompatible, and tolerable in SC tumor therapy [55].

Dextran in Hyperlipidemia Treatment. Apheresis systems have been used in patients with hyperlipidemia to reduce the low-density lipoproteins (LDL) levels. While many apheresis systems are very complicated and expensive treatments, a new LDL apheresis system based on dextran sulfate was developed at a lower cost [56]. Fifty patients with hyperlipidemia were treated with the new apheresis system. Typically, 600 mL of plasma was collected by apheresis, treated with dextran sulfate and calcium chloride solutions that serve as LDL absorber and catalyzer, respectively. Dextran sulfate selectively bound LDL cholesterol under catalysis by calcium ion, and this LDL–dextran sulfate complex was removed by centrifugation, and the excess calcium was removed by cation exchange column and the treated plasma was transfused back into the patients. After treatment an acute mean reduction of LDL cholesterol was found to be 97%, and a corresponding total cholesterol and total triglyceride reduction were found to be 55.2 and 69.5%, respectively. No significant reduction in high-density lipoprotein cholesterol and albumin, suggesting this new dextran-sulfate-based apheresis system is a safe, effective, and inexpensive means for treating patients with hyperlipidemia.

6.1.7 Safety of Dextran

While the clinical applications of dextrans are growing, the safety concern of dextrans used in a particular application needs to be addressed. Use of 32% dextran 70 to prevent postsurgical pelvic adhesion has been suggested as an

adjuvant therapy. In a study of 234 women who received an antiadhesion regimen of intraperitoneal 32% dextran 70, no anaphylactic reactions, peritonitis, or wound infection were experienced by any woman, suggesting intraperitoneal dextran 70 as a safe, well-tolerated antiadhesion adjuvant for gynecological operations [57]. High-molecular-weight dextrans induce generation of antidextran antibodies, leading to anaphylactoid reactions in some patients. These antibodies can be counteracted with monovalent haptens such as dextran 1. Dextran 1 is well tolerated in humans. A pharmacokinetic study of dextran 1 was reported [58]. No symptoms of anaphylactoid reactions were observed after dextran 1 injection in volunteers with dextran antibodies, suggesting an IV preinjection of dextran 1 can prevent antibody-mediated side effects after infusions with clinical dextrans. Studies on the effects of hapten inhibition with dextran 1, which is used for the prevention of severe dextran-induced anaphylactic reactions (DIAR) caused by the immune system revealed that the incidence of severe DIAR (grades III–V) to clinical dextran after prophylactic use of hapten inhibition was approximately one in 200,000 patients receiving dextran 1. Side effects of dextran 1 were mostly mild and were not antibody mediated, suggesting that dextran with hapten inhibition is possibly the safest plasma substitute in clinical practice [59]. In vitro studies on mitogenic lymphocytes from normal volunteers showed no transformations in response to the presence of dextran sulfate at concentrations of 0.8–8000 μg/mL, suggesting clinical dextrans of average molecular weight 40,000–150,000 are not B- or T-cell mitogens [60].

Patients with liver cirrhosis and tense ascites showed better mobilization of ascitic fluid when treated with large volume paracentesis (LVP) and infusion of low-molecular-weight dextran in one week compared to the minimal mobilization of ascitic fluid in patients receiving diuretics aldactone and furosemide. No hypovolumia after complete mobilization of ascites was noted in patients treated with LVP and dextran, suggesting dextran infusion is safe, low-cost, and effective replacement therapy for cirrhotic ascites treatment by LVP [61]. Low-molecular-weight dextran is used commonly in clinical microsurgery. A study in rabbits that underwent bilateral 2-mm arterial inversion graft construction in femoral arteries infused with IV dextran 40 at 2.1 mL/h showed 85% of dextran grafts were patent at one week compared to only 48% of control grafts that received no infusion. Scanning electron micrographs revealed a decrease in platelet and fibrin deposition in patent dextran graft showing marked diminution in microvascular thrombosis supporting the IV dextran 40 usage in clinical microsurgery [62]. The National Kidney Foundation has published guidelines stating that intravenous iron therapy when used regularly will prevent iron deficiency and promote erythropoiesis better than oral iron therapy in end stage renal disease (ESRD) patients who are undergoing hemodialysis despite some incidences of adverse events associated with this mode of iron supplementation. In a 6-month study of 62 patients with ESRD, only one patient experienced adverse events of hypotension and chest pain. No patient developed anaphylactoid reaction, suggesting safety of IV

iron–dextran during hemodialysis in patient with ESRD [63]. In another study on iron–dextran safety, 20 per 100,000 doses caused the suspected adverse drug events including dyspnea, hypotension, and neurological symptoms as common major adverse drug events. Also these adverse drug events were 8.1-fold more common among patients who received Dexferrum compared with those who received InFed, suggesting serious adverse reactions to IV iron–dextran are rare in clinical practice and are formulation dependent [64].

6.1.8 Toxicity of Dextran

The polycationic derivative of dextran, diethylaminoethyl–dextran (DEAE–dextran) is used as a transfection agent in gene delivery. In order to exploit its potential in gene therapy, its toxicity should be studied. High doses of (500 mg) of DEAE–dextran were reported to induce acute renal failure both morphologically and functionally in rats [65]. High blood urea levels and severe epithelial cell necrosis of proximal tubules was found 48 h postinjection, which is absent in animals injected with equal doses of neutral dextran. Renal mitochondrial respiration rate declined in state 3, state 4, and 2,4-Dinitrophenol (DNP)-uncoupled respiration in DEAE–dextran rats compared to the rate of sham control rats, while an identical dose of neutral dextran showed no effect on mitochondrial respiratory parameters compared to controls. The same results were also found in in vitro studies with isolated mitochondria, suggesting cationic charge implicated the renal and mitochondrial toxicities of DEAE–dextran.

Clinical use of dextran as a plasma expander to improve blood flow and as thromboprophylaxis is also associated with untoward side effects because of immune responses. Prophylactic treatment with dextran 1 before infusion of dextran 70 has reduced the incidence of dextran-induced anaphylactic reactions. But still it was reported that in spite of prophylactic pretreatment with dextran 1 before caesarean section, there was a mild reaction and the child was born with serious brain damage, suggesting dextran 70 should not be given during pregnancy and during caesarean section before delivery of the child [66]. Another case of a death from myocardial infarction with a high titre of dextran-reactive antibodies was also reported in the same study [66]. Dextran–drug conjugates improve drug solubility, life span of drug in vivo by controlled release, and thus increasing the efficacy of treatment. While long-term retention of adriamycin when administered as adriamycin–dextran conjugate was reported to induce acute hepatotoxicity in rats, the free drug was, although cardiotoxic, not hepatotoxic itself. The hepatotoxicity of the conjugate was attributed to the long-term retention of the drug [67]. Iron–dextran that is used as an IV iron supplement was reported to induce exacerbation of synovitis, along with an increase in lipid peroxidation products in synovial fluid but to a small extent in serum. Iron–dextran in vitro stimulated lipid peroxidation, but dextran alone had no effect, suggesting iron–dextran worsens synovial inflammation by promoting lipid peroxidation [68].

6.2 PENTOSAN POLYSULFATE

6.2.1 Introduction

Pentosan polysulfate (PPS) or simply pentosan sulfate is a polysaccharide made up of a sulfated pentose sugar, predominantly xylose. This polymer of β-D-(1,4)-xylopyranose contains a 4-methylglucopyranosyluronic acid linked to the 2 position of the main chain at every tenth xylopyranose unit on average [69]. It contains approximately 15–17% sulfur in the form of approximately 1.5–1.9 covalently bound sulfate groups per sugar unit. PPS structurally and chemically resembles glycosaminoglycans. It is generally used as a sodium or calcium salt (Fig. 6.9). It is also called SP54. The molecular weight range is approximately 1500–6000.

6.2.2 Characterization of PPS

A method using a capillary zone electrophoresis (CZE) to characterize the different PPS sodium was reported that can give the fingerprint profiles of each batch of products capable of indicating heterogeneous mixtures probably containing other polysaccharides as well [69]. To characterize and estimate PPS, a sensitive assay was reported in which PPS was coupled with methylated bovine serum albumin (MBSA) and injected in rabbits to generate anti-PPS antibodies. Anti-MBSA antibodies were removed by passing the sera through MBSA-sepharose immunoadsorbent. With the enzyme-linked immunosorbent assay (ELISA) test, at least 50 ng/mL of PPS can be detected, with a minimal cross reactivity by heparin, which can be eliminated by sample pretreatment with heparinase without affecting PPS [70].

6.2.3 Production of Pentosan Polysulfate

PPS is derived semisynthetically from plant material (beechwood hemicellulose) followed by sulfation of free hydroxyl groups at the 2 and 3 positions in the pentose units. Bene-Arzneimittel GmbH, Germany, remains the world's sole manufacturer of Food and Drug Administration (FDA) approved clinical-grade pentosan polysulfate sodium.

6.2.4 Pharmacokinetics of PPS

Studies on pentosan polysulfate degradation in human vascular endothelial cells using a radiolabeled drug showed that it was associated with the cellular fraction and incorporated into the subendothelial matrix. Major catabolic products are high-molecular-weight desulfated carbohydrate chains, and it was not degraded further, suggesting the desulfation is the major catabolic step in the catabolism of pentosan polysulfate [71]. Pharmacokinetics of pentosan polysulfate after oral and IV administration of ^{3}H-pentosan polysulfate in rats showed that after IV administration the drug accumulates in connective tissue,

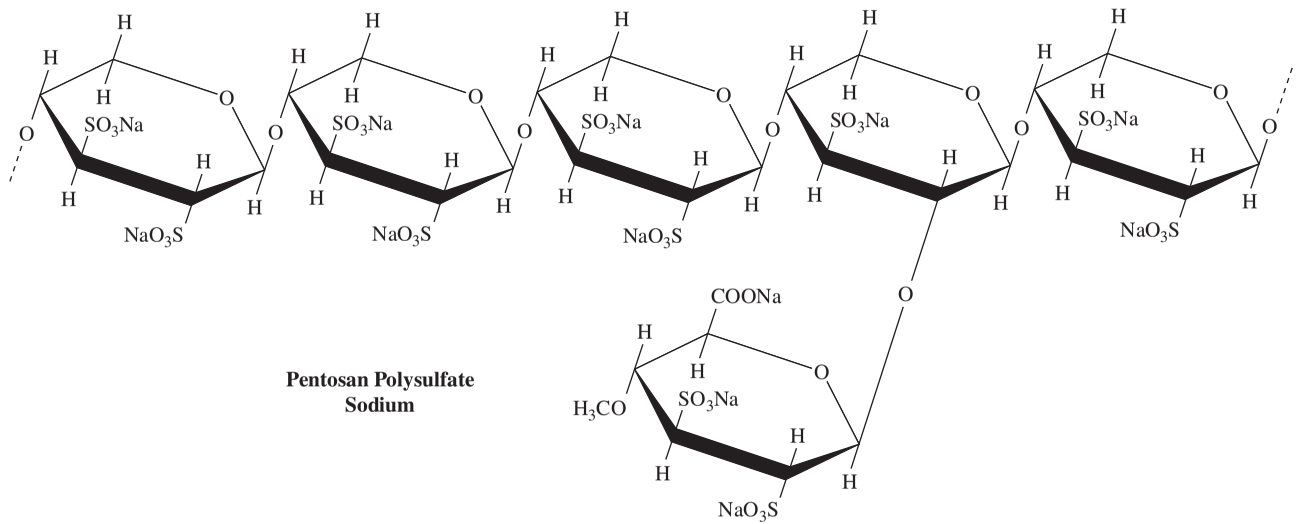


FIGURE 6.9 Structure of pentosan polysulfate sodium.

while bone and cartilage showed low levels. High radioactivity is found in urine and the preferential localization of the drug in the pelvis, ureter, and bladder. A similar but lower distribution was found after oral administration [72].

6.2.5 Clinical Applications of PPS

PPS is structurally and chemically similar to glycosaminoglycans. Hence it mimics some of the pharmacological activities of glycosaminoglycans such as heparin sulfate, and condroitin sulfate such as anticoagulant or fibrinolytic action. Popular brand names of pentosan polysulfates are Elmiron (IVAX Research Inc) in human and Cartrophen (Arthropharm) in veterinary uses.

PPS in Prion Disease. Prion diseases, also called transmissible spongiform encephalopathies, or simply "Scrapie," are a group of progressive conditions that affect the brain and nervous system of animals. It is caused when a normal prion protein PrPC is defectively misfolded into an abnormal conformer PrPSc, also called scrapie protein. The mental and physical abilities deteriorate and tiny holes appear in the brain cortex, causing it to appear like a sponge under a microscope. Pentosan polysulfate given IP was found to prolong the incubation period of the disease in hamsters infected with the 263K strain of scrapie by the intraperitoneal or the intracerebral route [73]. Later it was found that PPS inhibited the association of protease-resistant scrapie protein (PrPres) with sulfated glycosaminoglycans or proteoglycan such as heparin sulfate, which is observed in amyloid plaques of scrapie-infected brain tissue [74]. PPS was found to stimulate endocytosis of the cellular isoform of the prion protein, PrPC, from the cell surface, causing redistribution of the protein from the plasma membrane to the cell interior and change in ultrastructural localization of PrPC, suggesting sulfated glycans inhibit prion production by altering cellular localization of the PrPC precursor and metabolism of PrPC and PrPSc, the scrapie isoform [75] (Fig. 6.10). A recent study in mice showed that treatment with pentosan polysulfate, despite its initial toxic mortality in some mice, had no detectable PrPres in the spleen and increased the lifetime of the treated mice by 185 days than controls [76]. However, the study has suggested that more in vivo animal studies are needed before initiating human trials in treating the disease. (For a review on prion disease see Liberski [77].)

PPS in Amyloid Disease. PPS was reported to be effective in attenuating the neurotoxicity of β-amyloid, fragment 10−40 and fragment 25−35, indicating that the glycosaminoglycan interaction is occurring also with the 25−35 fragment, suggesting an interaction of the sulfated compounds with the β-sheet structure of the amyloid protein [78].

PPS in Cartilage Protection and Osteoarthritis. In an animal study using rabbit, pentosan polysulfate given intraarticular combined with hydrocortisone was found to prevent the hydrocortisone-induced loss of hyaluronic acid and

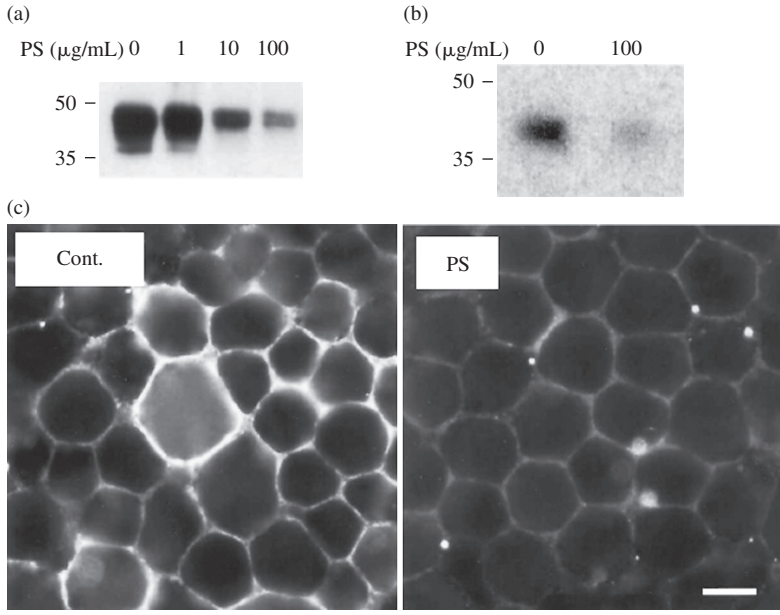


FIGURE 6.10 Pentosan sulfate (PS) reduces the amount of PrP released and amount of cell surface PrP protein. (a) A26 cells were incubated in Opti-MEM containing the indicated concentrations of PS for 12 h. Cells were then treated with phosphatidylinositol-specific phospholipase C (PIPLC) for 2 h at 4°C to cleave cell surface PrP. Proteins were methanol precipitated from the PIPLC incubation medium, and immunoblotted using anti-chPrP antibodies. (b) Untransfected N2a cells were incubated in Opti-MEM in the presence or absence of 100 μg/mL PS for 12 h. Cells were then surface iodinated at 4°C using lactoperoxidase and mouse PrP immunoprecipitated from cell lysates and analyzed by SDS-PAGE. (c) A26 cells were incubated for 12 h at 37°C in Opti-MEM with (PS) or without (Cont.) 0.1 mg/mL pentosan sulfate. They were then rinsed in PBS, incubated with anti-chPrP antibodies at 4°C for 2 h, and fixed for 5 min in methanol at −20°C. Fluorescein isothiocyanate-conjugated secondary antibodies were then applied, and the cells were viewed by fluorescence microscopy. Scale bar = 30 μm. (*Source:* Shyng et al., *J. Biol. Chem.* 1995;**270**:30221–30229.)

proteoglycans from the cartilage of rabbit joints and also prevented the elevation of keratin sulfate in serum to control levels, suggesting the cartilage-protecting effect of pentosan polysulfate [79]. Aggrecan is a proteoglycan and a major structural component of articular cartilage. Its excessive degradation by proteases called aggrecanases is leading to osteoarthritis, a degenerative joint disease. In an experiment using ^{35}S-labeled aggrecan in bovine articular cartilage explant culture, pentosan polysulfate calcium was shown to inhibit the reduction in tissue level of aggrecan stimulated by retinoic acid and recombinant human interleukin-1α, comparable to that of heparin and heparin

sulfate. Also PPS calcium inhibited the degradation of aggrecan by soluble aggrecanase activity and did not adversely affect the chondrocyte metabolism, suggesting potential influence of sulfated glycosaminoglycan on aggrecan catabolism [80]. Another recent study showed that pentosan polysulfate calcium interacts with the noncatalytic spacer domain of ADAMTS-4 and the cysteine-rich domain of ADAMTS-5, the two enzymes responsible for aggrecan breakdown, and blocking the proteolytic activity on their natural substrate aggrecan with an IC_{50} (half maximal inhibitory concentration) value of 10–40 nM but only weakly inhibiting the nonglycosylated recombinant aggrecan. Furthermore, it was shown that the PPS calcium increased the cartilage level of tissue inhibitor of metallo-proteinases-3 (an endogenous inhibitor of ADAMTS-4 and -5), suggesting the potential of calcium pentosan polysulfate as a prototypic disease-modifying agent for osteoarthritis [81]. (For a review on aggrecanases see Fosang et al. [82].)

PPS in Interstitial Cystitis. Interstitial cystitis is a urinary bladder disease of unknown cause characterized by urinary frequency, urgency, pressure, and pain in the bladder and pelvis. Pentosan sulfate sodium (Elmiron) at a dose of 100 mg three times a day for 4 months in a total of 62 patients was reported to show better improvement in pain, urgency, frequency, and nocturia [83]. PPS inhibited histamine secretion from bladder mast cells in a dose-dependent manner and decreased the intracellular calcium levels, suggesting this mechanism for the protective effect of PPS in interstitial cystitis [84]. PPS modified by the attachment with lactose moiety showed better binding with the bladder epithelium than the unmodified PPS, mediated by the interaction of endogenous bladder galactins and nonreducing galactose terminals in the lactose, suggesting an improved adherence of PPS and retention in the bladder for the improved treatment of interstitial cystitis [85]. (For reviews on interstitial cystitis and its treatment with PPS see Bhavanandan et al. [86] and Anderson and Perry [81].)

Anti-HIV Activities of PPS. Pentosan polysulfate was shown to inhibit the reverse transcriptase activity of several retroviruses in vitro, except the bovine leukosis virus that was insensitive, suggesting PPS as one of the most active in vitro inhibitors of retrovirus-specific reverse transcriptases [88]. Furthermore, PPS was reported to be the most potent inhibitor of HIV-1 in vitro with a ED_{50} (effective dose 50%) of 0.19 μg/mL in MT-4 cells, and in HUT-78 cells it inhibited HIV-1 antigen expression with a ED_{50} of 0.02 μg/mL, while showing no toxicity to MT-4 cells upto 2500 μg/mL, suggesting its potent anti-HIV activity in vitro [89] (Table 6.2). Further research showed that PPS and dextran sulfate inhibit the HIV-1 infectivity by blocking the binding of virions to various target $CD4^+$ T lymphocytes and inhibit syncytia formation, suggesting PPS and dextran sulfate as prototype anti-HIV drugs [90]. But later it was found that PPS was not interacting with $CD4^+$ at all and hence must inhibit HIV infection by a totally CD4-independent mechanism [91]. Further research suggested that the antiviral activity of pentosan sulfate and other sulfated polyanions toward HIV

TABLE 6.2 Anti-HIV-1 Activity and Anticoagulant Activity of Polysaccharides

Compound	ED_{50}[a] (μg/mL)	CD_{50}[b] (μg/mL)	Anticoagulant activity (U/mg)
Pentosan polysulfate (MW 3100)	0.19 ± 0.12	>2500	14.4
Fucoidan	1.4 ± 0.43	1060 ± 210	2.6
Dextran sulfate (MW 5000)	0.30 ± 0.10	>2500	14.7
Dextran (MW 90000)	>625	>2500	< 0.01
Heparin (MW 11000)	0.58 ± 0.14	>2500	177
N-desulfated heparin(MW 8800)	>625	>2500	0.6
Dermatan sulfate	>625	>2500	<0.01
Chondroitin sulfate	230 ± 14	>2500	0.5
ι-Carrageenan	12 ± 1.0	>625	3.2
κ-Carrageenan	2.5 ± 0.30	>625	2.9
λ-Carrageenan	0.54 ± 0.02	>625	4.2

[a] 50% Antiviral effective dose, based on the inhibition of HIV-1 induced cytopathogenicity in MT-4 cells. Data represent mean values with standard deviations for three separate experiments.
[b] 50% Cytotoxic dose, based on the reduction of the viability of mock-infected MT-4 cells. Data represent mean values with standard deviations for three separate experiments.

Source: Baba et al., *Antiviral Res.* 1988;**9**:335–343.

infection of lymphocyte might be by disruption of the CD4-gp120 interaction, while their inability to do so in monocytes as a reason for the unaffected HIV infection and gp120 binding by virions in monocytes [92]. Although PPS was seen as a promising candidate for HIV treatment, its further development was superseded by nucleoside-based antiretroviral agents such as zidovudine.

Antiviral Activities of PPS. Concurrent to research on PPS and other sulfated polyanions as anti-HIV drugs, their effect on other viruses was also studied. PPS was shown to inhibit adsorption of African swine fever virus to Vero cells in vitro [93] and to inhibit human cytomegalovirus to human embryonic lung cell in vitro [94]. PPS was also reported to show inhibition of the herpes simplex virus in vitro [95], the sandfly fever Sicilian virus (Phlebovirus) replication in vitro [96], and *Chlamydia trachomatis* infectivity in human epithelial cells in vitro, although it lacked protective efficacy in an in vivo murine model of chlamydial genital tract infection [97]. A recent study showed that PPS inhibited the infectivity of the dengue virus and the encephalitic flavivirus in vitro [98], and active research on PPS for development of possible antiviral agent is evident.

Effect of PPS on Immune System. PPS was reported to have reduced lymph node entry of lymphocytes and increased their blood level in the mouse in vivo, indicating PPS slowed down the circulation of lymphocytes through the marginal zone and red pulp of the spleen, suggesting their role in controlling lymphocyte traffic [99]. Klegeris et al. [100] reported that PPS inhibited complement activation in vitro in a concentration range of 1–1000 μg/mL.

PPS in Cancer. PPS was found to inhibit the proliferation of rabbit aortic smooth muscle cell in vitro. The inhibitory effects of PPS persisted even after removal, suggesting that it exerts the antiproliferative effect by binding to the cell surface and not by interacting with growth factors [101]. Its antitumor activity was evaluated in several animal models and human tumor cell lines owing to the inhibition of basic fibroblast growth factor (bFGF) binding to cell surface receptors and bFGF-stimulated angiogenesis. But a phase I study of PPS sodium in patients with advanced stage metastatic cancers did not show any evidence of objective response [102].

6.2.6 Toxicity of PPS

PPS was reported to induce thrombocytopenia and thrombosis in two patients during treatment with PPS, marked by platelet aggregation and serotonin release, suggesting platelet counts should be monitored periodically during PPS treatment [103].

REFERENCES

1. Ingelman, B. Chemistry of dextrans and some dextran products. *Acta Acad. Regiae Sci. Upsaliensis* 1969;**12**:9–23.
2. Jarl, K. and C. Engblom. Dextran production by microorganisms or enzymes. European Patent, EP 83-850040 19830216, 1983.
3. Hashem, A. M. Utilization of sugar cane molasses for the production of dextran by Leuconostoc mesenteroides. *Bull. Faculty Pharm. (Cairo University)* 1993;**31**:121–124.
4. Day, D. F. and D. Kim. Process for the production of dextran polymers of controlled molecular size and narrow molecular size distributions. U.S. Patent, 91-664755 19910305, 1993.
5. Kim, D. and D. F. Day. Isolation of a dextranase constitutive mutant of Lipomyces starkeyi and its use for the production of clinical size dextran. *Lett. Appl. Microbiol.* 1995;**20**:268–270.
6. Barker, P. E., G. Ganetsos, and N. J. Ajongwen. A novel approach to the production of clinical-grade dextran. *J. Chem. Tech. Biotech.* 1993;**57**:21–26.
7. Kim, D., D. Y. Jhon, K. H. Park, and D. F. Day. Mixed culture fermentation for the production of clinical quality dextran with starch and sucrose. *Biotech. Lett.* 1996;**18**:1031–1034.
8. Chen, S., L. Liu, J. Lu, Z. Han, Y. Xu, and H. Mo. Clinical dextran purified by electric ultrafiltration coupling with solvent crystallization. *Comp. Rend. Chim.* 2008;**11**:80–83.
9. Kim, D., J. F. Robyt, S. Y. Lee, J. H. Lee, and Y. M. Kim. Dextran molecular size and degree of branching as a function of sucrose concentration, pH, and temperature of reaction of Leuconostoc mesenteroides B-512FMCM dextransucrase. *Carbohydr. Res.* 2003;**338**:1183–1189.

10. Neubauer, H., A. Bauche, and B. Mollet. Molecular characterization and expression analysis of the dextransucrase DsrD of Leuconostoc mesenteroides Lcc4 in homologous and heterologous Lactococcus lactis cultures. *Microbiology* 2003;**149**: 973–982.
11. Kakuchi, T., A. Kusuno, M. Miura, and H. Kaga. Cationic ring-opening polymerization of 1,6-anhydro-2,3,4-tri-O-allyl-α-D-glucopyranose as a convenient synthesis of dextran. *Macromol. Rapid Comm.* 2000;**21**:1003–1006.
12. Kusuno, A., T. Kakuchi, M. Miura, and H. Kaga. Facile synthesis of dextran by cationic ring-opening polymerization of 1,6-anhydro-2,3,4-tri-O-allyl-α-D-glucopyranose. *Polym. Prep.* 2000;**41**:146–147.
13. Sgouras, D. and R. Duncan. Methods for the evaluation of biocompatibility of soluble synthetic-polymers which have potential for bio-medical use. 1. Use of the tetrazolium-based colorimetric assay (MTT) as a preliminary screen for evaluation of in vitro cytotoxicity. *J. Mater. Sci.-Mater. Med.* 1990;**1**:61–68.
14. Vercauteren, R., E. Schacht, and R. Duncan. Effect of the chemical modification of dextran on the degradation by rat-liver lysosomal-enzymes. *J. Bioactive Compatible Polym.* 1992;**7**:346–357.
15. Ercan, M. T., M. Schneidereit, R. Senekowitsch, and H. Kriegel. Evaluation of ^{99m}Tc-dextran as a lymphoscintigraphic agent in rabbits. *Eur. J. Nucle. Med.* 1985;**11**:80–84.
16. Salem, C. E., C. K. Hoh, A. M. Wallace, and D. R. Vera. A preclinical study of prostate sentinel lymph node mapping with [^{99m}TC]diethylenetetramine pentaacetic acid-mannosyl-dextran. *J. Urol.* 2006;**175**:744–748.
17. Hifumi, H., S. Yamaoka, A. Tanimoto, D. Citterio, and K. Suzuki. Gadolinium-based hybrid nanoparticles as a positive MR contrast agent. *J. Am. Chem. Soc.* 2006;**128**:15090–15091.
18. Chanimov, M., S. Berman, M. L. Cohen, M. Friedland, J. Weissgarten, Z. Averbukh, M. Herbert, J. Sandbank, Z. Haitov, and M. Bahar. Dextran 40 (Rheomacrodex) or polygeline (Haemaccel) as an epidural patch for post dural puncture headache: a neurotoxicity study in a rat model of Dextran 40 and polygeline injected intrathecally. *Eur. J. Anaesthesiol.* 2006;**23**:776–780.
19. Johansson, H., M. Goto, A. Siegbahn, G. Elgue, O. Korsgren, and B. Nilsson. Low molecular weight dextran sulfate: A strong candidate drug to block IBMIR in clinical islet transplantation. *Am. J. Transplant.* 2006;**6**:305–312.
20. Videm, V. and T. E. Mollnes. Human complement activation by polygeline and dextran 70. *Scand. J. Immunol.* 1994;**39**:314–320.
21. Hahn, R. G. Dextran 70 and blood loss during transurethral resection of the prostate. *Acta Anaesthesiol. Scand.* 1996;**40**:820–823.
22. Strand T. Evaluation of long-term outcome and safety after hemodilution therapy in acute ischemic stroke. *Stroke; J. Cereb. Circul.* 1992;**23**:657–662.
23. Hiroyoshi, K., N. Yasuaki, and O. Yoshitaka. Dextran sulfate as a leukocyte-endothelium adhesion molecule inhibitor of lung injury in pediatric open-heart surgery. *Perfusion* 2005;**20**:77–82.
24. Rosenberg, S. M. and J. A. Board. High-molecular weight dextran in human infertility surgery. *Am. J. Obst. Gynecol.* 1984;**148**:380–385.

25. Roberts, T. S., C. L. Nelson, C. L. Barnes, D. W. Boone, E. J. Ferris, and J. L. Holder. Low dose dextran 40 in reconstructive hip surgery patients. *Orthopedics* 1989;**12**:797–801.
26. Reddy, C. M., S. K. Kathula, S. A. Ali, R. Bekal, and M. Walsh. Safety and efficacy of total dose infusion of iron dextran in iron deficiency anaemia. *Int. J. Clin. Practice* 2008;**62**:413–415.
27. Auerbach, M., M. Chaudhry, H. Goldman, and H. Ballard. Value of methylprednisolone in prevention of the arthralgia-myalgia syndrome associated with the total dose infusion of iron dextran: A double blind randomized trial. *J. Lab. Clin. Med.* 1998;**131**:257–260.
28. Burns, D. L. and J. J. Pomposelli. Toxicity of parenteral iron dextran therapy. *Kidney Int. Suppl.* 1999;**69** (Erythropoietin and Iron):S119–S124.
29. Sav, T., B. Tokgoz, M. H. Sipahioglu, M. Deveci, I. Sari, O. Oymak, and C. Utas. Is there a difference between the allergic potencies of the iron sucrose and low molecular weight iron dextran? *Renal Failure* 2007;**29**:423–426.
30. Feng, W., H. Garrett, D. P. Speert, and M. King. Improved clearability of cystic fibrosis sputum with dextran treatment in vitro. *Am. J. Resp. Crit. Care Med.* 1998;**157**:710–714.
31. Candinas, D., F. Largiader, U. Binswanger, D. E. R. Sutherland, and R. Schlumpf. A novel dextran 40-based preservation solution. *Transplant Int.* 1996;**9**:32–37.
32. Muller, C., H. Furst, H. Reichenspurner, J. Briegel, J. Groh, and B. Reichart. Lung procurement by low-potassium dextran and the effect on preservation injury. *Transplantation* 1999;**68**:1139–1143.
33. Domb, A. J., G. Linden, I. Polacheck, and S. Benita. Synthesis and biological activity of nystatin-dextran conjugates. *Proc. Int. Symp. Control. Release Bioactive Mater.* 1995;**22**:744–745.
34. Tu, J., S. Zhong, and P. Li. Studies on acyclovir-dextran conjugate: Synthesis and pharmacokinetics. *Drug Develop. Indust. Pharm.* 2004;**30**:959–965.
35. Inoue, K., E. Kumazawa, H. Kuga, H. Susaki, N. Masubuchi, and T. Kajimura. CM-dextran-polyalcohol-camptothecin conjugate: DE-310 with a novel carrier system and its preclinical data. *Adv. Exper. Med. Biol.* 2003;**519** (Polymer Drugs in the Clinical Stage):145–153.
36. Chau, Y., F. E. Tan, and R. Langer. Synthesis and characterization of dextran-peptide-methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase II and matrix metalloproteinase IX. *Bioconj. Chem.* 2004;**15**:931–941.
37. Roseeuw, E., V. Coessens, A. Balazuc, M. Lagranderie, P. Chavarot, A. Pessina, M. G. Neri, E. Schacht, G. Marchal, and D. Domurado. Synthesis, degradation, and antimicrobial properties of targeted macromolecular prodrugs of norfloxacin. *Antimicro. Agents Chemother.* 2003;**47**:3435–3441.
38. Chimalakonda, K. C., H. K. Agarwal, A. Kumar, K. Parang, R. Mehvar. Synthesis, analysis, in vitro characterization, and in vivo disposition of a lamivudine-dextran conjugate for selective antiviral delivery to the liver. *Bioconjug. Chem.* 2007;**18**:2097–2108.
39. Vyas, S., P. Trivedi, S. C. Chaturvedi. Ketorolac-dextran conjugates: Synthesis, in vitro and in vivo evaluation. *Acta Pharma.* 2007;**57**:441–450.

40. Kim, S. H., and C. C. Chu. In vitro release behavior of dextran-methacrylate hydrogels using doxorubicin and other model compounds. *J. Biomater. Appl.* 2000;**15**:23–46.

41. Giannuzzo, M., F. Corrente, M. Feeney, L. Paoletti, P. Paolicelli, B. Tita, F. Vitali, and M. A. Casadei. pH-Sensitive hydrogels of dextran: Synthesis, characterization and in vivo studies. *J. Drug Target.* 2008;**16**:649–659.

42. Senthilkumar, K. S., K. S. Saravanan, G. Chandra, K. M. Sindhu, A. Jayakrishnan, and K. P. Mohanakumar. Unilateral implantation of dopamine-loaded biodegradable hydrogel in the striatum attenuates motor abnormalities in the 6-hydroxydopamine model of hemi-parkinsonism. *Behav. Brain Res.* 2007;**184**: 11–18.

43. Larionova, N. I., N. A. Moroz, N. G. Balabushevitch, and O. V. Polekhina. Carbohydrate-containing derivatives of aprotinin for receptor-mediated targeting and longevity. *Proc. Int. Symp. Control. Release Bioactive Mater.* 1996;**23**: 879–880.

44. Zhao, Q., E. Blomquist, H. Bolander, L. Gedda, P. Hartvig, J. C. Janson, H. Lundqvist, H. Mellstedt, S. Nilsson, M. Nister, A. Sundin, V. Tolmachev, J. E. Westlin, and J. Carlsson. Conjugate chemistry, iodination and cellular binding of mEGF-dextran-tyrosine: preclinical tests in preparation for clinical trials. *Int. J. Mol. Med.* 1998;**1**:693–702.

45. Gregory, L. G., R. P. Harbottle, L. Lawrence, H. J. Knapton, M. Themis, and C. Coutelle. Enhancement of adenovirus-mediated gene transfer to the airways by DEAE dextran and sodium caprate in vivo. *Mol. Therapy* 2003;**7**:19–26.

46. Anchordoquy, T.J., T. K. Armstrong, and M. D. C. Molina. Low molecular weight dextrans stabilize nonviral vectors during lyophilization at low osmolalities: Concentrating suspensions by rehydration to reduced volumes. *J. Pharma. Sci.* 2005;**94**:1226–1236.

47. Mitsuya, H., D. J. Looney, S. Kuno, R. Ueno, F. Wong-Staal, and S. Broder. Dextran sulfate suppression of viruses in the HIV family: Inhibition of virion binding to CD4+ cells. *Science* 1988;**240**:646–649.

48. Abrams, D. I., S. Kuno, R. Wong, K. Jeffords, M. Nash, J. B. Molaghan, R. Gorter, and R. Ueno. Oral dextran sulfate (UA001) in the treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Ann. Int. Med.* 1989;**110**:183–188.

49. Hartman, N. R., D. G. Johns, and H. Mitsuya. Pharmacokinetic analysis of dextran sulfate in rats as pertains to its clinical usefulness for therapy of HIV infection. *AIDS Res. Human Retrovir.* 1990;**6**:805–812.

50. Busso, M. E. and L. Resnick. Anti-human immunodeficiency virus effects of dextran sulfate are strain dependent and synergistic or antagonistic when dextran sulfate is given in combination with dideoxynucleosides. *Antimicrob. Agents Chemother.* 1990;**34**:1991–1995.

51. Pearce-Pratt, R. and D. M. Phillips. Sulfated polysaccharides inhibit lymphocyte-to-epithelial transmission of human immunodeficiency virus-1. *Biol. Reproduc.* 1996;**54**:173–182.

52. Suemasu, K. and S. Ishikawa. Inhibitive effect of heparin and dextran sulfate on experimental pulmonary metastases. *Gann* 1970;**61**:125–130.

53. Tsang, K. S., C. K. Li, A. P. Wong, Y. Leung, T. T. Lau, K. Li, M. M. Shing, K. W. Chik, and P. M. Yuen. Processing of major ABO-incompatible bone marrow for transplantation by using dextran sedimentation. *Transfusion* 1999;**39**: 1212–1219.

54. Hagiwara, A., C. Sakakura, M. Shirasu, T. Togawa, Y. Sonoyama, J. Fujiyama, Y. Ebihara, T. Itoh, and H. Yamagishi. Intraperitoneal injection of dextran sulfate as an anti-adherent drug for the prevention of peritoneal metastasis of cancer shows low toxicity in animals. *Anti-Cancer Drugs* 2000;**11**:393–399.

55. Zhai, Y., X. Wang, X. Wang, H. Xie, and H. Gu. Acute toxicity and irritation of water-based dextran-coated magnetic fluid injected in mice. *J. Biomed. Mater. Res., Part A* 2008;**85A**:582–587.

56. Zhao, Y.H., Y. G. Zou, Q. J. Sun, D. Xi, and C. Y. Xing. Treatment of hyperlipidemia with a modified low density lipoprotein apheresis system with dextran sulfate. *Therap. Apher. Dialy*. 2007;**11**:249–254.

57. Magyar, D. M., M. F. Hayes, N. J. Spirtos, M. E. Hull, and K. S. Moghissi. Is intraperitoneal dextran 70 safe for routine gynecologic use? *Am. J. Obst. Gynecol.* 1985;**152**:198–204.

58. Schwarz, J. A., W. Koch, V. Buehler, and S. Kaumeier. Pharmacokinetics of low molecular (monovalent) dextran (Dx 1) in volunteers. *Int. J. Clin. Pharm. Ther. Toxicol.* 1981;**19**:358–367.

59. Ljungstrom, K. G. Safety of dextran in relation to other colloids—ten years experience with hapten inhibition. *Infusionstherap. Transfusion*. 1993;**20**:206–210.

60. Cunnington, P. G., R. M. Blackshaw, and I. K. Sykes. Clinical dextrans lack mitogenic activity for normal human lymphocytes in vitro. *Int. Arch. Aller. Appl. Immunol.* 1980;**63**:195–200.

61. Acharya, S. K., S. Balwinder, A. K. Padhee, S. Nijhawan, and B. N. Tandon. Large volume paracentesis and intravenous dextran to treat tense ascites. *J. Clin. Gastroenterol.* 1992;**14**:31–35.

62. Rothkopf, D. M., B. Chu, S. Bern, and J. W. May, Jr. The effect of dextran on microvascular thrombosis in an experimental rabbit model. *Plastic Reconstruct. Surg*. 1993;**92**:511–515.

63. Hood, S. A., M. O'Brien, and R. Higgins. The safety of intravenous iron dextran (Dexferrum) during hemodialysis in patients with end stage renal disease. *Nephrol. Nurs. J.: J. Am. Nephrol. Nurses' Assoc*. 2000;**27**:41–42.

64. Fletes, R., J. M. Lazarus, J. Gage, and G. M. Chertow. Suspected iron dextran-related adverse drug events in hemodialysis patients. *Am. J. Kidney Dis.: Offic. J. Nat. Kidney Found.* 2001;**37**:743–749.

65. Simmons, C. F., Jr., H. G. Rennke, and H. D. Humes. Acute renal failure induced by diethylaminoethyl dextran: Importance of cationic charge. *Kidney Int.* 1981;**19**:424–430.

66. Berg, E. M., S. Fasting, and O. F. Sellevold. Serious complications with dextran-70 despite hapten prophylaxis. Is it best avoided prior to delivery? *Anaesthesia* 1991;**46**:1033–1035.

67. Kawabata, Y., N. Ando, H. Koshiba, J. Kashihara, T. Sonoda, M. Nohara, C. Wakamatsu, H. Tsubota, K. Munechika, and M. Iwai. Acute hepatotoxicity of

adriamycin-oxidized dextran (ADM-OXD) in rat. *J. Toxicol. Pathol.* 1993;**6** (Suppl.):1–12.

68. Blake, D. R., J. Lunec, M. Ahern, E. F. Ring, J. Bradfield, and J. M. Gutteridge. Effect of intravenous iron dextran on rheumatoid synovitis. *Ann. Rheum. Dis.* 1985;**44**:183–188.
69. Degenhardt, M., P. Ghosh, and H. Wätzig. Studies on the structural variations of pentosan polysulfate sodium (NaPPS) from different sources by capillary electrophoresis. *Arch. Pharma.* 2001;**334**:27–29.
70. Callahan, H. J., D. Shupp-Byrne, M. Pizzo, C. L. Parsons, and S. G. Mulholland. The production of antibodies to pentosan polysulfate (ELMIRON, SP-54). *J. Immunol. Meth.* 1991;**136**:53–59.
71. Dawes, J. and D. S. Pepper. Human vascular endothelial cells catabolize exogenous glycosaminoglycans by a novel route. *Thromb. Haemostasis* 1992;**67**:468–472.
72. Odlind, B., L. Dencker, and A. Tengblad. Preferential localization of ^{3}H-pentosanpolysulfate to the urinary tract in rats. *Pharma. Toxicol.* 1987;**61**:162–166.
73. Ladogana, A., P. Casaccia, L. Ingrosso, M. Cibati, M. Salvatore, Y. G. Xi, C. Masullo, and M. Pocchiari. Sulfate polyanions prolong the incubation period of scrapie-infected hamsters. *J. Gen. Virol.* 1992;**73**:661–665.
74. Caughey, B., K. Brown, G. J. Raymond, G. E. Katzenstein, and W. Thresher. Binding of the protease-sensitive form of prion protein PrP to sulfated glycosaminoglycan and Congo red. *J. Virol.* 1994;**68**:2135–2141.
75. Shyng, S. L., S. Lehmann, K. L. Moulder, and D. A. Harris. Sulfated glycans stimulate endocytosis of the cellular isoform of the prion protein, PrPC, in cultured cells. *J. Biol. Chem.* 1995;**270**:30221–30229.
76. Larramendy-Gozalo, C., A. Barret, E. Daudigeos, E. Mathieu, L. Antonangeli, C. Riffet, E. Petit, D. Papy-Garcia, D. Barritault, P. Brown, and J. P. Deslys. Comparison of CR36, a new heparan mimetic, and pentosan polysulfate in the treatment of prion diseases. *J. Gen. Virol.* 2007;**88**:1062–1067.
77. Liberski, P. P. Prion protein as a target for therapeutic interventions. *Pure Appl. Chem.* 2004;**76**:915–920.
78. Sadler, I. I. J., S. R. Hawtin, V. Tailor, M. S. Shearman, and S. J. Pollack. Glycosaminoglycans and sulfated polyanions attenuate the neurotoxic effects of β-amyloid. *Biochem. Soc. Trans.* 1995;**23**:106S.
79. Kongtawelert, P., P. M. Brooks, and P. Ghosh. Pentosan polysulfate (Cartrophen) prevents the hydrocortisone-induced loss of hyaluronic acid and proteoglycans from cartilage of rabbit joints as well as normalizes the keratan sulfate levels in their serum. *J. Rheumatol.* 1989;**16**:1454–1459.
80. Munteanu, S. E., M. Z. Ilic, and C. J. Handley. Highly sulfated glycosaminoglycans inhibit aggrecanase degradation of aggrecan by bovine articular cartilage explant cultures. *Matrix Biol.* 2002;**21**:429–440.
81. Troeberg, L., K. Fushimi, R. Khokha, H. Emonard, P. Ghosh, and H. Nagase. Calcium pentosan polysulfate is a multifaceted exosite inhibitor of aggrecanases. *FASEB J.* 2008;**22**:3515–3524.
82. Fosang, A. J., and C. B. Little. Drug insight: Aggrecanases as therapeutic targets for osteoarthritis. *Nature Clin. Practice Rheumatol.* 2008;**4**:420–427.
83. Parsons, C. L. and S. G. Mulholland. Successful therapy of interstitial cystitis with pentosanpolysulfate. *J. Urol.* 1987;**138**:513–516.

84. Chiang, G., P. Patra, R. Letourneau, S. Jeudy, W. Boucher, M. Green, G. R. Sant, and T. C. Theoharides. Pentosanpolysulfate inhibits mast cell histamine secretion and intracellular calcium ion levels: An alternative explanation of its beneficial effect in interstitial cystitis. *J. Urol.* 2000;**164**:2119–2125.

85. Muthusamy, A., D. R. Erickson, M. Sheykhnazari, and V. P. Bhavanandan. Enhanced binding of modified pentosan polysulfate and heparin to bladder--a strategy for improved treatment of interstitial cystitis. *Urology* 2006;**67**:209–213.

86. Bhavanandan, V. P., D. R. Erickson, N. Herb, M. Sheykhnazari, and S. Ordille. Use of glycosaminoglycans in the treatment of interstitial cystitis: A strategy to improve efficacy. *Int. Cong. Series* 2001;**1223** (New Developments in Glycomedicine):227–237.

87. Anderson, V. R. and C. M. Perry. Pentosan polysulfate: A review of its use in the relief of bladder pain or discomfort in interstitial cystitis. *Drugs* 2006; **66**:821–835.

88. Sydow, G., R. Kloecking, and H. P. Kloecking. Effect of pentosan polysulfate (SP 54) on the reverse transcriptase activity of several retroviruses. *Biomed. Biochim. Acta* 1987;**46**:527–530.

89. Baba, M., M. Nakajima, D. Schols, R. Pauwels, J. Balzarini, and E. De Clercq. Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent in vitro. *Antiviral Res.* 1988;**9**:335–343.

90. Mitsuya, H., D. J. Looney, S. Kuno, R. Ueno, F. Wong-Staal, and S. Broder. Inhibition of virion binding to CD4+ cells: Suppression of human immunodeficiency viruses by anionic polysaccharides. *UCLA Symp. Mol. Cell. Biol., New Series* 1989;**100**(Mech. Action Ther. Appl. Biol. Cancer Immune Defic. Disord.):331–341.

91. Parish, C. R., L. Low, H. S. Warren, and A. L. Cunningham. A polyanion binding site on the CD4 molecule. Proximity to the HIV-gp120 binding region. *J. Immunol.* 1990;**145**:1188–1195.

92. Lynch, G., L. Low, S. Li, A. Sloane, S. Adams, C. Parish, B. Kemp, and A. L. Cunningham. Sulfated polyanions prevent HIV infection of lymphocytes by disruption of the CD4-gp120 interaction, but do not inhibit monocyte infection. *J. Leukocyte Biol.* 1994;**56**:266–272.

93. Garcia-Villalon, D. and C. Gil-Fernandez. Antiviral activity of sulfated polysaccharides against African swine fever virus. *Antiviral Res.* 1991;**15**:139–148.

94. Neyts, J., R. Snoeck, D. Schols, J. Balzarini, J. D. Esko, A. Van Schepdael, and E. De Clercq. Sulfated polymers inhibit the interaction of human cytomegalovirus with cell surface heparan sulfate. *Virology* 1992;**189**:48–58.

95. Andrei, G., R. Snoeck, P. Goubau, J. Desmyter, and E. De Clercq. Comparative activity of various compounds against clinical strains of herpes simplex virus. *Eur. J. Clin. Microbiol. Infect. Dis.* 1992;**11**:143–151.

96. Crance, J. M., D. Gratier, J. Guimet, and A. Jouan. Inhibition of sandfly fever Sicilian virus (Phlebovirus) replication in vitro by antiviral compounds. *Res. Virol.* 1997;**148** :353–365.

97. Su, H. and H. D. Caldwell. Sulfated polysaccharides and a synthetic sulfated polymer are potent inhibitors of Chlamydia trachomatis infectivity in vitro but lack protective efficacy in an in vivo murine model of chlamydial genital tract infection. *Infect. Immun.* 1998;**66**:1258–1260.

98. Lee, E., M. Pavy, N. Young, C. Freeman, and M. Lobigs. Antiviral effect of the heparan sulfate mimetic, PI-88, against dengue and encephalitic flaviviruses. *Antiviral Res*. 2006;**69**:31–38.

99. Freitas, A. A. and M. De Sousa. Control mechanism of lymphocyte traffic. A study of the action of two sulfated polysaccharides on the distribution of ^{51}Cr- and [^{3}H]-adenosine-labeled mouse lymph node cells. *Cell. Immunol*. 1977;**31**:62–76.

100. Klegeris, A., E. A. Singh, and P. L. McGeer. Effects of C-reactive protein and pentosan polysulphate on human complement activation. *Immunology* 2002;**106**:381–388.

101. Paul, R., J. M. Herbert, J. P. Maffrand, J. Lansen, G. Modat, J. M. Pereillo, and J. L. Gordon. Inhibition of vascular smooth muscle cell proliferation in culture by pentosan polysulfate and related compounds. *Thrombosis Res*. 1987;**46**:793–801.

102. Lush, R. M., W. D. Figg, J. M. Pluda, R. Bitton, D. Headlee, D. Kohler, E. Reed, O. Sartor, and M. R. Cooper. A phase I study of pentosan polysulfate sodium in patients with advanced malignancies. *Ann. Oncol*. 1996;**7**:939–944.

103. Tardy-Poncet, B., B. Tardy, F. Grelac, J. Reynaud, P. Mismetti, J. C. Bertrand, and D. Guyotat. Pentosan polysulfate-induced thrombocytopenia and thrombosis. *Am. J. Hematol*. 1994;**45**:252–257.

CHAPTER 7

ARABINOGALACTAN IN CLINICAL USE

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7.1 INTRODUCTION

Polysaccharides are the most abundant natural products produced by plants. Polysaccharide materials have been used for various purposes since time immemorial: wood for housing and fire, vegetables, fruits, and seeds for food, fibers for clothing, paper production, and for curing various diseases. Earlier, the low-molecular-weight plant compounds were used in pharmacy and medicine. It was also believed that only substances exhibiting biological activity are able to cure diseases. Polysaccharides are recognized as food; the fibrous polysaccharides were particularly appreciated by modern society in our diet. They reduce blood cholesterol level and regulate the blood flow through the body. They reduce the glucose uptake from the intestines and also reduce the uptake of mutagens from the food we eat. Other parts of plants are rich in polysaccharides and provide different types of pharmaceutical usages over the years and still do.

Some of them are used as:

- Thickeners and stabilizers of solutions
- Filling material, adhesives, and swelling agents in tablet production
- Lubricants in cough mixtures

Polysaccharide-based therapeutics were used to control various disorders, including cancer, viral infections, and immune dysfunctions. Long-chain polysaccharides have been used to cure different diseases. Various herb plants were used in traditional medicine in different parts of the world. One such natural plant-derived polysaccharide exhibiting potent biological activity is the arabinogalactan [1].

Clinically used high-grade larch arabinogalactan is composed of 98% arabinogalactan. It is a dry, free-flowing powder, with a slight pinelike odor and sweetish in taste. It is 100% water soluble and produces low-viscosity solutions. Since it is soluble in water and having a mild taste, It's powder mites readily in water and juices for any administration. A naturopathic physician,

Peter D'Adamo was the first to introduce larch arabinogalactan into clinical practice.

Some common important features of arabinogalactan type I and II are:

- They have a high degree branching backbone.
- They have highly branched side chains.

These features help to make the molecules of the backbone reasonably hard with side chains giving an overall shape and a surface for the receptors involved in the biological systems studied so far. More than one binding site is necessary for the activity as well as when immunoglobulins are involved. Few polysaccharides have been studied in detail with regard to the important structural features responsible for their biological activity. More energy and involvement should be put into polysaccharide research. Research in various polysaccharides could lead to new carbohydrate-based drugs with few side effects [1].

7.1.1 Occurrence

Arabinogalactans (AG) is a long densely branched polysaccharide. In nature it is found in different plants such as leek seeds, carrots, radish, black gram beans, pear, maize, wheat, red wine, Italian ryegrass, tomatoes, ragweed, sorghum, bamboo grass, coconut meat and milk, and several microbial systems especially acid-fast mycobacteria. Arabinogalactans are a class of polysaccharides found in a wide range of plants. They are abundantly found in plants of the genus *Larix* [2]. Biological activity, immune-enhancing properties, peculiar solution properties of this unique dietary fiber received increasing attention as a clinically useful nutraceutical agent. Although the larch arabinogalactan is extracted from either western larch or mongolian larch, their primary source is the larch tree (*Larix* sp.). Commercially available arabinogalactan is produced from the western larch.

Biologically active arabinogalactan was first isolated from the roots of *Angelica acutiloba* [3]. This was called AGIIa, which activates the complement system. Later various plants (*Coix lacryma-jobi* var. *Ma-yuen*, *Viscum album*, *Calendula officinalis*, *Malva verticillata*, *Arnica montana*, *Plantago major*, and *Atractylodes lancea*) showed the presence of biologically active arabinogalactans [4, 5]. Many edible and inedible plants are rich sources of arabinogalactan that occur in the form of glycoconjugates—glycoproteins and proteoglycans, the arabinogalactan proteins (AGPs).

7.1.2 Strucure

Arabinogalactans are in the class of branched polysaccharides that have molecular weights ranging from 10,000 to 120,000 [6]. Based on their structure, arabinogalactans fall into two groups, namely arabinogalactan type I and type II. The important structural difference between these two types of arabinogalactans is the

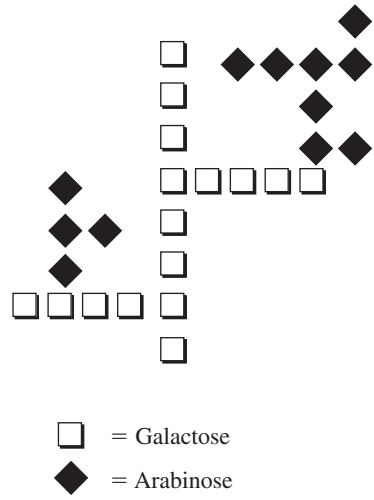

FIGURE 7.1 Model arabinogalactan II chain. (From [1].)

galactose units, which are (1→4) linked in type I and (1→3) and (1→6) linked in type II. Most of the arabinogalactans involved in biological activity belong to the arabinogalactan type II [1].

One of the methods used to distinguish between these two types of arabinogalactans is their precipitation in the Yariv reagent. Only AGII form a red precipitate with the Yariv reagent. Yariv reagent is frequently used to confirm the presence of AGII in bioactive polymers and also used for the quantitative estimation of such polymers (Fig. 7.1).

7.1.3 Biochemistry

In nature, arabinogalactans are found in several microbial systems, especially acid-fast mycobacteria, where it is complexed between peptidoglycans and mycolic acids as a component of the cell wall and influences monocyte–macrophage immuno reactivity of tubercular antigen [7]. Edible and nonedible oil plants are the rich sources of arabinogalactans, in glycoprotein form, bound to a protein spine of either threonine, proline, or serine. These plants include leek seeds, carrots, radish, black gram beans, pear, maize, wheat, red wine, Italian ryegrass, tomatoes, ragweed, sorghum, bamboo grass, and coconut meat and milk. Herbs such as *Echinacea purpurea*, *Baptisia tinctoria*, *Thuja occidentalis* [8], *Angelica acutiloba* [9], and *Curcuma longa* [10] also contain significant amounts of arabinogalactans with well-established immune-enhancing properties (Table 7.1).

TABLE 7.1 Properties of Arabinogalactan Computed from Structure

Molecular weight	500.49144 (g/mol)
Molecular formula	$C_{20}H_{36}O_{14}$
XlogP3AA	−4.2
H bond donor	7
H bond accepter	14
Rotatable bond count	8
Exact mass	500.210506
Monoisotopic mass	500.210506
Topological polar surface area	206
Heavy atom count	34
Formal charge	0
Complexity	626
Isotope atom count	0
Defined atom stereocenter count	0
Undefined atom stereocenter count	14
Defined bond stereocenter count	0
Undefined bond stereocenter count	0
Covalently bonded unit count	1

Source: NCBI PUBCHEM.

7.1.4 Chemical Nature

Arabinogalactans isolated from *Larix* species are readily water soluble. They are nitrogen-free polysaccharides of the 3,6-β-D-galactan types. Experimental analysis show they are highly branched molecules with a 3,6-galactan backbone. Side chains consist of combinations of single galactose sugars, and longer side chains comprised of 3-, 4-, 6-; 2-, 3-, 6-; 3-, 6-; 3-, 4-; and 3-linked β-galactose and β-arabinose residues. The galactose and arabinose units (consisting of β-galactopyranose, β-arabinofuranose, and β-arabinopyranose) are in a molar ratio of approximately 6 : 1 and comprise more than 99% of the total glycosyl content. A trace amount of glucuronic acid is also found with them [2, 11].

7.1.5 Pharmacokinetics

Pharmacokinetics of oral arabinogalactan has not been well elaborated in humans. The absolute concentration of orally absorbed arabinogalactan is unclear; however, nonabsorbed arabinogalactan is fermented vigorously by gastrointestinal microflora [12]. Injected arabinogalactan in animal models is cleared with a half-life of 3.8 min from blood. After 90 min of injection, the concentrations found in liver is highest (52.5%) and in urine (30%), with a

hepatic clearance following first-order kinetics with a half-life of 3.42 days [11]. Arabinogalactans from western larch extracted bind in vitro *and* in vivo with liver asialoglycoprotein receptors. On reaching to the liver via portal circulation arabinogalactan is rapid and specifically internalized within hepatocytes by receptor-mediated endocytosis. A higher percentage of arabinogalactan in liver suggested an ideal carrier for drug delivery to the liver [11].

7.2 NATURAL KILLER (NK) CYTOTOXICITY

Human NK cytotoxicity is "the ability of spontaneous cytotoxicity against a variety of tumor cells and virus-infected cells without prior sensitization by antigen and restriction by products of the major histocompatibility gene complex" [6]. Several new adjuvant approaches to cancer treatment include the use of biological response modifiers to modulate immune function. The most promising approach to immune modulation is the utilization of biotherapeutic agents for natural killer cell cytotoxicity.

In general, NK cell activity is an exceptional functional marker of health. The literature reports indicate that decreased NK cell activity is related to the variety of chronic diseases including cancer [13], chronic fatigue syndrome (CFS) [14], autoimmune diseases such as multiple sclerosis [15], and viral hepatitis [16]. In CFS NK cell activity restoration is associated with clinical recovery [17], whereas in multiple sclerosis a relationship between NK cell activity reductions and the development of active lesions has been reported [15]. In prostate cancer, changes in NK activity were associated with both the metastasis and tumor response to therapy. In fact, it was reliable as the specific tumor markers reflect prostate cancer [18]. Some plant origin oligo- or polysaccharide NK cell activity inducers enhance human NK cytotoxicity against cancer cells. The rhamnogalacturonan from mistletoe (*V. album*) is an example of a plant saccharide with known NK cytotoxicity-enhancing properties [19, 20]. Arabinogalactans that stimulate the NK cell cytotoxicity against K562 tumor cells in cell cultures have been investigated. Under experimental conditions, human peripheral blood mononuclear cell cultures, preseparated, peripheral nonadherent cell cultures, and monocytes showed the enhancement of natural killer cytotoxicity against K562 tumor cells on pretreatment with arabinogalactan for 2–3 days. Arabinogalactan-mediated NK cytotoxicity is not directly initiated but the cytokine network governed it.

Generally, arabinogalactan increased the release of interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1-β), and interleukin-6 (IL-6) before treatment. However, the increase in IFN-γ is responsible for the enhancement of NK cytotoxicity [6]. Initial observations showed that the arabinogalactan interacted with the same receptor for an NK-cytotoxicity-enhancing oligosaccharide from *V. album*. No synergism in the NK-cytotoxicity-stimulating activity was found with *V. album* oligosaccharide and arabinogalactan in combination. Both promote NK cytotoxicity; however, neither component added

a significant effect on NK cytotoxicity induced by the other compound. As compared to the other oligosaccharides, arabinogalactan consistently demonstrated a greater ability to induce IFN-γ. A cell culture study was also conducted on healthy individuals. Although all the individuals did not respond to NK cytotoxicity stimulation by arabinogalactan, 63% of responded, out of which 33% showed a high response, that is, double the NK cytotoxicity after pretreatment with arabinogalactan [21].

Spontaneous cytotoxicity of donors prior to the treatment was unpredictable. However, the arabinogalactan pretreatment response was also variable. More predictable response might be obtained if the studies had consisted of individuals with a variety of chronic diseases. In general, one would expect these individuals to have a lower initial NK cytotoxicity.

7.3 ARABINOGALACTAN PROTEINS (AGPS)

Arabinogalactan proteins (AGPs) are highly glycosylated hydroxyproline-rich glycoprotiens (HRGPs) analogous to animal proteoglycans. They are widely distributed in each cell of every plant. These proteoglycans are mainly present at cell surfaces and play an important role in plant growth and development. AGPs can be detected in different plant organs by staining with the synthetic phenylglycoside known as Yariv reagent, which binds to and precipitate AGPs [22, 23].

AGPs are highly heterogeneous in their size range and reactivity with anti-AGP monoclonal antibodies. Each organ and cell is associated with a specific subset of AGPs. The AGP molecule is enclosed in 95% by weight of carbohydrate such as galactopyranose and arabinofurinose residues. The sugar groups are O-linked to hydroxy amino acids in the core protein, which is usually rich in Ser, Ala, Gly, and Hyp residues [24]. AGPs are mainly divided into two classes depending on their core protein: classical and nonclassical AGPs. Classical AGPs are core proteins containing Hyp, Ala, Ser, Thr, and Gly as the major amino acid constituents, whereas nonclassical AGPs have carbohydrate moieties attached to the core proteins, for example, Hyp-poor AGPs, Cys-rich AGPs, and Asn-rich AGPs [23].

7.3.1 Molecular Shape and Aggregation of AGPs

The transmission electron microscopic (TEM) image of AGPs suggests some AGPs are globular whereas others are rod shaped. Two models for AGP structure are recognized on the basis of their shapes. One is the "wattle blossom" model and the other is the "twisted hairy rope" model. In the wattle blossom model, the polysaccharide chains are folded into globular units to decorate the core proteins in a spheroidal shape, whereas in the twisted hairy rope model, the polysaccharide chains and oligoarabinosides wrap around the rodlike core protein. These wattle blossom and twisted having rare models, may prove the different molecular shapes of AGP's. Hyp-poor carrot AGP and transmitting

tissue specific proline rich proteins (TTS proteins) appeared spheroidal, whereas gum arabic AGP appeared rodlike. Moreover these models will also modify. For example, oligoarabinoside chains may exist along with polysaccharide chains, as predicted by the Hyp-contiguity hypothesis, within the general content of the wattle blossom model. AGPs generally aggregate in vitro and sometime in vivo as well. Such aggregations were observed in Hyp-poor carrot AGP and TTS proteins through electron microscopy. These self-associations are consistent with the adhesive behavior of AGPs, related to its crosslinking [24].

7.3.2 Commercial Applications of AGPs

In addition to the several functions of AGPs in plants, they have a commercial importance also. AGPs are significant components of plant gums or exudates and confer special properties of the plant products. For example, gum arabic has been harvested from wounded *Acacia senegal* trees, which represents one of the most commercially important gums. The gum arabic ability is used to suspend flavorings and colorings with low viscosity to make it a valuable additive in the food industry. Moreover, gum arabic is also used in the candy industry to lower the hardening process in the manufacture of hard candy and as an adhesive in the stamp industry. A major question, which still remains unsolved, is the extent to which AGPs in arabic gum are responsible for these remarkable properties. AGPs and type II arabinogalactans may be used in medicine. Both of these molecules reportedly stimulate animal immune systems, in some cases by activating the complement system and by enhancing the cytotoxic activity of natural killer cells (Table 7.2) [24].

7.4 MEDICINAL APPLICATIONS

Some of the important medicinal applications of arabinogalactan are discussed in the following sections.

7.4.1 Arbinogalactan: A Potential MRI Agent

In clinical medicine, magnetic resonance imaging (MRI) is used as a diagnostic technique. This technique allows researchers and doctors to image the body in a noninvasive manner [25]. Administration of contrast agents enhances the image quality by decreasing the relaxation time of the tissue water. Thus, it is much more conspicuous than the surrounding tissues. At present, more than 35% of all MRI examinations are accompanied by the administration of contrast agents [26]. Until now, four kinds of gadolinium complexes (gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA), gadolinium diethylenetriaminepentaacetic acid bismethylamide (Gd-DTPA-BMA), gadolinium-1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (Gd-DOTA), and 1, 4, 7-tris[carboxymethyl]-10-[2′hydroxypropyl]-1,4,7,10-tetraazacyclododecane (Gd-HP-DO3A)) have been

TABLE 7.2 Developmentally Regulated Expression of AGP Epitopes as Revealed by a Variety Antibodies

Species	Organ/Tissue	Antibody	Labeling[a]	Cell type(s)	Localization
Daucus carota		MAC207	IF	Protoplasts	Plasma membrane
Beta vulgaris		MAC207	IF	Protoplasts	Plasma membrane
Daucus carota	Root	MAC207	IF	Cortex	Cell surface
Pisum sativum	Root	MAC207	IF	Cortex	Cell surface
Allium cepa	Root	MAC207	IG	Cortex	Plasma membrane
Cucurbita pepo	Hypocotyl	MAC207	IG	Mesophyll cell	Plasma membrane
Beta vulgaris	Anther	MAC207	IG	Pollen vegetative cell	Plasma membrane
Daucus carota		MAC207	IG	Cultured cells	Plasma membrane
Hyacinthoides non-scriptus	Leaf	MAC207	IG	Mesophyll cell	Plasma membrane
Daucus carota	Root	JIM4	IF	Pericycle cells	Cell surface
Pisum sativum	Embryogenic apex (meristems, primordia)	MAC207	IF	Cells of future stamen filaments, petal primordia, outer pollen sac boundary	Plasma membrane
	Stamen	MAC207	IF	Pollen vegetative cell	Plasma membrane
	Carpel	MAC207	IF	Cells of integuments and heart- and torpedo-stage embryos	Plasma membrane
Daucus carota	Hypocotyl	JIM4	IF	Epidermal cells and vascular cylinder cells	Cell surface
	Preembryogenic mases (PEMs)	JIM4	IF	Surface cells	Cell surface
	PEM embryos:				
	Globular stage	JIM4	IF	Surface layers center on the shoot end	Cell surface
	Early heart stage	JIM4	IF	Two groups of internal cells (reflect cotyledonary ridges)	Cell surface
	Late heart stage	JIM4	IF		Cell surface

(*Continued*)

TABLE 7.2 (Continued)

Species	Organ/Tissue	Antibody	Labeling[a]	Cell type(s)	Localization
				Epidermal cells at the shoot apical end	
	Early torpedo stage	JIM4	IF	Provascular tissue cells	Cell surface
	Late torpedo stage	JIM4	IF	Provascular tissue cells, isolated epidermal cells and future shoot apex cells	Cell surface
Daucus carota		JIM8	IF	Protoplasts	Plasma membrane
Brassica napus	Anther	JIM8	IF	Endothecium, middle layer, tapetum, microspore tetrads, vegetative cells and sperm cells	Plasma membrane
	Ovule	JIM8	IF	Nucellus, synergid cells and egg cell	Plasma membrane
	Embryo	JIM8	IF	Zygote, embryo proper and suspensor cells	Plasma membrane
	Anthers	JIM8	IG	Vegetative cell and sperm cell is pollen	Outer face of the plasma membrane
Daucus carola		MAC207 and JIM4	IF	Plasmolysed cultured cells	Outer face of the plasma membrane
	Root	JIM4	IF	Future pericycle cell	Cell surface
	Root	JIM13	IF	Epidermal cells, developing xylem elements, root cap cells	Cell surface
	Root	JIM14	IF	All cells	Cell surface
	Root	JIM15	IF		Cell surface

				All cells except epidermal and future xylem cells	
	Root	JIM16	IF	All cells	Cell surface
		MAC207	IG	Plasmolysed	Outer face of the plasma membrane
				Cultured cells	
		JIM4	IG	Cultured cells	Cell wall and plasma membrane
Nicotiana tabacton	Flowers	JIM8 and MAC207	IG	Pollen grains	Intine, cytoplasm including vesicles. Golgi apparatus
	Flowers	JIM8 and MAC207	IG	Generative cell	Cell wall and cytoplasm including vesicles
	Flowers	JIM8 and MAC207	IG	Pollen tube	Periodically along the outer wall of the tube except the tip
Arabidopsis thaliana	Root	JIM13	IF	Initial of cental metaxylem vessels other pre-metaxylem elements, parenchyma cells, endodermal and pericycle cells)	Cell surface
	Root	JIM13	IG	Differentiating metaxylem vessel elements	Outer face of plasma membrane and cell wall
Zea mays	Coleoptiles	MAC207	IG	All cells	Plasma membrane
	Coleoptiles	JIM13	IG	Maturing sclerenchyma cells	Plasma membrane Invaginations. Multivesicular bodies
				Differentiating tracheid cells	Secondary wall thickenings

(*Continued*)

TABLE 7.2 (Continued)

Species	Organ/Tissue	Antibody	Labeling[a]	Cell type(s)	Localization
	Coleoptiles	JIM14	IG	Maturing sclerenchyma cells	Innermost wall layer
Daucus carota		JIM8	IF	Cultured cells	Cell suface
Arabidopsis thaliana	Root	CCRC-M7	IF	Columellar root cap cells epidermal cells, cortical, endodermal and pericycle cells	Cell suface
	Root	CCRC-M7	IG	Endodermal, pericycle and phloem cells	Cell wall
Lilium longiflorum	Flowers	JIM13	IF	Pollen tube	Tube tip
		JIM13	IF	Stylar transmitting tract epidermal cells (TTEs)	Cell suface
	Flowers	LM2	IF	All cells in the style except the TTEs	Cell suface
	Flowers	JIM13	IG	Pollen tube	Cell wall, plasma membrane and cytoplasmic vesicles
	Flowers	JIM13	IG	Generative cell	Plasma membrane
Lilium longifiorum	Flowers	JIM13	IG	(β-D-glucosyl), Yariv reagent-teated pollen tubes	Secretory vesicles, plasmalemma, electron-translucent areas within the expanded periplasm

Amaranthus hypochondriacus	Young flowers	MAC207	IF	All cells but nuclear cells	Cell suface
	Young flowers	JIM8	IF	All cells with nucellus selectively labeled on the micropylar cells	Cell suface
	Ovule with mature embryo sac	JIM8	IF	Synergid cells with filiform apparatus, integument and micropylar nucellus cells	Cell suface
	Ovule with mature embryo sac	MAC207	IF	Micropylar nucellus cells, filiform apparatus and integument cells	Cell suface
	Ovule with young embryo	JIM8	IF	Embryo proper, suspensor cells and filiform apparatus	Cell suface
	Ovule with young embryo	MAC207	IF	Filiform apparatus	Cell suface
	Ovule with young globular embryo	JIM8	IF	Micropylar nucellus cells, embryo proper, suspensor and integument cells	Cell suface

Source: From [24].

used worldwide for intravenous administration. These small molecules enhance the imaging of the brain and the central nervous system. However, these small hydrophilic complexes are nonspecific extracellular contrast agents that are excreted quickly via the kidneys. It may restrict their use in other parts of the body. Hence, there is a need to investigate certain agents that target the specific organs, regions of the body, or diseased tissue to gain the greatest diagnostic value [27–29]. Recently, liver-specific contrasting agents with high relaxivities and kinetic stability have been developed [30, 31]. Some of them enter to hepatocytes through hepatic asialoglycoprotein receptor (ASGP-R), an organ-specific lectin [32].

Polysaccharides and their derivatives have been known as carriers to deliver drugs to hepatocytes via this receptor [33]. Plant polysaccharide arabinogalactan is specifically absorbed by hepatocytes via the ASGP-R. Numerous terminal galactose residues and a high degree of branching of arabinogalactan are responsible for its bindings to the ASGP-R. A spin-labeled arabinogalactan and arabinogalactan-stabilized small paramagnetic iron oxide (AG-USPIO) are reported as liver-specific agents for MRI by targeting hepatocyte ASGP-R (Fig. 7.2) [34].

Overall, the synthesis and characterization of new macromolecular conjugates consisting of arabinogalactan and Gd-DTPA with higher T1 relaxivity as MRI contrast agents has been demonstrated. MR imaging showed that signal intensities from the livers of healthy rats injected with lower doses of Gd-DTPA-CMAG-A2 were remarkably enhanced for a longer period. An in vivo and in vitro study has suggested that Gd-DTPA-CMAG-A2 could be a potential liver-specific MRI contrast agent [34].

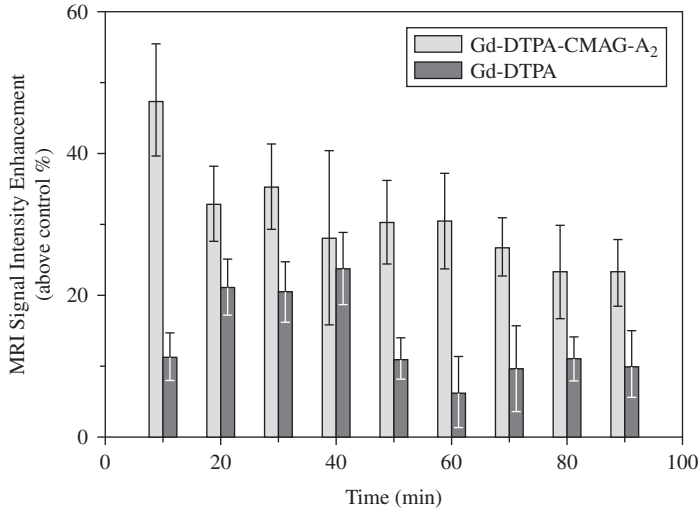

FIGURE 7.2 Mean percentage enhancement of liver versus time postintravenous administration of Gd-DTPA-CMAG-A2 (light gray bar) and Gd-DTPA (gray bar experiments). (From [34].)

7.4.2 Arabinogalactan Protein Carrier in Drug Formulations

In the past few years, life-threatening mycotic infections have increased in humans caused by various fungi, mainly in immuno-compromised hosts, such as cancer and AIDS patients who have undergone surgery [35]. Chemotherapy is a common strategy for them. A large number of antifungal drugs are also used to control the fungal infections. Azoles and their derivatives are found to be most effective and predominant [36, 37]. However, the fungal infection represents a major therapeutic challenge owing to the increasing prevalence of organisms resistant to commonly used azoles [38, 39]. Moreover, the high cost of azoles and their derivatives restrict their use. Novel drugs development and/or treatment strategies to fight against these infections are critical and have led to the development of azoles with enlarged spectra and to the discovery of other novel, broad-spectrum fungicidal drugs. Among the nonazole drugs amphotericin B (AmB) and its derivatives is the drug of choice for the treatment of mycotic infection caused by a wide range of fungi. In contrast, amphotericin B (AmB) is inexpensive, highly fungicidal against most pathogenic fungi, and found to be free of clinically meaningful resistance so far [40]. However, the use of AmB has decreased due to its dose-related toxicity, mainly to the kidneys, central nervous system, and liver, the frequency of which may be very high [41], and its side effects, such as nausea, fever, and shivering. One of the approaches for improving drug performance and reducing toxicity is conjugation to a polymeric carrier. In the recent past, the arabinogalactans have generated highly water-soluble conjugates that were found to be much safer and effective than the commonly used AmB-DOC formulation. The glycoconjugates, mainly the polysaccharides and polysaccharide–protein complexes, could be a suitable alternative due to their high water solubility.

Arabinogalactan proteins are plant-derived glycoconjugates with an established history of pharmaceutical and other industrial applications, such as emulsifiers [42]. AGPs from various crop and medicinal plants have been isolated and their structures were elucidated [43, 44]. Both AGP and its purified fraction are highly water soluble, posses a high degree of biocompatibility, and are used in several pharmaceutical and neutraceutical preparations. Water-soluble injectable conjugate of amphoptericin B–arabinogalactan (AmB–AG) was used as a commercial preparation of AmB and AG [33]. This conjugate increases the solubility and stability of AmB in aqueous solution and significantly reduces its toxicity [45, 46].

The two-step Schiff base method has been used for the conjugation of AmB with arabinogalactan. A water-soluble injectable AGP–AmB conjugate has been prepared without affecting its antifungal activity. A reduction of aggregation by complexation with AGP explains the lesser cytotoxicity of AGP–AmB as compared with AmB. Thus, it is clear that the AGP conjugation with AmB significantly reduces its toxicity and does not have any adverse effect on its antifungal activity. Therefore, AGP could serve as novel potent carrier for AmB drug formulation. Since the AGPs from various sources found to have

immunostimulatory activity and the fungal infections are more common in immunocompromised host, it is speculated that the conjugation of AmB to AGP could be advantageous by having both antifungal as well as immunostimulatory properties. However, this is merely a speculation, unless AmB is conjugated to AGP and the conjugate tested for antifungal and immunostimulatory activity [47].

7.4.3 Antiulcer Activity of Arbinogalactan

Industrial soybean oil extractions by products are protein and polysaccharides rich in proteins and polysaccharides. Some of their structures were partly elucidated during the 1960s. The major component of the hot-water extraction in this study was an arabinogalactan. The soybean arabionogalactan is derived as type I. Plant polysaccharides have been reported to have antiviral, antitumor, immunostimulating, antiinflammatry, anticomplementary, anticoagulant, hypoglycemic, and antiulcer activities [48]. The last has been attributed to pectic polysaccharides from *Panax ginseng* and *Bupleurum falcatum*, which have a high galacturonic acid content, acidic heteroxylans from *Maytenus ilicifolia* and *Phyllanthus niruri* [49], and type II arabinogalactans from *Cochlospermum tinctorium* and *M. ilicifolia* [50]. Although arabinogalactans have an antiulcer protective effect, the isolation and characterization of the type I arabinogalactan of soybean (AG) has been done and its protective antiulcer activity evaluated.

Studies showed the type II arabinogalactans also possesses antiulcer protective effects [48, 50], but this property has not yet been described for type I arabinsogalactans. To determine the AG antiulcer activity, 10, 30, and 100 mg/kg AG was administered orally to female Wistar rats. EtOH-induced gastric lesions were reduced by 33, 48, and 71%, respectively, with ED50 of 35 mg/kg. Omeprazole (40 mg/kg) control showed a 47% reduction of the lesions (Fig. 7.3). Results indicate the potential activity of AG to act as a direct cytoprotective agent. Possible mechanisms for antiulcer effects of polysaccharides involved its ability to (1) bind the mucosal surface to work as a protective coating, (2) reduce secretory activities of acid and pepsin, and (3) protect the mucosa by increasing mucus synthesis and/or scavenging radicals.

Overall, the highly purified type I arabinogalactan from soybean inhibits ethanol-induced gastric lesions, indicating that it could be an effective gastroprotective agent [51].

7.4.4 Antioxidant Activity of Carotenoid–Arbinogalactan Complexes

Carotenoid is a class of naturally occurring pigments. These essential nutrients are synthesized by plants and microorganisms, hence abundantly found in vegetables, fruits, and fish. Polyene chain and various terminal substituents present in carotenoid molecules determine their redox properties and the location inside the lipid layers in biological media. In photosynthesis processes, their role is

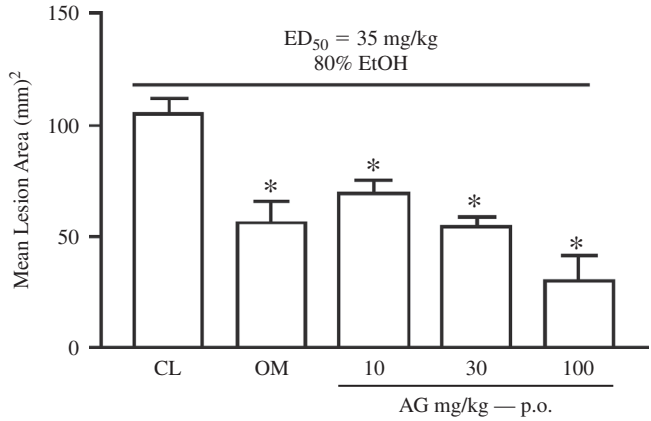

FIGURE 7.3 Protective effect of AG (10, 30, and 100 mg/kg, p.o.) against ethanol-induced gastric lesions (CL: control, water 0.1 mL/100 g, p.o.; OM: omeprazole 40 mg/kg, p.o.). The results are expressed as means ± SEM ($n = 6$), with $*p < 0.05$ when compared to control group. (From [51].)

related to energy and electron transfer. Recently, more attention has been focused on the reactions between carotenoids and free radicals [52–54] to prevent the development of diseases caused by toxic free radicals. At the same time, wide applications of carotenoids as antioxidants are substantially hampered by their hydrophobic properties, instability in the presence of oxygen, and high photosensitivity. Artificial solar cell application of carotenoids is restricted by their instability toward reactive oxygen species and metal ions, especially in the presence of water. A majority of carotenoids are lipophilic in nature. Carotenoids used in pharmaceutical application require a chemical delivery system to overcome the problems of parenteral administration of a highly lipophilic, low-molecular-weight compound. To avoid these disadvantages, a search for complexing agents is being continued. The increased aqueous solubility of such a carotenoid complex can be introduced into mammalian cell culture systems that depend on liposomes or toxic organic solvents [55].

The complex formation between carotenoids and natural polysaccharide arabinogalactan has been described. Arabinogalactan has potential therapeutic benefits as an immune-stimulating agent and cancer protocol adjunct. The study describe an AG that increases the production of the short-chain fatty acids butyrate and propionate. These small fatty acids are essential for the health of the colon. AG also acts as a food supply for "friendly" bacteria, such as bifidobacteria and lactobacillus, while eliminating "bad" bacteria. AG has a beneficial effect upon the immune system as it increases the activity of natural killer cells and other immune system components, helping the body to fight against infection. Complexes of carotenoids II and III with AG prepared mechanochemically in

stoichiometric proportion 1 : 1 is 5 mM in a water solution. The carotenoid–arabinogalactan complexes maintain their original color and show significant changes in the absorption spectra. The Ultraviolet–Visible (UV–Vis) spectrum of aqueous solutions of a canthaxanthin–AG complex has the same maximum absorption as the spectrum of a canthaxanthin solution in 30% ethanol. However, the preparation of a concentrated solution of this complex using traditional methods was not successful. An important result of this study is the increase in yield and stability of the radical cation of canthaxanthin in a solid-state complex of AG. An increased stability of the carotenoid radical cation imbedded into a polysaccharide host opens many possibilities for the application of these complexes in the designing of artificial light harvesting, photoredox, and catalytic devices [56].

7.4.5 Larch Arabinogalactan for the Treatment of Corneal Lesions

Corneal abrasions occur because of cutting, scratching, or abrading the thin, protective, clear coat of the anterior ocular epithelium portion. These injuries cause pain, tearing, photophobia, foreign body sensation, and a gritty feeling [57]. Abrasions may be caused by several factors including toxic and mechanical and by altering the physiologic functions. Contact lenses are a specific and uncommon source of corneal abrasions. Approximately, 100 million people wear contact lenses worldwide. Thus, the risk should not be underestimated. Hence, the number of corneal lesions is speedily increasing [58, 59], Tissue repairing involves cell attachment and adhesion to specific extracellular matrix substrates, through recognized specific membrane receptors called integrins. Since integrin recognition is affected by natural or synthetic polysaccharides. Both polysaccharides and glycosaminoglycans influence the cell adhesion and wound-healing process [60]. Continuous search for compounds favoring cell adhesion and promoting ocular wound healing prompted the investigation of a natural polysaccharide. The U.S. Food and Drug Administration (FDA) has already approved larch arabinogalactan as a dietary fiber source. Its benefits are therapeutic as an immune-stimulating agent [61]. The test was carried out on eye tolerance of AG and its protective action against experimentally induced dry-eye conditions and corneal abrasions.

The preliminary results showed AG produces Newtonian, nonviscous solutions, possessing mucoadhesive properties used in the retention of eye surface. A dry-eye model exerted a protective effect against the appearance of dry spots on the corneal epithelium, and significantly increased the healing rate of corneal wounds, with respect to other polymers, commonly used as adjuvants in ophthalmic vehicles of AG formulations. These findings suggest the potential of AG in dry-eye conditions, and for the prevention and treatment of corneal wounds. AG solutions also benefit contact lens wearers because of their tolerability, prolonged permanence, and noninterference with vision owing to low viscosity. Further research for verification of AG compatibility with contact lens and microscopy studies, aimed at a thorough survey of its involvement at

TABLE 7.3 Composition and Properties of Formulations for in Vivo Studies[a]

Formulation	Polymer (% w/w)	Mannitol (% w/w)	pH	Viscosity (η, mPa · s)
AG-Sol	5.00	4.4	6.5	1.21
TSP	0.50	5.0	6.4	9.16
HA 0.2%	0.20	5.0	6.5	24.40
TSP 0.04%	0.04	5.0	6.0	1.22
HA 0.00144%	0.00144	5.0	6.6	1.26

Abbreviations: AG, arabinogalactan; TSP, tamarind seed polysaccharide; HA, hyaluronic acid.
Source: From [62].

tissue level, is in progress. Finally, clinical trials will definitely need to verify the transferability of these results to humans (Table 7.3) [62].

7.4.6 Arabinogalactan in Tuberculosis

Mycobacterium tuberculosis, is the slow growing bacterium that causes tuberculosis (TB). It is a respiratory transmitted disease affecting approximately 32% of the world's population. Among infected individuals, about 2 million people die each year from this disease. Every year 95% of TB cases are found in developing countries [63]. About 1 million young women are victimized every year by this disease. The spread of this disease is linked to dense population, poor nutrition, and poor sanitation [64, 65]. India, with 2% of the world's land area and 15% of the its population, thus has 30% of the TB burden. In India, TB kills 14 times more people than all tropical diseases. Approximately 50% of India's population is reported to be tuberculin test positive [66] and one person dies from TB every minute [67]. TB was rare until the second half of the nineteenth century. Concomitant with the growing population density caused by industrialization, the incidence of TB has increased progressively since then [68, 69].

Arabinogalactan in Mycobacterial Cell Wall. Arabinogalactan is one of the cell wall components in mycobacteria. It forms a complex with peptidoglycan and mycolic acids for the appearance of the cell wall mycolyl–arabinogalactan–peptidoglycan complex (MAPc).

The basic structure of the mycobacterial cell wall core is shown in Figure 7.2. The core consists of a highly impermeable outer mycolic acid layer of (C70–C90 lipids) and an inner peptidoglycan layer. These two layers are linked through polysachharide arabinogalactan. The major mycobacterium cell wall is made up of a branched AG chain with the arabinose AG attached to the muramic acid via a phosphodiester linkage. The polymer is unique in its elemental sugar comprised of a few distinct structural motifs. The galactan polymer is linked to the sixth position of the peptidoglycan muramic acid residues α-L-Rhap-(1-3)-α-D-GlcNAc-(1-P) "linker region." The galactan itself

is linear, consisting of about 30 alternating 5- and 6-linked β-D-Gal*f* residues. The arabinan chains are attached to the fifth carbon of some of the 6-linked Gal*f* residues to reduce the end of the polymer.

However, some galactan polymers are not arabinosylated, forming a large unbranched galactan chain. The arabinan chains are mainly composed of 5-linked α-D-Ara*f* with branching introduced by 3,5-α-D-Ara*f* residues. Non-reducing termini of the arabinan have a [β-D-Ara*f*-(1-2) α-D-araf]$_2$ −3,5-α-D Ara*f*-(1-5)-α-D-Ara*f* motif and mycolic acids (long-chain α-alkyl β-hydroxyl fatty acids) are located in clusters of four on two-thirds of the terminal arabinofuranosides (Fig. 7.4) [70].

Arabinogalactan Biosynthesis in Mycobateria Tuberculosis. The biosynthesis process of arabinogalactan peptidoglycan complex in *Mycobacterium* takes place in three steps. Synthesis of polyprenyl phosphate, peptidoglycan, and arabinogalactan.

Synthesis of arabinogalactan in *M. tuberculosis* begins with the transfer of a GlcNAc-1-phosphate to prenyl phosphate followed by an addition of rhamnose (Rha) from dTDP-Rha. It form the linker region of the AG. RmlA, RmlB, RmlC, and RmlD are responsible for the synthesis of dTDP from

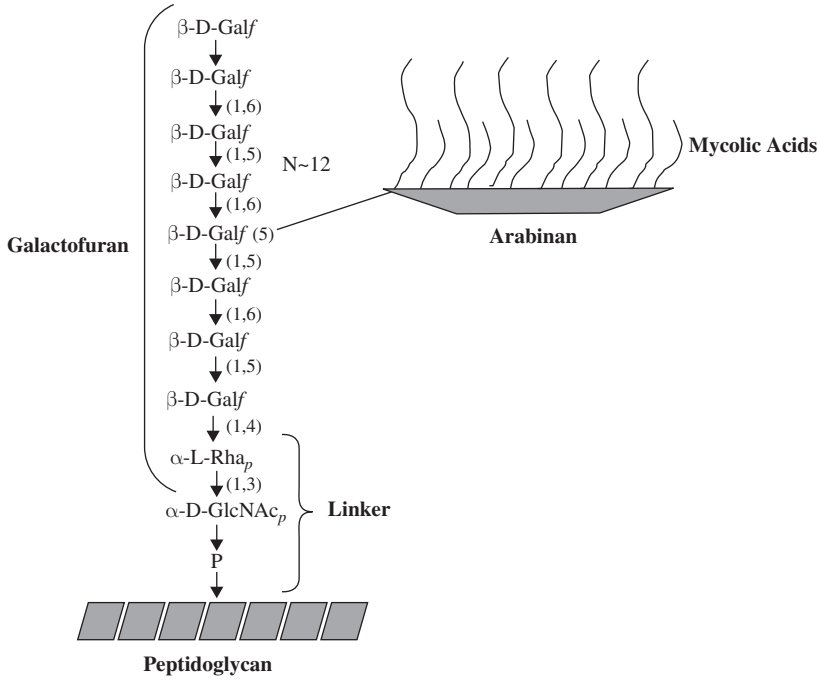

FIGURE 7.4 *Mycobacteria* cell wall structure. (From [70].)

glucose-1-phosphate encoding an α-D-glucose-1 phosphate thymidylyl transferase, dTDP-D-glucose 4,6-dehydratase, dTDP-4-keto-6deoxy D-glucose 3,5 epimerase, and dTDP rhamnose synthetase, respectively. Rhamnosyl transferase encoded by reading frame Rv3265c adds Rha to the prenyl diphosphoryl GlcNAc.

UDP-galactofuranose (UDP-uridinediphosphategalactofuranose) is the donor of galactofuranose residues of the galactan. The first reaction in the synthesis of UDP-Gal*f* is the conversion of UDP-glucose to UDP-galactopyranose (UDP-Gal*p*) catalyzed by UDP-galactopyranose epimerase. The enzyme encoded by *glf* then converts UDP-Gal*p* to UDP-gal*f*, which is the donor of the gal*f* residues of the galactan in *M. tuberculosis*. Subsequently, in the formation of the linker region, the Gal*f* and Ara*f* residues are added to form the mature lipid linked AG. It further transferred to peptidoglycan en block by a ligase, which releases decaprenyl phosphate. Enzyme Gal*f* transferase catalyse for the formation of 1-5 and 1-6 linkages [70].

7.4.7 Larch Arabinogalactan as a Dietary Fiber

The U.S. FDA approved arabinogalactan as an excellent source of dietary fiber. The production of short-chain fatty acids (SCFAs) is increased with a decrease in the generation and absorption of ammonia [71]. Human consumption of arabinogalactan has a significant effect on enhancing gut microflora, specifically increasing anaerobes such as *Bifidobacteria* and *Lactobacillus*, while decreasing *Clostridia* [72]. Carbohydrate fermentation into SCFAs by intestinal microflora is important for the large bowel function and help in hepatic as well as peripheral tissue metabolism [71]. Butyrate plays an important role in colon health. It is the suitable substrate for energy generation by colonic epithelial cells [73]. It protects the mucosa against a variety of intestinal diseases and protects these cells against cancer-promoting agents [74]. The ability of arabinogalactan to increase concentrations of butyrate suggests some significant health benefits subsequent to oral administration of this polysaccharide.

Arabinogalactan fermented strongly by gastrointestinal microflora results in the production of SCFAs. High production of SCFA suggests that complete fermentation takes place by gut microflora of any nonabsorbed arabinogalactan. Intestinal bacteria used an in vitro fecal incubation system to study the metabolism of complex carbohydrates. Arabinogalactan increased the yield of SCFAs and acetate in all substrates at all times. Fecal homogenates incubated with cellulose showed no large SCFA production than in the controls. Backbone and side-chain sugars of arabinogalactan were co-utilized as fermentation substrates; however, the sugars were broken down more slowly than starch or pectin. Although starch was the most effective polysaccharide in generating high butyrate concentrations, arabinogalactan had significantly more butyrate-generating activity than pectin or xylan [71]. Arabinogalactan found to have clinical value in the treatment of portosystemic encephalopathy because of its ability to

lower the generation and subsequent absorption of ammonia. Arabinogalactan promotes the increase in bifidobacteria, particularly *B. longum*, which more specifically ferment the arabinogalactan [75]. A controlled study of larch arabinogalactan in human volunteers has demonstrated its ability to promote an increase of bifidobacteria, as well as other anaerobes such as *Lactobacillus* [72].

7.4.8 Reticuloendothelial and Complement Activation

Low- to middle-molecular-weight (5000–50,000) arabinogalactan polysaccharides showed the strong immuno-stimulating properties, including the ability to activate phagocytosis and potentiate reticuloendothelial system action. The number of arabinogalactans also showed the anticomplement activity [76, 77], although arabinogalactan was not utilized in these studies. In fact, recent findings demonstrated arabinogalactan ingested by human volunteers had a significant dose-dependent in vivo effect. It enhances the function of the mononuclear portion of the immune system [78].

7.4.9 Larch Arabinogalactan and Pediatric Otitis Media

Otitis media is a common pediatric problem. Children suffering from chronic otitis media need a powerful immune system along with reestablishing gut microflora balance. This is a reasonable therapeutic goal. Decrease in frequency and severity of pediatric otitis media with arabinogalactan was clinically proved and reported. The combination of known immune-enhancing properties and effects on gut microflora might indeed produce good clinical outcomes. High solubility of arabinogalactan in fluids such as water or juice and the long-term administration of this substance to pediatric patients might be accomplished with a great deal less difficulty than other available immune-modulating herbal or nutritional substances [21].

7.4.10 Arabinogalactan and Cancer

Arabinogalactan possesses several interesting properties; thus it is used as an ideal adjunctive supplement in cancer protocols. Experimental studies indicate that arabinogalactan stimulates natural killer cell cytotoxicity, enhances the immune system, and inhibits the metastasis of tumor cells to the liver. All of these activities have significant utility as strategies to support conventional cancer treatment [21].

Antimetastatic Activity. Metastatic diseases are commonly spread to the liver, in comparison of other organ sites. This results because of the reaction between the galactose-based glycoconjugate on the metastatic cells and a hepatic-specific lectin receptor (e.g., the D-galactose-specific hepatic binding protein) found in liver parenchyma. Several compelling studies showed that arabinogalactan inhibits this reaction. Modified citrus pectin (MCP) has been

also found to be a possible antimetastatic agent in cancer [79]. However, arabinogalactan showed the same antimetastatic mechanism of action as MCP. It also offers immune-modulating activity, which is not offered by other natural substances. In a study, the effects of arabinogalactan were investigated in a syngeneic tumor–host system of one animal. The study includes the systemic treatment with D-galactose and arabinogalactan as well as cell pretreatment with arabinogalactan and two other glycoconjugates. Although all the test animals finally succumbed to liver metastasis, treatment with arabinogalactan significantly reduced the amount of liver metastasis and prolonged the survival times of the animals. This was to be an arabinogalactan blockade effect of potential liver receptors, covering galactose-specific binding sites [80]. In another study, pretreatment and regular application of arabinogalactan as a receptor-blocking agent completely prevented the settling of sarcoma L-1 tumor cells in the liver of experimental animals. Other galactans, dextrans, and phosphate-buffered saline showed no effect. The results showed that as lectinlike liver receptors are blocked with the competitive polysaccharide-like arabinogalactan, tumor metastasis may be prevented [81]. An other study also demonstrated that arabinogalactan significantly reduced the colonization process of highly metastatic Esb lymphoma cells [82].

7.5 SIDE EFFECTS AND TOXICITY

Arabinogalactan is FDA approved for use in food applications. Toxicity tests on rats indicate arabinogalactan is less toxic than methylcellulose. In acute toxicity studies, mice and rats showed no signs or symptoms of toxicity at a dose of 5000 mg/kg, while in prolonged toxicity studies, doses of 500 mg/kg for 90 days resulted in no toxicity. Clinical feedback suggests an occasional reaction of bloating and flatulence in less than 3% of individuals. This side effect might be secondary to the effect of arabinogalactan, beneficially altering gut microflora [21].

7.6 CONCLUSIONS

Outcome studies on novel polysaccharide clinical applications have not been conducted to date. Physiological properties of polysaccharides imply a broad range of potential therapeutic applications. As a dietary fiber, larch arabinogalactan possesses several beneficial properties, including promoting the growth of friendly bacteria, increasing the production of SCFAs, and decreasing the generation of ammonia. As most of the diets are deficient in dietary fiber, arabinogalactan enhance the immune activity.

Larch arabinogalactans also offer substantial promise as a biological response modifier. Documented effects on NK cytotoxicity, along with effects on mononuclear portion of the immune system, have been studied. An array of

clinical uses was suggested both in preventive medicine and in clinical medicine, as a therapeutic agent to decrease NK activity. As a therapeutic agent, larch arabinogalactans reduced NK activity and chronic viral infections, such as CFS and viral hepatitis. The greatest potential of this polysaccharide is to support conventional cancer treatments as a biological response modifier. Combination of its immune-enhancing properties along with antimetastatic activity fulfills two therapeutically desirable goals. Modified citrus pectin has received considerable attention in both conventional and alternative medical journals for its antimetastatic activity. Larch arabinogalactan works in the same manner, by inhibiting the attachment of metastatic cells to liver parenchyma via competitive binding to liver hepatic galactose receptors. Larch arabinogalactan, however, offers the additional advantage of enhancing immune function. Larch arabinogalactan not only offers antimetastatic activity but also shows positive effects on gut microflora and SCFAs, which was not found with *V. album*.

REFERENCES

1. Paulsen, B. S. Biologically active polysaccharides as possible lead compounds. *Phytochem. Rev.* 2002;**1**:379–387.
2. Odonmazig, P., A. Ebringerova, E. Machova, and J. Alfoldi. Structural and molecular properties of the arabinogalactan isolated from Mongolian larchwood (*Larix dahurica L.*). *Carbohydr Res.* 1994;**252**:317–324.
3. Yamada, H., H. Kiyohara, J. C. Cyong, and Y. Otsuka. Studies on polysaccharides from Angelica acutiloba IV. Characterisation of anti-complementary arabinogalactans from *Angelica acutiloba* Kitagawa. *Mol. Immunol.* 1985;**22**:295–304.
4. Yamada, H. and H. Kiyohara. Complement activating polysaccharides from medicinal herbs. In *Immunomodulatory Agents from Plants*. H. Wagner (Ed.). Birkhäuser: Basel, 1999, pp. 161–202.
5. Yu, K. W., H. Kiyohara, T. Matsumoto, H. C. Yang, and H. Yamada. Structural characterization of intestinal immune system modulating new arabino-3,6-galactan from rhizomes of Atractylodes lancea DC. *Carbohydr. Polym.* 2001;**46**:147–156.
6. Hauer, J. and F. A. Anderer. Mechanism of stimulation of human natural killer cytotoxicity by arabinogalactan from *Larix occidentalis*. *Cancer Immunol Immunother*. 1993;**36**:237.
7. McNeil, M., S. J. Wallner, S. W. Hunter, and P. J. Brennan. Demonstration that the galactosyl and arabinosyl residues in the cell wall arabinogalactan of Mycobacterium leprae and Mycobacterium tuberculosis are furanoid. *Carbohydr. Res.* 1987;**166**:299–308.
8. Egert, D. and N. Beuscher. Studies on antigen specificity of immunoreactive arabinogalactan proteins extracted from Baptisia tinctoria and Echinacea purpurea. *Planta. Med.* 1992;**58**:163–165.

9. Kiyohara, H., J. C. Cyong, and H. Yamada. Relationship between structure and activity of an anticomplementary arabinogalactan from the roots of Angelica acutiloba Kitagawa. *Carbohydr. Res.* 1989;**193**:173–192.
10. Gonda, R., M. Tomoda, N. Ohara, and K. Takada. Arabinogalactan core structure and immunological activities of ukonan C, an acidic polysaccharide from the rhizome of Curcuma longa. *Biol. Pharm. Bull.* 1993;**16**:235–238.
11. Groman, E. V., P. M. Enriquez, C. Jung, and L. Josephson. Arabinogalactan for hepatic drug delivery. *Bioconjug. Chem.* 1994;**5**:547–556.
12. Vince, A. J., N. I. McNeil, J. D. Wager, and O. M. Wrong. The effect of lactulose, pectin, arabinogalactan, and cellulose on the production of organic acids and metabolism of ammonia by intestinal bacteria in a faecal incubation system. *Br. J. Nutr.* 1990;**63**:17–26.
13. Parra, S., R. Pinochet, R. Vargas, et al. Natural killer cytolytic activity in renal and prostatic cancer. *Rev. Med. Chil.* 1994;**122**:630–637.
14. Levine, P. H., T. L. Whiteside, and D. Friberg, et al. Dysfunction of natural killer activity in a family with chronic fatigue syndrome.*Clin. Immunol. Immunopathol.* 1998;**88**:96–104.
15. Kastrukoff, L. F., N. G. Morgan, and D. Zecchini, et al. A role for natural killer cells in the immunopathogenesis of multiple sclerosis. *J. Neuroimmunol.* 1998;**86**:123–133.
16. Corado, J., F. Toro, and H. Rivera, et al. Impairment of natural killer (NK) cytotoxic activity in hepatitis C virus (HCV) infection. *Clin. Exp. Immunol.* 1997;**109**:451–457.
17. Uchida, A. Therapy of chronic fatigue syndrome. *Nippon Rinsho.* 1992;**50**:2679–2683.
18. Kastelan, M., K. Kovacic, and R. Tarle, et al. Analysis of NK cell activity, lymphocyte reactivity to mitogens and serotest PSA and TPS values in patients with primary and disseminated prostate cancer, PIN and BPH. *Anticancer Res.* 1997;**17**:1671–1675.
19. Mueller, E. A. and F. A. Anderer. A *Viscum album* oligosaccharide activating human natural cytotoxicity is an interferon gamma inducer. *Cancer Immunol. Immunother.* 1990;**32**:221–227.
20. Mueller, E. A., K. Hamprecht, and F. A. Anderer. Biochemical characterization of a component in extracts of *Viscum album* enhancing human NK cytotoxicity. *Immunopharmacology* 1989;**17**:11–18.
21. Kelly G. S. Larch arabinogalactan: Clinical relevance of a novel immune-enhancing polysaccharide, *Altern. Med. Rev.* 1999;**4**(2):96–103.
22. Sawka, A. M. and E. A. Nothnagel. The multiple roles of arabinogalactan proteins in plant development. *Plant Physiol.* 2000;**122**:3–9.
23. Schultz, C. J., K. L. Johnson, G. Currie, and A. Bacic. The classical arabinogalactan protein gene family of arabidopsis. *Plant Cell.* 2000;**12**:1751–1768.
24. Showalter, A. M. Arabinogalactan-proteins: Structure, expression and function, *Cell. Mol. Life Sci.* 2001;**58**:1399–1417.
25. Rinck, P. A. *Magnetic Resonance Imaging*, 4th ed. Blackwell Science: Berlin, 2001, p. 149.

26. Aime, S., S. G. Crich, E. Gianolio, G. B. Giovenzana, L. Tei, and E. Terreno. *Coord. Chem. Rev*. 2006;**250**:1562–1579.
27. Barkhausen, J., W. Ebert, J. F. Debatin, and H. J. Weinmann. *J. Am. Colloid Cardiol*. 2002;**39**:1392–1398.
28. Runge, V. M. *J. Magn. Reson. Imaging* 2000;**12**:205–213.
29. Bottrill, M., L. K. Nicholas, and N. J. Long. *Chem. Soc. Rev*. 2006;**35**:557–571.
30. Anelli, P. L., L. Lattuada, V. Lorusso, G. Lux, A. Morisetti, P. Morosini, M. Serleti, and F. J. Uggeri. *Med. Chem*. 2004;**47**:3629–3641.
31. Thompson, M. K., B. Misselwitz, L. S. Tso, D. M. J. Doble, H. Schmitt-Willich, and K. N. Raymond. *J. Med. Chem*. 2005;**48**:3874–3877.
32. Prata, M. I. M., A. C. Santos, S. Torres, J. P. Andre, J. A. Martins, M. Neves, M. L. Garcia-Martin, T. B. Rodrigues, P. Lopez-Larrubia, S. Cerdan, and C. F. G. C. Geraldes. *Contrast Med. Mol. Imaging* 2006;**1**:246–258.
33. Falk, R., A. J. Domb, and I. Polacheck. *Antimicrob. Agents Chemother*. 1999;**43**:1975–1981.
34. Weisheng, L., L. Zhongfeng, J. Fengying, D. Yuefeng, W. Lai, L. Peiqiu, Y. Xiangguang, L. Xiaojing, P. Fengkui, W. Xuxia, and L. Hao. *Carbohydr. Res*. 2008;**343**:685–694.
35. Groll, A. H., and T.J. Walsh. Uncommon opportunistic fungi: New nosocomial threats. *Clin. Microbiol. Infect*. 2001;**7**:8–24.
36. Johnson, M. D., and J.R. Perfect. Caspofungin: First approved agent in a new class of antifungals. *Expert Opin. Pharmacother*. 2003;**4**:807–823.
37. Donnelly, J. P. and B.E. De Pauw. Voriconazole—a new therapeutic agent with an extended spectrum of antifungal activity. *Clin. Microbiol. Infect*. 2004;**10**:107–117.
38. Singh, N. Changing spectrum of invasive candidiasis and its therapeutic implications. *Clin. Microbiol. Infect*. 2001;**7**:1–7.
39. Ostrosky-Zeichner, L., J. H. Rex, P. G. Pappas, R. J. Hamill, R. A. Larsen, H. W. Horowitz, W. G. Powderly, N. Hyslop, C. A. Kauffman, J. Cleary, J. E. Mangino, and J. Lee. Antifungal susceptibility survey of 2000 blood stream Candida isolates in the United States. *Antimicrob. Agents Chemother*. 2003;**47**:3149–3154.
40. Barrett, J. P., K. A. Vardulaki, C. Conlon, J. Cooke, P. Daza-Ramirez, E. G. Evans, P. M. Hawkey, R. Herbrecht, D. I. Marks, J. M. Moraleda, G. R. Park, S. J. Senn, and C. Viscoli. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. *Clin. Ther*. 2003;**25**:1295–1320.
41. Razzaque, M. S., M. A. Hossain, N. Ahsan, and T. Taguchi. Lipid formulations of polyene antifungal drugs and attenuation of associated nephrotoxicity. *Nephron*. 2001;**89**:251–254.
42. Classen, B., K. Witthohn, and W. Blaschek. Characterization of an arabinogalactan protein isolated from pressed juice of *Echinacea purpurea* by precipitation with the β-Glucosylyariv reagent. *Carbohydr. Res*. 2000;**327**:497–504.
43. Y. Gasper, K. L. Johanson, J. A. McKenna, A. Bacic, and C. J. Schultz. The complex structure of arabinogalactan proteins and the journey towards understanding function. *Plant Mol. Biol*. 2001;**47**:161–176.
44. Showalter, A. M. Arabinogalactan-proteins: Structure, expression and function. *Cell. Mol. Life Sci*. 2001;**58**:1399–1417.

45. Ehrenfreund-Kleinman, T., T. Azzam, R. Falk, I. Polacheck, J. Golenser, and A. J. Domb. Synthesis and characterization of novel water soluble amphotericin B-arabinogalactan conjugates. *Biomaterials* 2002;**23**:1327–1335.
46. Folk, R., G. Jacob, A. Hoffmann, J. D. Abraham, and L. Polacheck. Distribution of amphoptericin B-arabinogalactan conjugate in mouse tissue and its therapeutic efficacy against murine aspergillosis. *Antimicrob. Agents Chemother*. 2004; **48**:3006–3009.
47. Parveen, S., A. D. Gupta, and R. Prasad. Arabinogalactan protein from *Arachis hypogaea*: Role as carrier in drug-formulations, *Int. J. Pharm*. 2007;**333**:79–86.
48. Nergard, C. S., D. Diallo, K. Inngjerdingen, T. E. Michaelsen, T. Matsumoto, and H. Kiyohara, et al. Medicinal use of Cochlospermum tinctorium in Mali:Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *J. Ethnopharm*. 2005;**96**:255–269.
49. Cipriani, T. R., C. G. Mellinger, L. M. Souza, C. H. Baggio, C. S. Freitas, and M. C. A. Marques, et al. Acidic heteroxylans from medicinal plants and their anti-ulcer activity. *Carbohydr. Polym*. 2008;**74**:274–278.
50. Cipriani, T. R., C. G. Mellinger, L. M. Souza, C. H. Baggio, C. S. Freitas, and M. C. A. Marques, et al. Polysaccharide from a tea (infusion) of Maytenus ilicifolia leaves with anti-ulcer protective effects. *J. Nat. Prod*. 2006;**69**:1018–1021.
51. Cipriani, T. R., C. G. Mellinger, M. L. C. Bertolini, C. H. Baggio, C. S. Freitas, M. C. A. Marques, P. A. J. Gorin, G. L. Sassaki, and M. Iacomini. Gastroprotective effect of a type I arabinogalactan from soybean meal. *Food Chem*. 2009;**115**:687–690.
52. Al-Agamey, A., G. M. Lowe, D. J. McGarvey, A. Mortensen, D. M. Phillip, T. G. Truscott, and A. J. Young. *Arch. Biochim. Biophys*. 2004;**430**:37.
53. Palozza, P., G. Calviello, S. Serini, N. Maggiano, P. Lanza, F. O. Ranelletti, and G. M. Bartoli. *Free Radical Biol. Med*. 2001;**30**:1000.
54. Polyakov, N. E., A. I. Kruppa, T. V. Leshina, T. A. Konovalova, and L. D. Kispert. *Free Radical Biol. Med*. 2001;**31**:43.
55. Lockwood, S. F., S. O'Malley, and G. L. Mosher. *J. Pharm. Sci*. 2003;**92**:922.
56. Polyakov, N. E., T. V. Leshina, E. S. Meteleva, A. V. Dushkin, T. A. Konovalova, and L. D. Kispert. Water soluble complexes of carotenoids with arabinogalactan. *J. Phys. Chem. B*. 2009;**113**:275–282.
57. Wilson, S. A. and A. Last. Management of corneal abrasions. *Am. Fam. Physician* 2004;**70**:123–128.
58. Jalbert, I. and F. Stapleton. The corneal stroma during contact lens wear. *Contact Lens Ant. Eye* 2005;**28**:3–12.
59. Casser, L. and N. J. Lingel. *Diseases of the cornea*. In *Clinical Ocular Pharmacology*. J. D. Bartlett, and S. D. Jaanus, (Eds.). Butterworth-Heinemann: Boston, 1995, pp. 679–745.
60. Burgalassi, S., L. Raimondi, R. Pirisino, et al. Effect of xyloglucan (tamarind seed polysaccharide) on conjunctival cell adhesion to laminin and on corneal epithelium wound healing. *Eur. J. Ophthalmol*. 2000;**10**:71–76.
61. Gregory, S. and N. D. Kelly. Larch arabinogalactan: Clinical relevance of a novel immune-enhancing polysaccharide. *Altern. Med. Rev*. 1999;**4**:96–103.

62. Burgalassi, S., N. Nicosia, D. Monti, G. Falcone, E. Boldrini, and P. Chetoni. Larch; Arabinogalactan for dry eye protection and treatment of corneal lesions: Investigation in rabbits. *J. Ocular Pharma. Therapeu.* 2007;**23**(6):541–549.

63. (a) World Health Organisation. Global Tuberculosis Control, WHO Report 2001. (b) World Health Organisation. Geneva, Switzerland, WHO/CDS/TB/2001, 287. (c) World Health Organisation. Tuberculosis Fact Sheet, No. 104. 2000, http://www.who.int/inf-fs/en/fact104.html/.

64. Dony, J. F., J. Ahmad, and Y. Khen Tiong. Epidemiology of tuberculosis and leprosy, Sabah, Malaysia. *Tuberculosis* 2004;**84**(1–2):8–18.

65. Singh, M. M., T. Bano, D. Pagare, N. Sharma, R. Devi, and M. Mehra. Knowledge and attitude towards tuberculosis in a slum community of Delhi. *J. Commun. Dis.* 2002;**34**(3):203–214.

66. Dhingra, V. K., S. Rajpal, D. K. Taneja, D. Kalra, and R. Malhotra. Health care seeking pattern of tuberculosis patients attending an urban TB clinic in Delhi. *J. Commun. Dis.* 2002;**34**(3):185–192.

67. Singh, M. M. tuberculosis—Triumph and tragedy. *J. Indian Med. Assoc.* 2003;**101**(3):190–194.

68. Ramachandran, R., R. Balasubramanian, and M. Muniyandi. Socio-economic impact of TB on patients and families in India. *Int. J. Tuberc. Lung Dis.* 1999;**3**:869–877.

69. Mahadev, B. and P. Kumar. History of tuberculosis control in India. *J. Indian Med. Assoc.* 2003;**101**(3):142–143.

70. Crick, D. C., S. Mahapatra, and P. J. Brenan. Biosynthesis of the arabinogalactan-peptidoglycan complex of Mycobacterium tuberculosis. *Glycobiology* 2001;**11**:107R–118R.

71. Englyst, H. N., S. Hay, and G. T. Macfarlane. Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiol. Ecol.* 1987;**95**:163–171.

72. Slavin, J., J. Feirtag, R. Robinson, and J. Causey. Physiological effects of arabinogalactan (AG) in human subjects. Unpublished research.

73. Roediger, W. E. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* 1989;**83**:424–429.

74. Tsao, D., Z. Shi, A. Wong, and Y. S. Kim. Effect of sodium butyrate on carcinoembryonic antigen production by human colonic adenocarcinoma cells in culture. *Cancer Res.* 1983;**43**:1217–1222.

75. Crociani, F., A. Alessandrini, M. M. Mucci, and B. Biavati. Degradation of complex carbohydrates by Bifidobacterium spp. *Int. J. Food Microbiol.* 1994;**24**:199–210.

76. Yamada, H., H. Kiyohara, J. C. Cyong, and Y. Otsuka. Studies on polysaccharides from Angelica acutiloba IV. Characterization of an anticomplementary arabinogalactan from the roots of Angelica acutiloba Kitagawa. *Mol. Immunol.* 1985; **22**:295–304.

77. Yamada, H., H. Kiyohara, J. C. Cyong, and Y. Otsuka. Structural characterization of an anticomplementary arabinogalactan from the roots of Angelica acutiloba Kitagawa. *Carbohydr. Res.* 1987;**159**:275–291.

78. Causey, J. L., R. R. Robinson, and J. M. Feirtag, et al. Effects of larch arabinogalactan on human peripheral blood mononuclear cells: Results from in vivo and *in vitro* human trials. Unpublished results.
79. Kidd, P. A new approach to metastatic cancer prevention: Modified citrus pectin (MCP), a unique pectin that blocks cell surface lectins. *Altern. Med. Rev.* 1996;**1**:4–10.
80. Hagmar, B., W. Ryd, and H. Skomedal. Arabinogalactan blockade of experimental metastases to liver by murine hepatoma. *Invasion Metastasis* 1991;**11**:348–355.
81. Beuth, J., H. L. Ko, K. Oette, et al. Inhibition of liver metastasis in mice by blocking hepatocyte lectins with arabinogalactan infusions and d-galactose. *J. Cancer Res. Clin. Oncol.* 1987;**113**:51–55.
82. Beuth, J., H. L. Ko, V. Schirrmacher, et al. Inhibition of liver tumor cell colonization in two animal tumor models by lectin blocking with d-galactose or arabinogalactan. *Clin. Exp. Metastasis* 1988;**6**:115–120.

PART IV

BIODEGRADABLE POLYMERS OF NATURAL ORIGIN: POLYESTERS

CHAPTER 8

POLYHYDROXYALKANOATE

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CONTENTS

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8.1 INTRODUCTION

The concept of biocompatibility has evolved on par with the continuing development of materials used in medical devices. Biocompatibility can be defined as the quality of not having toxic or injurious effects on biological systems. A biomaterial is regarded as a material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body [1]. Biomaterials also have to be easily processable, sterilizable, and capable of controlled stability or degradation in response to biological conditions. The biocompatibility of a medical implant will be influenced by a number of factors, including the toxicity of the material used, form and design of the implant, technique of inserting the device, in situ dynamics, and movement of the implant based on the surrounding matrix (e.g., soft or hard tissue, cardiovascular system, etc.).

Among various well-known biomaterials, polyhydroxyalkanoate (PHA) has been attracting much attention with regard to its unique properties that enables it to be processed as biodegradable biomaterials. PHA are naturally occurring carbon and energy storage compounds found in various bacteria, and their formation is known to be induced by unfavorable growth conditions [2, 3]. To date, approximately 150 different constituents of PHA have been identified as either homopolymers or copolymers [4]. However, only several polymers are available in sufficient quantities for application research. They include homopolymers of poly(3-hydroxybutyrate) [P(3HB)], poly(4-hydroxybutyrate) [P(4HB)], and poly(3-hydroxyoctanoate) [P(3HO)]; and copolymers of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)], poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HHx)], and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)].

After years of research and development, PHA and its composites have been tailored as suitable biomaterials, which are currently being evaluated in various pharmaceutical and medical applications. Their applications will be discussed in the following sections of this chapter. Processes involved in the production of PHA have been well documented. Bacterial and plant production systems have been established and evaluated for the production of different types of PHA polymers. The types of polymer produced depend on the host organism and carbon substrates. This is due to different substrate specificity of the genes involved

in PHA biosynthesis. The mechanical properties, biocompatibility and rate of degradation, can be controlled by changing the composition of PHA polymers. PHAs are renewable by nature as they can be produced from renewable resources.

This chapter will discuss current applications of PHA in the medical field. Results from recent in vitro and in vivo efficacy studies are highlighted, and potential applications of PHA in medical sciences are discussed. Insights on toxicity and biocompatibility of PHA polymers are revealed. Well-established PHA productions systems alongside characterization of the resulting polymers have been demonstrated.

8.2 POLYHYDROXYALKANOATE

PHA was first discovered and characterized in the 1920s [5]. Initially recognized as inclusion bodies/granules in the cytoplasm, PHA was later identified to be an intracellular reserve for carbon and energy [6]. These granules can be stained specifically with Sudan black or light fluorescent stains such as Nile blue and Nile red [7–10]. P(3HB) was the very first constituent of PHA to be identified. Since then, PHA has been thoroughly investigated. More than 90 genera of archea and eubacteria (both gram positive and gram negative) have been identified as PHA producers [11]. The following are some of the well-known PHA producers: *Cupriavidus necator* (formerly known as *Wautersia eutropha, Ralstonia eutropha,* or *Alcaligenes eutrophus*), *Alcaligenes latus*, *Aeromonas caviae*, *Bacillus* spp., *Burkholderia cepacia*, *Delftia acidovorans* (formerly known as *Comamonas acidovorans*), *Pseudomonas* spp., and recombinant *Escherichia coli*. To date, approximately 150 different constituents of PHA have been identified as either homopolymers or copolymers [4]. Monomers with straight, branched, saturated, unsaturated and also aromatic monomers have been reported (for a review see Wiltholt and Kessler [12]). PHA is categorized into three classes: short-chain-length PHA (SCL_{PHA}, carbon numbers of monomers ranging from C3 to C5), medium-chain-length PHA (MCL_{PHA}, C6–C14), and long-chain-length PHA (LCL_{PHA}, more than C14). Polymers consisting of SCL_{PHA}–MCL_{PHA} have also been reported.

The properties of PHA resemble some of the properties of commodity plastics. Most PHA are thermoplastics that can be tailored into stiff packing materials or highly elastic elastomers and are completely biodegradable in the natural environment [2, 13]. The chemical and physical properties of resulting PHA polymers is influenced by the functionalized groups in the side chain of monomers such as halogen, carboxyl, hydroxyl, epoxyl, and phenoxy [14, 15]. The production and characterization of PHA have been the subjects of interest since they are targeted as substitutes for petrochemical-based plastics. Currently, a variety of bioplastics from microbial fermentation of plant sugars and oils under various trademarks such as Biomer, Mirel, Biogreen, Biocycle, and Biopol are being produced at commercial-scale amounts [13].

8.2.1 PHA Biosynthesis Pathways

In general, PHA biosynthesis involves the uptake and conversion of carbon sources into precursor molecules that are subsequently polymerized by the enzyme PHA synthase. Naturally occurring metabolic pathway of PHA biosynthesis varies according to the genus of the bacterium. On the other hand, genetically engineered pathway enables the production of desired monomer type and composition. There are three well-known PHA biosynthetic pathways [16, 17]. Among the most well-established pathways are the ones found in *C. necator* (pathway I) and *Pseudomonas* spp. (pathways II and III). Pathway I is the most common pathway and can be found in a wide range of bacteria (Fig. 8.1). P(3HB) synthesis in this pathway occurs in a three-step reaction: condensation, reduction, and polymerization, which is catalyzed by three different enzymes [18, 19]. In the first step, two acetyl-CoA derived from the

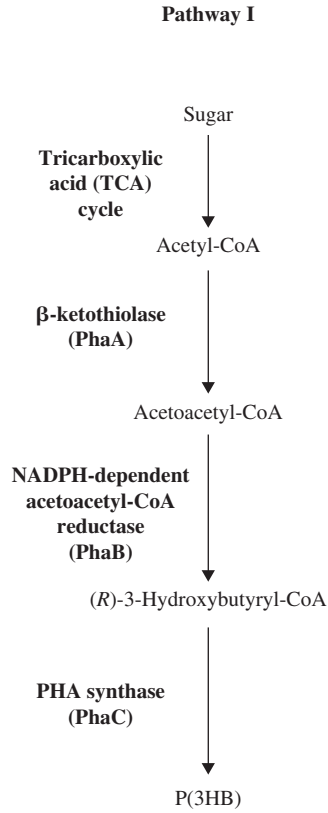

FIGURE 8.1 Most well-established P(3HB) biosynthesis pathway. This pathway is generally present in all microorganisms having the ability to synthesize P(3HB).

tricarboxylic acid (TCA) cycle is condensed to form acetoacetyl-CoA by the enzyme β-ketothiolase (PhaA). Then, acetoacetyl-CoA is reduced by acetoacetyl-CoA reductase (PhaB) to form 3-hydroxybutyryl-CoA. Finally, the PHA synthase (PhaC), which is the key enzyme in PHA biosynthesis, catalyzes the polymerization via esterification of 3-hydroxybutyryl-CoA into P(3HB). The ability of a microbe to produce varying 3HA monomers depends on the substrate specificity of its PHA synthase enzyme. The PHA synthase can be divided into four classes based on its substrate specificity and subunit structure (for a review see Rehm [20] and Nomura and Taguchi [21]).

PHA is also synthesized from pathways involved in fatty acid metabolism. Different hydroxyalkanoate units besides 3HB are derived from these pathways. Pathways II and III are also known as the fatty acid β-oxidation pathway and the fatty acid biosynthesis pathway, respectively (Fig. 8.2). These pathways are well studied in *Pseudomonas* spp., namely, *P. putida*, *P. oleovorans,* and *P. aeruginosa,* which belong to the ribosomal ribonucleic acid (rRNA) homology group I. The intermediate substrates generated from fatty acid metabolism can be efficiently polymerized by these microbes. *Pseudomonas* spp. can synthesize MCL_{PHA} from various alkanes, alkenes, and alkanoates but trace or no SCL_{PHA}. The intermediates in these pathways are effectively converted to (R)-3HA-CoA monomers by specific enzymes. The monomer composition is much wider and related to the carbon source used. As shown in Figure 8.2, the (R)-specific enoyl-CoA hydratase (PhaJ) and (R)-3-hydroxyacyl-ACP-CoA transferase (PhaG) are capable of supplying (R)-3HA-CoA from *trans*-2-enoyl-CoA and (R)-3HA-ACP, respectively [17]. In *A. caviae*, *trans*-2-enoyl-CoA is converted to (R)-3HA-CoA by PhaJ [22, 23]. In pathway III, sugars such as glucose, fructose, and sucrose are converted to (R)-3HA-CoA intermediates from (R)-3HA-ACP by PhaG. Both PhaJ and PhaG are the key links between fatty acid metabolism and PHA biosynthesis.

Microbes that cannot accumulate PHA naturally have been successfully engineered by introducing PHA biosynthesis pathways from known producers. PHA synthase gene (*phaC*) and monomer supplying genes such as *phaA*, *phaB*, *phaG,* and *phaJ* have been cloned and expressed in recombinant strains. The substrate specificity of the PHA synthase is generally quite broad. For example, the PHA synthase of *C. necator* has been shown to polymerize HA monomers of 3C atoms (3-hydroxypropionate [3HP]) up to 12C atoms (3-hydroxydodecanoate), although the MCL monomers are less efficiently polymerized relative to SCL monomers. Thus, the monomer-supplying pathway available in a microbe determines the type of PHA that can be produced. So, when the PHA synthase of *C. necator* is expressed in *A. caviae,* it is active toward C4–C6 monomers while in *Pseudomonas* it is active toward C4–C12 monomers. Thus, monomers synthesized by the recombinant can be controlled by expressing selected PHA biosynthesis genes in a particular host strain, and this has been proven in previous studies [24–27]. Genetic engineering technique has therefore proven to be a powerful tool in creating recombinant strains that can produce tailor-made PHA efficiently.

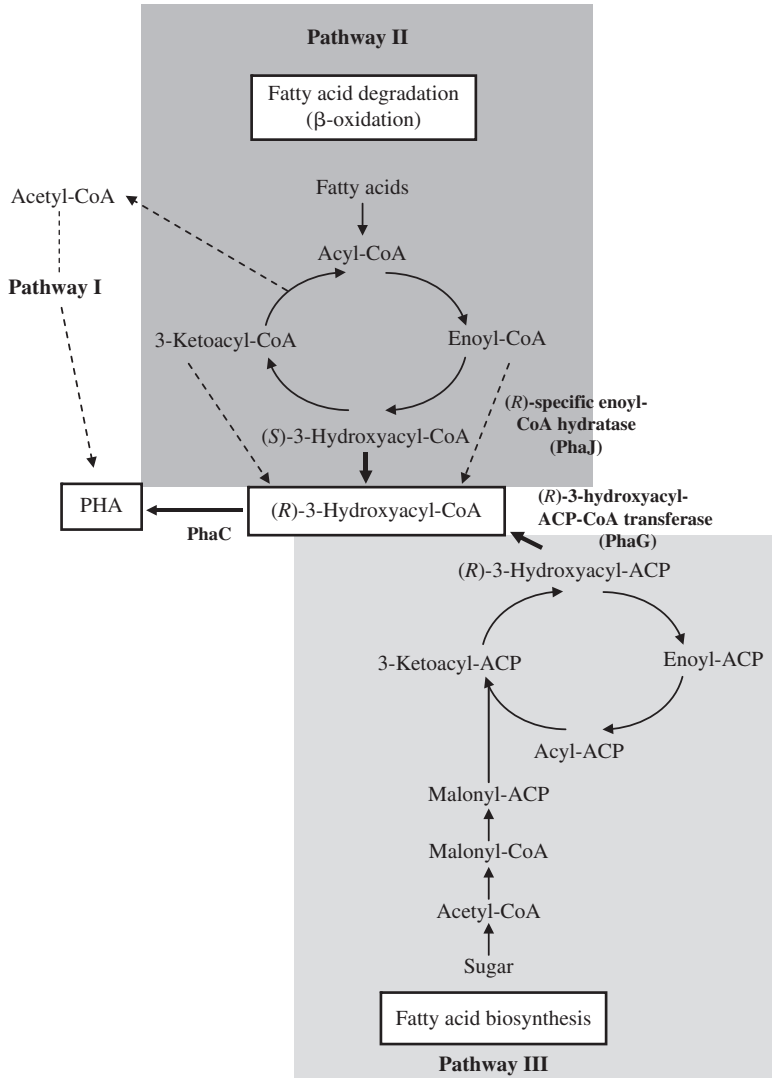

FIGURE 8.2 Biosynthesis of PHA from fatty acid metabolism. The fatty acid β-oxidation and fatty acid biosynthesis pathways have been well studied in *Pseudomonas*.

8.2.2 Production and Characterization

PHA production systems in various microbes and organisms have been documented. The bacterial production system is the most well-established form of PHA production. Besides that, an attempt to initiate PHA accumulation in plants was carried out in the 1990s [28, 29]. For example, *Arabidopsis*

thaliana was transformed with the PHA synthase of *C. necator* to induce polymerization of the intermediates derived from the plant's endogenous 3-ketothiolase and acetoacetyl-CoA reductase. As a result, PHA accumulation was identified in the cytoplasm. Several other attempts to produce PHA in a cost effective manner via transgenic plants are currently in progress [30–32]. Besides plants, other potential production systems in insect cells, yeast, and cyanobacteria have been also evaluated (for a review see Sudesh et al. [33]). Nevertheless, large-scale PHA production processes for commercialization purposes are currently derived from bacterial fermentations.

Bacterial synthesis of PHA is normally carried out in batch or fed-batch cultures. To initiate PHA accumulation, the growth medium is prepared in such a way that one nutrient (generally nitrogen) limits growth while the carbon source is in excess. The depletion of the selected nutrient acts as a trigger for the metabolic shift to PHA biosynthesis. Two different approaches have been developed in batch cultivation: one-stage cultivation and two-stage cultivation. In one-stage cultivation, growth of cells and PHA accumulation occurs simultaneously. While two-stage cultivation consists of a cell growth phase that is carried out in a separate nutrient-enriched medium. The cells are then transferred into a nutrient-limited medium for PHA accumulation phase.

Usually, the cultivation period ranges from 24 to 96 h. During the period of cultivation, cells will go through a sequence of growth phases, such as lag phase, exponential phase, PHA production, stationary phase, and finally death phase. In a fed-batch culture, the cells are continuously fed with selected carbon source after it has entered the late exponential phase. Normally, large-scale or industrial-scale production systems use the fed-batch cultivation mode [34, 35]. The fed-batch method generally yields high cell densities that consequently reduces the overall production cost [36–38]. Other modes of cultivation have also been evaluated, such as pH-stat-based cultivation whereby a carbon source is fed based on the fluctuation of pH [39], and the chemo-stat method whereby culture medium is continuously exchanged with sterile growth medium [40].

A carbon source is one of the key factors that can reduce the cost of PHA production. The type of PHA synthesized depends on the carbon substrates, monomer supplying pathways, and the specificity of PHA synthases. Sugars such as glucose and sucrose are the most common carbon sources for large-scale PHA production. Efforts have been devoted to identify cheaper carbon sources that can be utilized efficiently. Plant oils and fatty acid derivatives are being investigated as suitable carbon sources for PHA production. They are cheaper, renewable, and produce higher yields of polymer. It has been found from previous studies that P(3HB) production from plant oils was almost twofold higher (0.6–0.8 g/g) as compared to glucose (0.3–0.4 g/g) [41]. This is mainly due to the higher number of carbon atoms per gram of oil compared to sugar.

Major commodity plant oils such as soybean oil, palm oil, corn oil, and others have also been identified as possible carbon sources for PHA biosynthesis. Besides this, PHA is also produced from fatty acids. *Pseudomonas* spp. are

well-known PHA producers from fatty acids and its salt derivatives and MCL_{PHA} are normally produced. Copolymers of different PHA constituents are produced by adding co-substrates, which are also known as precursors. For example, P(3HB-*co*-3HV) copolymer is mostly produced by adding 3HV precursors such as valeric or propionic acids or their salt forms, while the P(3HB-*co*-4HB) copolymer is synthesized from the addition of 1,4-butanediol or γ-butyrolactone. These substrates can also be used as sole carbon sources to obtain the mentioned copolymers.

Aeromonas spp. are known to produce P(3HB-*co*-3HHx) copolymer, which are a combination of $SCL-MCL_{PHA}$ from fatty acids or oily substrates. The PHA synthase gene of *A. caviae* has been expressed heterologously in PHA synthase mutant of *C. necator* to produce better yields of this copolymer. Lately, terpolymers of different monomer types and compositions have been produced from various carbon sources. Normally, the terpolymers are combinations of both $SCL-MCL_{PHA}$ as well. Besides this, poly(3-hydroxyoctanoate-*co*-3-hydroxyhexanoate) [P(3HO-*co*-3HHx)], an MCL_{PHA}, has been produced in fed-batch cultures of *P. oleovorans* with sodium octanoate as the sole carbon source [42]. A random copolymer with 3HO, 3HHx, and poly(3-hydroxydecanoate) (3HD) monomers is biosynthesized. Higher compositions of 3HO (86 mol%) and 3HHx (11 mol%) is usually obtained.

PHA terpolymers are also being studied for their potential usages in the medical field. The terpolymers consist of either SCL_{PHA} or $SCL_{PHA}-MCL_{PHA}$. The common terpolymers produced includes P(3HB-*co*-3HV-*co*-3HHx), P(3HB-*co*-3HV-*co*-4HB), and P(3HB-*co*-3HV-*co*-3HHp). Most of the bacterial strains used for terpolymer production are recombinants; however, reports of wild-type producers have been published as well. Sugars, specific precursors, fatty acids, and palm kernel oil have been tested as potential substrates. The type of PHA polymer produced by some well-known bacterial strains and the carbon sources used are summarized in Table 8.1.

Agricultural by-products and food industrial wastes are also being considered as inexpensive carbon and nitrogen sources for PHA biosynthesis. Bioconversion of whey [67], molasses [68], starch [69], triacylglycerols [70], and other substrates from food industrial wastes or industrial by-products [71–73] to value-added PHA polymers has been investigated. Organic acids such as lactic acid, acetic acid, and propionic acid derived as by-products of anaerobic fermentation processes could be utilized for synthesizing P(3HB) and P(3HB-*co*-3HV) polymers [74–76]. Mixtures of organic acids derived from anaerobically treated palm oil mill effluent have also been evaluated [77]. Utilization of these types of cheap carbon sources for large-scale PHA production could reduce the cost of production.

The physical property of PHA polymers utterly depends on the type of monomer incorporated into the polymer chains. PHA polymers could be tailored by controlling the type and composition of monomer incorporated. P(3HB) is a biocompatible polymer that is optically pure and possesses piezoelectricity [3, 78]. This aids in the process of inducing osteogenesis. However,

TABLE 8.1 Production of PHA Polymers by Various Microorganisms

Bacterial strain	PHA	Carbon source	References
C. necator	P(3HB)	Glucose	3
		Soybean oil	34
	P(3HB-*co*-3HV)	Glucose + propionic acid	43
		Plant oils + sodium propionate/valerate	44
	P(3HB-*co*-4HB)	Fructose + γ-butyrolactone	45
	P(3HB-*co*-3HV-*co*-4HB)	Fructose + butyric acid + valeric acid	46
		Glucose + propionic acid + 4-hydrobutyric acid/ γ-butyrolactone	47
Cupriavidus sp.	P(3HB-*co*-4HB)	γ-butyrolactone, 4-hydrobutyric acid, 1,4-butanediol	48
		γ-butyrolactone, even carbon numbered fatty acids (C6–C12)	92
		γ-butyrolactone, 4-hydrobutyric acid	50
Recombinant *C. necator*	P(3HB-*co*-3HHx)	Hexanoate, octanoate	54
		Soybean oil	34
		Palm kernel oil	51
	P(3HB-*co*-3HV-*co*-3HHx)	Palm kernel oil + sodium valerate/sodium propionate	52
	P(3HB-*co*-3HV-*co*-3HHp)	Odd carbon numbered fatty acids (C5-C13)	53
		Pentanoate, nonanoate	54
D. acidovorans	P(3HB-*co*-4HB)	4-hydroxybutyric acid, 1,4-butanediol	55
Recombinant *D. acidovorans*		Glucose + 1,4-butanediol	56
		4-hydroxybutyric acid	57
A. caviae	P(3HB-*co*-3HHx)	Olive oil, sodium salts of fatty acids (C12-C18)	58
	P(3HB-*co*-3HV)	Fatty acids (C11-C17)	
Alcaligenes latus	P(3HB-*co*-3HP)	3-hydroxypropionic acid	59
Pseudomonas putida	MCL_{PHA}	Saponified palm kernel oil	60
P. aeruginosa 47T2	MCL_{PHA}	Oily substrates	61

(*Continued*)

TABLE 8.1 (Continued)

Bacterial strain	PHA	Carbon source	References
Bacillus sp.	P(3HB)	Glucose	62
	P(3HB-*co*-4HB)	Fructose, sucrose	
	P(3HB-*co*-3HV-*co*-4HB)	Gluconate	
B. cepacia	P(3HB)	Sucrose/gluconate	63
Chromobacterium violaceum	P(3HB)	Fructose, gluconate, propionate, hexanoate	64
	P(3HV)	Valerate	
Recombinant *E. coli*	P(3HB)	Glucose	65
	P(3HB-*co*-3HV)	Glucose + propionic acid	39
	P(3HB-*co*-3HV-*co*-3HHx)	Dodecanoic acid + valeric acid/heptanoic acid/nonanoic acid	66

P(3HB) homopolymer is a highly crystalline, brittle, and stiff material [3]. It was found that the physical properties of P(3HB) homopolymer could be improved by producing higher molecular weight polymers. This polymer was produced by heterologous expression of cloned PHA biosynthesis genes from *C. necator* in recombinant *E. coli* [79].

In contrast, P(4HB) homopolymer possesses almost similar characteristics as ultrahigh-molecular-weight polyethylene. It could be considered as a strong elastic material since its tensile strength and elongation at break is 104 MPa and 1000% as compared to P(3HB), which is only 40 MPa and 6%, respectively.

The introduction of secondary monomers is a common strategy to produce better and more processable polymers. PHA copolymer and terpolymer are less crystalline and more elastic materials. It could be observed that incorporation of other monomer in the 3HB polymer chain significantly improved the physical properties of resulting copolymers such as P(3HB-*co*-4HB), P(3HB-*co*-3HV), and P(3HB-*co*-3HHx). PHA terpolymers are known to be better materials compared to copolymers [46, 52, 53, 66, 80, 81]. P(3HO-*co*-3HHx), which is an MCL_{PHA}, is known to have high tensile strength and elongation to break due to the orientation of the amorphous rubbery chains [42].

Incorporation of more than one secondary monomer is assumed to result in improved properties. It has been demonstrated that, P(3HB-*co*-3HV-*co*-3HHx) terpolymer with higher amorphousness could be produced [52]. No melting peak was detected in the terpolymer with 39 mol% 3HV and 3 mol% 3HHx. P(3HB-*co*-3HV-*co*-4HB) with 93 mol% 4HB and 3mol% 3HV exhibited elongation of 430%, toughness of 33 MPa, and Young's modulus of 127 MPa, which is similar to low-density polyethylene, while terpolymer with 55 mol% 4HB and 34 mol% 3HV showed a Young's modulus of 618 MPa, similar to that of polypropylene [46].

The properties of PHA copolymers and terpolymers are almost similar to commercially available synthetic plastics. Physical properties of some well-known PHA polymers are compared with polypropylene and low-density polyethylene in Table 8.2. Formation of copolymer could be controlled at microstructure level as well, and the arrangement of monomer determines the property of a resulting copolymer [83]. The distribution of monomers is usually controlled via chemical synthesis by varying the feeding method of substrates involved. A copolymer could be classified as a random, block, or blend copolymer. This depends on the arrangement of monomers in the polymer chain. A random copolymer has identical alternating monomer arrangements throughout its length. On the other hand, a block copolymer consists of polymer chain containing two or more unique polymer regions covalently bonded together [84, 85]. A blend copolymer is made up by two or more distinct homopolymer or copolymer chains of the monomers present. The randomness of a copolymer is usually determined by ^{13}C-NMR spectroscopy based on dyad sequence analysis [86–88].

Besides the incorporation of secondary monomers, blends of PHA polymers with other biodegradable materials have also been generated. These blends are

TABLE 8.2 Comparison of Properties of Some PHA Copolymers and Terpolymers and Common Plastics

Polymer	T_m[a] (°C)	T_g[b] (°C)	Crystallinity (%)	Tensile Strength (MPa)	Elongation at Break (%)
P(3HB)	177	4	60	43	5
P(4HB)	60	−50	34	104	1000
P(3HB-*co*-20 mol% 3HV)	145	−1	56	20	50
P(3HB-*co*-16 mol% 4HB)	150	−7	45	26	444
P(3HB-*co*-10 mol% 3HHx)	127	−1	34	21	400
P(3HB-*co*-39 mol% 3HV-*co*-3 mol% 3HHx)	ND	−7.9	ND[c]	12	408
P(3HB-*co*-94 mol% 4HB-*co*-3 mol% 3HV)	54.8	−51.6	ND	14	430
Polypropylene	176	−10	50−70	38	400
Low-density polyethylene	130	−36	20−50	10	620

[a] Melting temperature.
[b] Glass transition temperature.
[c] ND = not detected.

Source: From [33, 46, 52, 82].

physical mixtures of structurally different polymers either as homogeneous or heterogeneous phases in amorphous regions on a microscopic scale at equilibrium [33]. The mixture may exist as a single phase (thermodynamically miscible) or two distinct phases (thermodynamically immiscible). The physical property of a blend depends on the phase structure. Examples of some PHA blends are poly(ethylene oxide), poly(vinyl alcohol), and poly(β-propiolactone) (for a review see Sudesh et al. [33]). PHA polymers can be designed and developed to suit specific needs by varying their physical properties. This idea has been realized from genetic, biological, and chemical synthesis approaches.

Biosynthesis of Poly(3-Hydroxybutyrate-co-4-Hydroxybutyrate) [P(3HB-co-4HB)]. The biosynthesis of P(3HB-*co*-4HB) copolymer by microorganisms can be achieved by employing different methods of carbon source feeding. The copolymer could be produced from mixtures of two different carbon sources such as butyric acid and 4-hydroxybutyric acid [55]. Second, utilization of a single carbon source that is structurally related to 4HB such as 4-hydroxybutyric acid, γ-butyrolactone, or 1,4-butanediol also results in the biosynthesis of P(3HB-*co*-4HB) [3]. Recombinant *E. coli* has been known to produce P(3HB-*co*-4HB) copolymer by using glucose as the sole carbon source [89]. This recombinant expresses succinic acid semialdehyde dehydrogenase, 4-hydroxybutyric acid dehydrogenase, and 4-hydroxybutyric acid-CoA transferase gene from *Clostridium kluyveri* with the PHA synthase from *C. necator*. The strategy of using the

mixtures of two different carbon sources has been the most preferred method because it can produce P(3HB-*co*-4HB) with a wide range of monomer compositions. For the generation of 3HB monomer, carbon sources such as fructose, sucrose, glucose, and butyric acid are commonly used. On the other hand, 4-hydroxybutyric acid, 1,4-butanediol, and γ-butyrolactone are used for 4HB generation.

Several bacteria possessing SCL_{PHA} substrate-specific synthase are capable of incorporating 4-hydroxybutyrate monomer [90]. The choice of carbon sources depends on the type of microorganisms used. To date, several wild-type bacteria have been identified to produce P(3HB-*co*-4HB). They are *C. necator*, *Cupriavidus* sp. USMAA1020, *Cupriavidus* sp. USMAA2-4, *A. latus*, *D. acidovorans*, *Comamonas testosteronii,* and *Hydrogenophaga pseudoflava*. Among these microorganisms, *D. acidovorans* seems to have the most suitable metabolic pathways for the biosynthesis of P(4HB) homopolymers as well as P(3HB-*co*-4HB) copolymers from two-stage cultivation [57]. Biosynthesis of P(3HB-*co*-4HB) copolymer involves different metabolic pathways with reference to the type of carbon source used in order to generate 3HB and 4HB monomers (Fig. 8.3).

P(4HB) and its copolymer P(3HB-*co*-4HB) are potentially the most well-established PHA in the field of medical sciences. The incorporation of 4HB monomers results in copolymers having various physical properties that range from highly crystalline to elastomeric materials [55]. The physical and mechanical properties are shown in Table 8.3. Based on the elongation at break values, P(3HB-*co*-4HB) copolymers with high 4HB fraction are more elastic materials compared to homopolymers of 4HB. Besides this, the copolymers are less crystalline with lowered melting temperatures. The decrease in crystallinity may be due to the disruption of crystallization by the 4HB monomers because it possesses a longer backbone than that of 3HB monomer [47]. These interesting elastomeric polymers are currently under development for medical applications [82].

8.2.3 Biodegradability

Intracellular and extracellular degradation of PHA has been investigated in detail in a number of microorganisms. PHAs are degraded internally by intracellular PHA depolymerases of PHA accumulating bacteria or extracellular PHA depolymerases of PHA degrading bacteria. Intracellular degradation occurs when the bacteria is stressed under carbon limitation conditions. Accumulated PHA granules are hydrolyzed by the cells as carbon and energy source [93, 94]. P(3HB) is broken down to 3-hydroxybutyric acid by the PHA depolymerase and oligomer hydrolase [95]. The 3-hydroxybutyric acid is then oxidized by a dehydrogenase to acetylacetate. Acetyl-CoA is generated once β-ketothiolase reacts upon acetylacetate and is utilized for cell regeneration. Since both PHA synthase and PHA depolymerase are present in PHA accumulating microorganisms, a study was done to identify the rate of polymer hydrolysis to synthesis. According to Doi et al. [96], the polymer hydrolysis rate

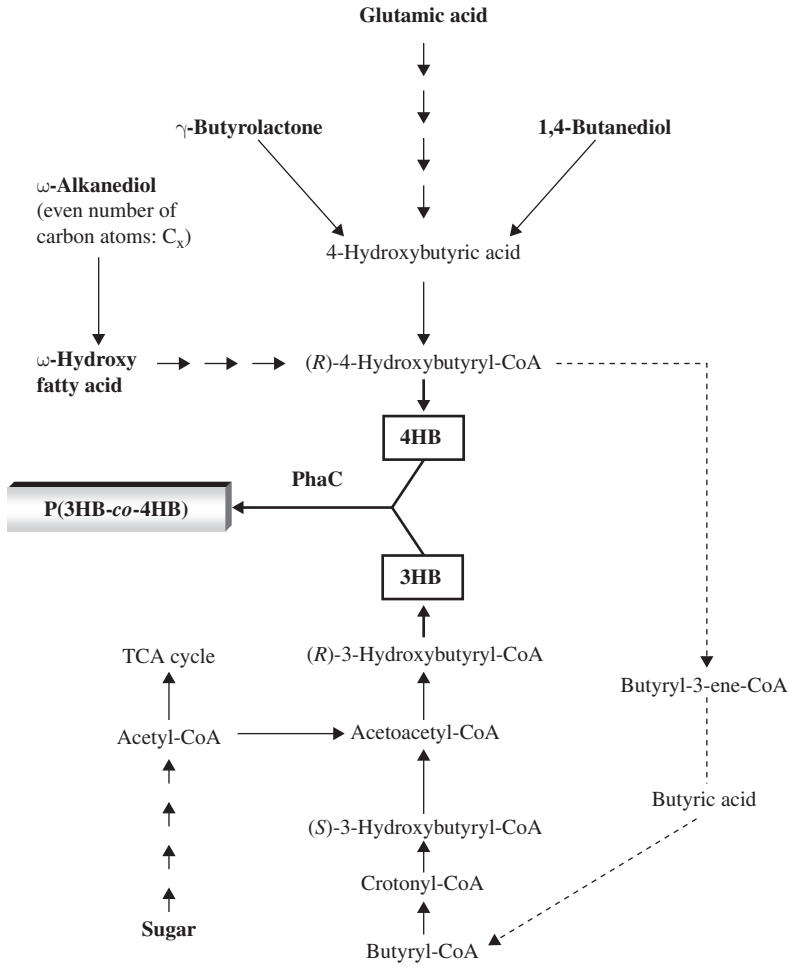

FIGURE 8.3 P(3HB-*co*-4HB) copolymer could be synthesized from different types of carbon sources. The P(3HB-*co*-4HB) biosynthesis pathway involves a combination of different metabolic pathways with reference to the carbon source used.

in *C. necator* is about 10 times lower compared to its synthesis in a nitrogen-free medium supplemented with an excess carbon source. Therefore, it is important to obtain optimum culture conditions in order to maximize PHA production with minimal hydrolysis.

In the natural environment, extracellular depolymerases are secreted by microorganisms to hydrolyze the PHA polymer. The soluble fraction after hydrolysis normally consists of mixtures of oligomers or monomers. These products are then taken up for cell metabolism via absorption through the microorganism's cell wall [3]. The PHA polymers are degraded via surface

TABLE 8.3 Physical and Mechanical Properties of P(3HB-*co*-4HB) Copolymers

Strain	4HB Monomer Composition (mol%)	T_m[a]	T_g[b]	M_n[c](kDa)	Crystallinity (%)	Tensile Strength (MPa)	Elongation at Break (%)	Reference
C. necator	16	130	−7	ND	45	26	444	91
D. acidovorans	78	49	−37	163	17	42	1120	55
	100	53	−48	339	34	104	1000	
Cupriavidus sp. USMAA1020	23	152	−7	590	ND[e]	13	626	92
	45	162	−16(−40)[d]	410	ND	3	268	
	75	51(157)[d]	−45	260	ND	16	526	

[a] Melting temperature.
[b] Glass transition temperature.
[c] Molecular weight.
[d] Minor peak.
[e] ND = not determined.

erosion and the molecular weight of the polymer remains unchanged during the course of degradation. The end products of PHA degradation in an aerobic environment are carbon dioxide and water. In an anaerobic condition, methane is also produced. The rate of biodegradation is influenced by a number of factors including the microbial population in a particular environment, temperature, and the physical properties of the polymers themselves [97]. Copolymers were found to degrade at a faster rate compared to P(3HB) homopolymer due to low crystallinity and porous surface [98, 99]. PHA film was found to degrade in less than 50 days when placed on soil surface under tropical conditions, and the same films when buried in soil or mangrove sediments show a faster rate of degradation [33, 100, 101]. Extracellular depolymerases have been purified from various microorganisms isolated from natural environments such as soil, lake water, and seawater [98, 102–106].

In vivo degradation of PHA polymers in living systems enables these polymers to be exploited as biomaterials. In fact, 3-hydroxybutyric acid and 4-hydroxybutyric acid were identified as normal constituent of blood at concentrations between 0.3 and 1.3 mM in the extract of brain tissues of rat, human, and pigeon [107–110]. Degradation of PHA results in the formation of D-hydroxyacids. Usually, D-3-hydroxyacids include 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 3-hydroxyhexanoate (3HHx), and 3-hydroxyheptanoate (3HHp) monomers. Among all the D-3-hydroxyacids, 3HB is the most common degradation product. In the human body, 3HB is one of the main ketone bodies that is primarily produced by degradation of long-chain fatty acids in liver [111] and during the process of starvation or disease [112]. In normal healthy adults, 3HB can be found at a concentration of 3–10 mg per 100 mL of blood [113]. The concentration of ketone body levels in the blood of mammals can be increased from nutritional or therapeutic compositions resources. Ketone body exists in a linear form, cyclic oligomers, or derivatives of 3-hydroxyacids. The 3HB can be in the form of linear oligomers other than linear homooligomers of 3HB if administered with mixtures of acetoacetate, cyclic oligomers of 3-hydroxyacids, esters of the linear or cyclic oligomers, and esters of 3-hydroxyacids (other than 3-hydroxybutyric acid) or others.

The biodegradation of various PHA-based biomaterials have been investigated in vivo. It is important to understand this mechanism because when PHA material is used as scaffold in tissue engineering applications, the rate of degradation should equal the regenerative rate of tissue, whereas, when it is used as a drug carrier, the rate of degradation will determine the drug release rate [114]. Many successful studies of PHA degradation in living organisms have been demonstrated. Borkenhagen et al. [115] implanted polymer tubular structures made of P(3HB-*co*-5 mol% 3HV)-diol and poly[glycolide-*co*-(ε-caprolactone)]-diol blends as nerve guidance channels. As the fraction of P(3HB-*co*-5 mol% 3HV) increased from 8, 17, to 41 wt%, the total weight loss of the blends decreased from 88, 74, to 33%, respectively, after 24 weeks of implantation. This result shows that P(3HB-*co*-5 mol% 3HV) is not readily

degraded in vivo [115]. Besides this, biodegradation of PHA materials in living organisms should not lead to the formation of toxic compounds. In a study done by Shishatskaya et al. [116] sutures made from P(3HB) and P(3HB-*co*-3HV) were implanted intramuscularly for a period of up to one year. The implantation did not cause any acute vascular reaction or adverse effect. The in vivo degradation rate of P(3HB-*co*-4HB) is relatively high compared to other PHAs and it can be controlled by varying the 4HB monomer composition [117]. Therefore, P(3HB-*co*-4HB) copolymer has gained interest in a wide range of medical applications [118, 119]. Besides being degraded by depolymerases, 4HB can be degraded by eukaryotic lipases and esterases as well [117, 120]. This further aids the biodegradation of biomaterials possessing 4HB monomers in living systems.

8.3 CURRENT APPLICATIONS OF PHA

8.3.1 Natural Occurrence and the Function of Monomers

Discovery of low-molecular-weight P(3HB) in the human body is the strongest evidence to indicate that PHA is compatible and absorbable in our system. This low-molecular-weight PHB (c-PHB) exists as a complex compound with other macromolecules and is widely distributed in many organisms from different phyla, including human beings (Table 8.4). The formation of this complex alters the physical and chemical properties of c-PHB, thus allowing it to solubilize in both aqueous and hydrophobic regions of the cell. A weak non-covalent bond is formed when c-PHB binds with polyphosphate. However, when it is bound with proteins, a stronger complex is formed. The ability of this compound to dissolve in chloroform depends on the strength of its association with the macromolecules. Normally, c-PHB consists of approximately 200 3HB units and the molecular weight is about 12,000 Da. This c-PHB compound is located in the cytoplasm, membranes, lipoproteins, and intracellular fluids of the cells. The changes in the c-PHB properties facilitate the diffusion through the hydrophobic regions of the cell. The ability of c-PHB to

TABLE 8.4 Occurrence and Distribution of c-PHB in Various Organisms

Organism	Location of c-PHB	Concentration	Reference
Human	Plasma, serum albumin, plaque	0.6–18.2 mg/L	121
Pig	Heart, liver	362.5–492.3 μg/g	
Cattle	Lung, brain	282.9–575 μg/g	
Sheep	Intestine	1703.2 μg/g	122
Yeast	Whole cell	1201.5 μg/g	
Bacteria [*E. coli* (JM 101)]	Whole cell	400 μg/g	

dissolve different salts and facilitate its transportation across the hydrophobic membrane demonstrates its role in the cell's physiological process [123]. These complexes are believed to have an important role in the transportation of Ca^{2+} across the cell membranes and also may be involved in the extracellular deoxyribonucleic acid (DNA) material uptake. Besides the presence of c-P(3HB) or 3-hydroxyacids, low-molecular-weight P(3HB) has also been identified in human tissues. The first finding of P(3HB) was made by Reusch and co-workers in blood serum with a concentration of 0.6–18.2 mg/L [121]. They were identified as a complex of low-density lipoproteins and with the carrier protein albumin. P(3HB) was also detected in the human aorta and is known to form ion channels in vivo when a complex of P(3HB)–polyphosphate is formed [121, 124].

8.3.2 PHA in Therapeutic Applications

The biocompatibility of PHA in medical applications is well known. The evaluation process had been started and well documented over the past 20 years. Ever since, much effort has been devoted to produce suitable polymers with improved biomaterial properties. Therapeutic applications of ketone bodies such as the degradation products of PHA, namely 3HB and 4HB, have been well documented. It was reported that increasing levels of ketone in the blood is useful to control seizures, metabolic disease, parenteral nutrition, appetite suppression, increase cardiac efficiency, reduce protein catabolism, treat diabetes and insulin-resistant states, and treat the effects of neurodegenerative disorders and epilepsy [125, 126]. 3HB has been also evaluated as an energy source (intravenous administration) in humans and piglets [127, 128]. Potential usage of this intermediate substrate has been suggested in ocular surgery as an irrigation solution to maintain the tissues [129]. In recent years, more therapeutic applications of 3HB have been discovered. It was shown that 3HB can provide partial protection against dopaminergic neurodegeneration and motor deficits in an experimental model of Parkinson's and Alzheimer's disease [130, 131]. In a separate study on neuronal disorder, 3HB aided in preserving neuronal integrity and stability during glucose starvation [132, 133]. Xiao et al. [134] through an in vitro study on glial cells found that the sodium salt derivatives of 3HB aided in reducing cell apoptosis and enabled mobilization of extracellular Ca^{2+}, which resulted in an increase in cytosolic Ca^{2+}, concentration. In a more recent study, 3HB and its derivative (3-hydroxybutyrate methyl ester) significantly improved the proliferation of neuroglial cells in vitro, and later mice treated with these compounds demonstrated enhanced learning and memory when subjected to the Morris water maze experiment [135].

In the medical field, 4HB is also known as γ-hydroxybutyrate (GHB) and was initially applied as an intravenous anesthetic agent. This is because this compound could rapidly cross the blood–brain barrier and induce a sleeplike state without disturbing cardiovascular stability [136, 137]. Subsequently, 4HB was used to treat narcolepsy as it helped to enhance slow-wave sleep (SWS)

and rapid-eye-movement (REM) sleep and containing them at night [138]. The outcome from this study highlighted the importance of 4HB and resulted in the approval by the Food and Drug Administration (FDA) of the United States to use 4HB in research such as narcolepsy trials. The application of 4HB was also extended in alcohol addiction treatment including alcohol withdrawal syndrome as it was found to increase brain dopamine levels [139–141]. Previous studies have proven that 4HB is able to protect the brain and peripheral tissues from damaging effects due to excessive energy substrate consumption, which could be caused by anoxia or excessive metabolic demand [142].

Sodium 4HB is used for therapeutic applications due to commercial unavailability of pure 4HB. Initially, oral application of 4HB salts was not possible due to strong hygroscopicity. To overcome this problem, development of nonhygroscopic salts was investigated. To date, 4HB has not yet been patented because it is a naturally occurring compound and has a relatively low cost of production [143]. The occurrence of 4HB has been detected in the mammalian living system, whereby it was found in the brain, lung, liver, heart, kidney, and muscle tissues [144]. Nevertheless, several methods describing the controlled release of 4HB for alcohol, heroin, as well as nicotine addiction has been reported, and the use of 4HB as an anesthetic agent has been evaluated.

Following are other possible therapeutic applications of 4HB as suggested by Williams [145]: chronic schizophrenia, catatonic schizophrenia, atypical psychoses, chronic brain syndrome, neurosis, Parkinson's disease, other neuropharmacological illnesses, hypertension, ischemia, circulatory collapse, radiation exposure, cancer, and myocardial infarction. Applications may also be extended for uses with growth hormone production, heightened sexual desire, anorectic effects, euphoria, smooth muscle relaxation, and muscle mass production.

8.3.3 Applications of PHA in the Medical Field

Biocompatibility and biodegradability are the main driving forces behind the development of medical devices from PHA material. Most of the products derived from PHA are channeled toward cardiovascular applications [146]. Cardiovascular diseases are increasing at an alarming rate, especially in most of the developed countries. In the United States alone, approximately 40,000 babies are born with fatal cardiovascular diseases [147]. Half of them die before they reach one year of age. In total, more than 1 million Americans suffer from hereditary cardiovascular defects. The modern lifestyle has resulted in uptake of unbalanced diet and less physical exercise. These have become major contributing factors of cardiovascular diseases. In cardiovascular treatments, PHA has been considered for use as heart valves, cardiovascular fabrics, pericardial patches, and vascular grafts.

PHA polymers are found to be potential materials to be used in wound management (suture, skin substitutes, nerve cuffs, surgical meshes, staples, and swabs), orthopedics (scaffolds for cartilage engineering, spinal cage, bone graft substitutes, meniscus regeneration, and internal fixation devices), and drug

delivery applications [146, 148]. P(3HB) and P(3HB-*co*-3HV) [116, 149–151] were the first polymers to be evaluated for medical purposes. However, their poor physical and mechanical properties have caused some limitations to further development to suit specific applications. Since the PHA family is rich and diverse with various monomers, the above limitation was overcome by either incorporating different monomers or increasing certain monomer compositions. This resulted in the investigation of P(3HO-*co*-3HHx), P(3HB-*co*-3HHx), P(3HB-*co*-4HB), and P(4HB), polymers for medical applications. Among these PHA polymers, some are already being produced on a commercial scale. However, limited supply of these polymers in medical grade has become a major setback for broad utilization in medical and pharmaceutical applications. P(4HB) is one of the most interesting polymer possessing very elastic and strong properties compared to other polymer. The mechanical and physical properties could be regulated by controlling the 4HB monomer composition. In terms of strength, P(4HB) possesses comparable value with commercially absorbable sutures such as Maxon and PDS II.

Tepha has been evaluating the biocompatibility of P(4HB) polymer as per a recommendation by the Office of Device Evaluation in a series of tests that includes cytotoxicity, sensitization, irritation and intracutaneous reactivity, hemacompatibility, endotoxin, and implantation [82]. The P(4HB) produced by Tepha is known to meet the standards set by the FDA for endotoxin levels even though it is produced by a gram-negative microorganism. A recent breakthrough was the approval of the FDA for the use of TephaFLEX bioabsorbable suture derived from P(4HB) (Tepha Medical Devices, www.tepha.com. Accessed March, 17, 2009). TephaFLEX monofilament suture is 30% stronger, more flexible, and has longer strength retention than currently marketed resorbable sutures.

8.3.4 Endotoxin Removal

Most of the commercially available PHA is produced by the large-scale fermentation of gram-negative bacteria. Since it is produced in bulk quantities, the quality of the polymers are of industrial grade. They might contain residual proteins, surfactants, and /or high levels of endotoxin which is a potent pyrogen (lipopolysaccharides present in gram-negative bacteria). In a previous study by Garrido [152], the presence of cellular debris in the industrial samples of P(3HB-*co*-3HV) was detected. On the other hand, Rouxhet and co-workers detected contaminants on the surface of PHA polymers by using X-ray photoelectron spectroscopy [153]. For medical usages, the PHA production and purification strategy has to meet specific requirements to obtain regulatory approval. Before the PHA material can be employed for medical applications, it is necessary to ensure that it is of a high level of purity in order to prevent immunogenic reactions and acute inflammatory effects. More than 120 endotoxin units per gram were detected in industrial-grade P(3HB) [1]. Previously, it was implemented that the endotoxin level should be maintained at a value of less than

20 US Pharmacopeia EU/mL. However, recently a more stringent regulation was set by the FDA, whereby the endotoxin level should not be more than 0.5 EU/mL. In case the biomaterial comes in contact with cerebrospinal fluid, the endotoxin level should be at a minimal value of 0.06 EU/mL [154, 155]. Oxidizing agents such as hydrogen peroxide and benzyl peroxide or sodium hydroxide are normally used to remove the endotoxins [156, 125]. The PHA produced from gram-positive bacteria are potentially more suitable material for biomedical applications since they lack endotoxin [62].

8.3.5 Sterilization of PHA Biomaterials

In the medical field, the sterilization process is very important in order to ensure the prevention of infections during the use of operation devices and implantation materials. The methods for the sterilization process normally depend on the type of devices or materials. Steam sterilization is the most common method of sterilization. Some PHA polymers possessing higher melting temperature ($>140^{\circ}C$) and found to be thermally stable at this temperature, can be subjected to steam sterilization. However, for polymers with lower melting temperatures that are heat sensitive such as P(4HB) or copolymers of P(3HO-*co*-3HHx), this method of sterilization is not suitable [146]. Cold-cycle sterilization has been proposed as a more suitable approach. Ethylene oxide is the most popular organic solvent used in this type of sterilization [157]. It is known that ethylene oxide does not cause any changes in the properties of these polymers. For example, P(3HO-*co*-3HHx) copolymer with low melting temperature is sterilized using a cold cycle for 8 h at $38^{\circ}C$ with 65% humidity over a 1-week period [157]. The residual ethylene oxide level detected was less than 1 ppm after this cycle.

Beside ethylene oxide, γ-irradiation was also reported as another method for sterilizing the PHA polymers such as P(3HB), P(3HO-*co*-3HHx), and P(3HB-*co*-3HV). Holmes [78] investigated γ-irradiation on P(3HB) at doses on the order 2.5 Mrad. It was stated that possible reduction in molecular weight might be observed after treatment. The mechanical properties of P(3HB) and P(3HB-*co*-3HV) were significantly affected when higher doses of γ-irradiation was applied (10–20 Mrad) [158]. Luo and Netravali [159] also made similar observations, whereby the mechanical properties and molecular weight of P(3HB-*co*-3HV) was affected when the same range of γ-irradiation doses were used. Molecular weight loss of approximately 17% was identified due to random chain termination followed by physical crosslinking of P(3HO-*co*-3HHx) copolymer, which was exposed to γ-irradiation at doses of 2.5 Mrad at room temperature [157]. In a recent study, P(3HB-*co*-4HB) films were subjected to 2.5 kGy γ-irradiation, and a drastic reduction in the molecular weight of 37% was observed. However, no changes were detected on the surface morphology of these films from scanning electron micrograph (SEM) [160]. Based on the above studies, it is shown that there are limitations according to different methods of sterilization. Therefore, proper sterilization methods

should be carefully selected based on the suitability and stability of the polymers.

8.4 IN VITRO TISSUE RESPONSE AND DEGRADATION

P(3HB) has been found as normal metabolite compounds in cellular membranes of animals [121] and in human blood [161] in small quantity. Toxicity testing according to USP XXII and ISO 10993 has revealed that it is suitable for use as implant material [162]. The in vitro cytotoxicity test of the P(3HB) microspheres on Wistar rat exhibited growth and metabolic activity of fibroblasts [163]. Recent genotoxicity tests on P(3HB) using *Salmonella* mutagenicity test revealed that P(3HB) is nongenotoxic and does not change the expression of protooncogenes and antiapoptotic genes on fibroblast MRC-5 cell lines such as p53, c-myc, and bcl-xl [164]. The mouse fibroblast L929 cellular response on the PHA oligomers showed that the oligomer concentration exceeding 40 mg/L reduced cell viability, increased cell apoptosis, cell death, and resulted in lower cell proliferation [165]. It was found that the presence of P(3HB) oligomers did not affect the viability of hamster V79 fibroblasts and murine melanoma B16 (F10) cells [166].

Better cell viability was detected on P(3HB) surfaces compared to poly-L-lactic acid (PLLA) when L929 cells were used [167]. Besides L929 cells, Chinese hamster lung (CHL) fibroblasts also showed superior cell compatibility on P(3HB) films. P(3HB) melt-spun fibers have been fabricated and tested with National Institutes of Health (NIH) 3T3 mouse fibroblasts. The cells remained highly viable on P(3HB) and 3HV copolymer films [150]. In a recent study, attachment efficacy, proliferation, and survival of adult rat Shwann cells (SC) and bone marrow stem cells on P(3HB) fibers and P(3HB) fibers coated with fibronectin, laminin, and collagen IV were examined in vitro. The effect of SC and bone marrow stem cells with/without the combination of the above extracellular matrix (ECM) on neurite outgrowth was also investigated (for a review see Tabesh et al. [168]).

Limited cell attachments and proliferation can be attributed to the hydrophobicity of the P(3HB) solution-cast film surfaces, which limits the absorption of water on the surfaces [167, 169]. In order to improve the surface absorption of water, implantation of the carboxyl ion and the oxygen plasma treatment have been tried [169, 170]. The adhesion of L929 cells on P(3HB) films was improved after modifications were done on the films, including ammonia plasma treatment [171] and coating of fibronectin [172]. Hydrolysis by lipases or alkaline solutions on unmodified P(3HB) films was found to increase the viability of L929 cells [118, 173, 174]. The cells viability can be further improved by lipase treatment on porous P(3HB) scaffolds. However, a contradicting observation was found on P(3HB) films coated with hyaluronic acid, even though the water absorption has been heightened by this modification [175]. Surface treatment with acidic or alkaline solutions were also found to improve the proliferation of P(3HB)-based wools [176, 177].

Blends of P(3HB) with other compounds have generally resulted in significant improvement of the biocompatibility of the P(3HB) polymer. Hydroxyapatite (HA) addition on the P(3HB) scaffolds contributed to better cell viability and alkaline phosphatase activity [178]. Besides that, various polysaccharides such as hyaluronic acid, chitosan, pectin, or alginic acid were individually immobilized into P(3HB) and P(4HB) matrices in order to promote more porous surfaces. The P(3HB)- and P(4HB)-blended cast films had excellent porous properties and thus significantly promoted human keratinocytes cell (HaCaT) growth. Cell growth was found to decrease according to the type of blend in the following order: P(4HB)/hyaluronic acid > P(4HB)/chitosan > P(3HB)/hyaluronic acid > P(3HB)/chitosan > P(3HB)/pectin > P(3HB)/alginic acid [179].

The P(3HB-*co*-3HV) copolymer is known to possess improved physical properties due to the incorporation of 3HV. The improvement in its properties was evaluated for usage in medical applications. P(3HB-*co*-3HV) films containing 7, 14, and 22 mol% of 3HV were found to persuade a mild toxic effect in the direct contact or agar diffusion tests [180]. P(3HB-*co*-22 mol% 3HV) in saline extracts has the capability to induce a noticeable hemolytic reaction [181]. However, a lower 3HV composition (5 mol%) did not exhibit any toxic effect in the bacterial bioluminescence test for 16 weeks [182]. In addition, P(3HB-*co*-3HV) with lower 3HV molar fraction was also found to be more compatible for proliferation of L929 fibroblasts and cytokine production [183]. P(3HB-*co*-3HV) with different 3HV compositions (15 and 28 mol%) was found suitable to promote better cell growth [150]. Regardless of the 3HV monomer composition, the proliferation rate of human scoliotic fibroblasts from spinal ligaments on the polymer surface was very low [181]. Recently, the use of P(3HB-*co*-3HV) as matrix material to induce bone formation has been investigated in vitro and was suggested as a suitable approach for bone tissue regeneration in vivo [184–186].

Some studies revealed that the attachment and proliferation of cells on P(3HB-*co*-3HV) copolymers were less compared to P(3HB). For example, the viability of human fibroblasts on P(3HB-*co*-7 mol% 3HV) surfaces was poorer as compared to P(3HB) [167]. Similarly, unmodified P(3HB-*co*-15 mol% 3HV) films contributed to limited NIH 3T3 fibroblasts adhesions and growth. To improve this situation, the copolymer was remedied by treatment with perchloric acid and potassium chlorate with the aim to introduce oxygen-based functional groups on the film surfaces [187]. Besides that, oxygen plasma treatment on the surface of P(3HB-*co*-8 mol% 3HV) films was found to enhanced the viability of rat bone marrow fibroblasts and alkaline phosphatase activity [185, 186]. Similar modification has also resulted in the enhanced attachment rate of human retinal pigment epithelium cells [188]. However, when plasticizer was added with P(3HB-*co*-3HV), it caused a decrease in compatibility of tested NIH 3T3 fibroblasts. This phenomenon occurred due to the leaching of the plasticizers [189]. Besides plasticizers, residue solvents left from film casting are attributable to potential leaching during in vitro cell cultures [162].

Other surface modifications of P(3HB-*co*-3HV) films such as hyaluronic acid coating, chitosan, and acrylic acid grafting also led to enhanced L929 fibroblast

attachment and proliferation. Results showed that chitosan grafting was superior in terms of cell attachment but poorer in cell growth as compared to hyaluronic acid modification [190]. Besides this, immobilization of collagen I with the aid of ozone treatment and methacrylic acid grafting on P(3HB-*co*-8 mol% 3HV) films attributed to increased mouse osteoblast and rat osteosarcoma cells growth [191].

A wide range of in vitro studies have been done with P(4HB) and P(3HB-*co*-4HB) to evaluate its level of biocompatibility. Under strict regulations by the FDA and careful examination by Tepha, P(4HB) has been known as an excellent biomaterial with high tolerance in vivo. Cell culture studies have proven P(4HB) to be a nontoxic material. For example, studies done using L929 mouse fibroblast and ovine vascular cells showed successful amount of cell proliferation and adhesion [192]. P(4HB) had been evaluated as a coating material for polyglycolic acid (PGA) matrices. The P(4HB) scaffold has been successfully seeded with various types of ovine and human cells. Cellular coagulation had been observed after P(4HB) and its copolymer comes in contact with human blood. In some studies, thrombus formation in vivo rabbit aorta and sheep aorta were reported by Optiz et al. [193, 194]. However, no such findings were made with PGA/P(4HB) heart valves or pulmonary artery patches in sheep [195, 196].

The degradation intermediate of 4HB is a natural metabolite present in the human brain, heart, lung, liver, kidney, and muscle [144]. This metabolite has a short half-life of just 35 minutes and is rapidly eliminated from the body via the Krebs cycle [197]. The identified pharmaceutical application of 4HB has been for treatment of cataplexy attacks in patients with narcolepsy. P(4HB) and P(3HB-*co*-4HB) degrades at a faster rate due to less crystallinity compared to P(3HB). Mass loss in the molecular weight of these films was detected after incubation in a buffer solution [125, 126]. Increasing amounts of 4HB composition in P(3HB-*co*-4HB) copolymer resulted in increased rate of hydrolysis. After 160 days of incubation in the buffer solution (37°C, pH 7.4), P(3HB-*co*-9 mol% 4HB) and P(3HB-*co*-16 mol% 4HB) films exhibited 60 and 40% loss in molecular weight [102, 198].

In a recent study, Siew and co-workers demonstrated the cytotoxicity of P(3HB-*co*-4HB) films with V79 and L929 cells using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT] assay, acridine orange/propidium iodide staining, and alkaline comet assay [199]. The P(3HB-*co*-4HB) resulted in comparable cell viability with the clinically used Polyglactin 910, and the alkaline comet assay revealed no genotoxic effects. It was reported that γ-irradiation of P(3HB-*co*-4HB) resulted in a decrease in molecular weight but no changes were observed in the morphology. The possibility of mutagenic and clastogenic effects from these poststerilized P(3HB-*co*-4HB) films were investigated [141]. Ames and *umu* tests (*S. typhimurium* strains) and Micronucleus assay (V79 fibroblasts) were carried out, and the results indicated that leachables of poststerilized P(3HB-*co*-4HB) caused no mutagenic and clastogenic effects. Similar studies on the toxicity effects of P(3HB-*co*-4HB) have

been also done and positive results were obtained by observing good cell viability [92, 119]. The influence of P(3HB-*co*-4HB) composition ratio and drug loading level (*Mitragyna speciosa* crude extracts) on the biocompatibility of P(3HB-*co*-4HB) were investigated, and a comparable result with poly(lactic-*co*-glycolic acid) (PLGA) (positive control) was obtained [119]. All the findings above provide strong evidence that P(3HB-*co*-4HB) has great potential to be further studied and developed as biomaterials.

SCL_{PHA}–MCL_{PHA}, P(3HB-*co*-3HHx) has been evaluated with L929 cells, rabbit stromal cells, and recently in HaCaT culture studies to determine its level of biocompatibility. Various experiments were carried out on surface modification of P(3HB-*co*-3HHx) films in order to improve cell viability. This includes ion implantation [169], treatment with lipases [118, 173, 174] and alkaline hydrolysis [118, 173]. However, in general, no significant differences were noticed between P(3HB) and P(3HB-*co*-3HHx). Surface hydrolysis using lipase or alkaline medium was found to improve cell viability. However, in these studies it was observed that surfaces having higher hydrophilicity and smoothness resulted in increased fibroblast viability.

Conversely, in a subsequent study, surface roughness of the P(3HB-*co*-3HHx) films were found to promote attachment and differentiation of osteoblasts-like cells [167]. Cell viability on P(3HB), P(3HB-*co*-3HHx), and PLLA films decreased in the following order: P(3HB-*co*-12 mol% 3HHx) > P(3HB-*co*-5 mol% 3HHx) > P(3HB) > P(3HB-*co*-20 mol% 3HHx) > PLLA. This was also observed in another study whereby porous scaffolds made by solution cast/salt-leaching improved the cell viability and differentiation of osteoblasts [200]. In vitro degradation study of P(3HB-*co*-12 mol% 3HHx) in simulated body fluid (SBF) (pH 7.4, 37°C) exhibited 7% mass loss compared to 3% for P(3HB) after 50 days of incubation [167].

Zheng et al. [49] tested rabbit articular-derived chondrocytes on solution-cast films of P(3HB), P(3HB-*co*-3HHx), and their blends. Blends containing equal amounts of both polymers had the highest adhesion of chondrocytes. The blends were found to possess high surface free energy and highest absorption of protein. Similar observation was made on porous blend scaffold material which showed higher amount of chondrocytes growth within 28 days [200, 202]. The chondrocytes were initially observed on the surface but later into the open pores on the scaffold. It was found that higher amounts of glycosaminoglycans and collagen were produced from polymer blends. P(3HO-*co*-3HHx) copolymer films were subjected to a mixed population of ovine cells in cell culture experiments [192, 203, 204]. A lower number of cell adhesion and lesser collagen deposition were found on the P(3HO-*co*-3HHx) films compared to PGA mesh but was almost similar with P(4HB) films.

In general, a slower rate of P(3HO-*co*-3HHx) degradation was identified in separate studies. Solution-cast films of P(3HO-*co*-18 mol% 3HHx) were found to be very resistant to hydrolysis after 20 weeks of incubation in a buffer solution (pH 10, 37°C). It was also evident in an enzymatic degradation study [205]. Marois et al. [206] investigated long-term (24 months) in vitro

degradation on solution-cast P(3HO-*co*-3 mol% 3HHx) films in water and buffer solution (pH 7.4). Only a simple hydrolytic degradation was observed with relatively slow molecular weight and mass loss.

PHA terpolymer of P(3HB-*co*-3HV-*co*-3HHx) has been recently investigated for cell viability [80]. The terpolymer was tested along with other polymers such as poly(lactic acid) (PLA), P(3HB), P(3HB-*co*-3HV), P(3HB-*co*-3HHx), and P(3HB-*co*-4HB). HaCat cells showed the strongest viability and the highest growth activity on the terpolymers compared with the others. P(3HB-*co*-3HV-*co*-3HHx) nanoparticles were prepared with size ranging from 200 to 350 nm, and it was found that the nanoparticles could increase the cellular activities by stimulating a rapid increase of cytosolic calcium influx in the cells. In a more recent study, human bone marrow mesenchymal stem cells (MSCs) showed greater cell number and adhesion compared to tissue culture plates (TCPs), PLA films, and P(3HB-*co*-3HHx) films [81]. The cell viability was statistically analyzed and the cell number on the terpolymer films was reported to be 115% higher than that on TCPs, 66% higher than on the P(3HB-*co*-3HHx) film, and 263% higher than on the PLA films ($p < 0.01$). The terpolymers were also found to support osteogenic differentiation of MSCs. The obtained results suggest that P(3HB-*co*-3HV-*co*-3HHx) could be fabricated as a suitable biomaterial for bone tissue engineering.

8.5 IN VIVO ASSESSMENT AND POTENTIAL APPLICATIONS

8.5.1 Poly(3-Hydroxybutyrate) [P(3HB)]

Wound Management and Tissue Regeneration. P(3HB) was first suggested for medical applications in 1962. There were ideas of using P(3HB) as biodegradable surgical sutures and as films to support tissue healing of injured arteries, blood vessels, prosthetic devices (tubes for healing severed blood vessel or ureter), and support devices for hernia repair. P(3HB) patch was used for the repair of soft-tissue defects by Behrend and co-workers [207]. Resorbable scaffolds of P(3HB) with a smooth surface on one side and porous on the other was used for the closure of lesions in the gastrointestinal tract in rats. The patch was first investigated in vitro, whereby moderate adhesion of mouse and rat intestine fibroblasts were observed on the patch. An in vivo study was later carried out on the stomachs of rats. P(3HB) patches were sutured over incisions in the stomachs. Better tissue regeneration was identified on the porous side of the implant as compared to Vicryl pathes [207].

A good cell compatibility of P(3HB) had been observed from various cell culture studies. NIH 3T3 and L929 mouse fibroblasts showed high cell viability on P(3HB) film surface while adhesion of L929 cells were detected on melt-spun P(3HB) fibers [150, 167, 208, 209]. Similar observation was also made with Chinese hamster lung fibroblasts [210]. Cell viability has been found to improve

greatly when the surface of P(3HB) films is pretreated with lipase or alkaline solution [118, 173]. It is evident that cell adhesion increases with porosity of the film surface, thus other methods of processing and modification have been adapted to increase the porosity, which include fibronectin coating [172] and ammonia plasma treatment [171].

Besides this, P(3HB) blends with β-butyrolactone and textile composites were fabricated in order to reduce stiffness and enhance hydrolysis [211]. The blend of P(3HB/at-3HB) at a 70/30 ratio was used for gastrointestinal patches to repair the bowel defect in rats. The patches were implanted onto the gastric wall of rats, and over 2 weeks time an interesting observation was made whereby messenger RNA (mRNA) encoding for pancreatic enzymes was detected [212]. Recently, Kunze et al. [213], implanted patches of P (3HB)/at-P(3HB) blends at different ratios in minipigs to support healing of dural defect. It was observed that the dura mater adhered onto the porous surface of the patch and the smooth surface helped to prevent brain tissue adhesion. P(3HB) was also fabricated into tubes for tissue healing of vessels and hollow organs. It was found that P(3HB) tubes could withstand stressful conditions due to constant secretion of the intestinal tract for a period of time before degrading.

Drug Delivery. Application of P(3HB) in drug delivery studies was initiated in the 1980s. The advancement in controlled-release drug delivery system has made it possible to maintain the drug concentration at a specified level for a preferred period of time. P(3HB) tablets were fabricated for controlled drug release applications. The release of a model drug [7-hydroxyethyltheophylline (HET)] from P(3HB) tablet was studied by Korsatko and co-workers [214, 215]. The tablets were prepared by homogeneously compounding and compressing P(3HB) with HET. The in vivo drug release was slower compared to the in vitro system. In a later study, it was found that the molecular weight of P(3HB) influenced the rate of drug release. The release of antihypertensive drug midodrin-HCl increased with increasing P(3HB) molecular weight [216]. In contrast, when P(3HB) was compressed with the β-blocker celiprolol-HCl, a small amount of higher molecular weight P(3HB) resulted in slow release of this drug. Later, Kharenko and Iordanskii [217] evaluated the in vitro release of vasodilador diltiazem from P(3HB) tablets containing about 45% vasodilador diltiazem. Here, the concentration of the drug loaded determined the rate of drug release.

Melt-pressed P(3HB) disks were also evaluated for drug release applications [218, 219]. For this purpose, a combination of 5-fluorouracil (5-FU) (10−50%) and P(3HB) was melt-pressed into disks, and these disks were subjected to in vitro examination. The rate of drug release depended on the loaded drug concentration. It was found that 50% of the drug was released from the disk after 5 days of incubation. In two separate studies by Collins et al. [220] and Deasy et al. [221], a compression-molded P(3HB) loaded with tetracycline was tested for dental application for the treatment of gingivitis.

After a 10-day study period, an improvement in the inflammation conditions was observed among the patients. Potential application of P(3HB) for gastrointestinal application was evaluated by developing a gastric retention drug delivery device that could extend the absorption period of a drug in the stomach [222]. However, rapid degradation of the device was found to affect its integrity and overall gastric retention potential. P(3HB) polymer was also evaluated for a hormone release study. Subcutaneous implantation of P(3HB) containing gonadotropin-releasing hormone (GnRH) was employed in a sheep model [223]. The hormone is responsible for the stimulation of luteinizing hormone (LH) secretion to promote preovulatory follicle growth and ovulation. In a recent study, tailor-made oligo-3-hydroxybutyrate was evaluated for MTT-based drug resistance on transformed hamster V79 fibroblasts and murine melanoma B 16(F10) cells [166]. The oligomers were found to be biocompatible and able to vectorize the drug in the form of chemical conjugates.

PHA was found to be a good material as a drug carrier because of its biodegradable characteristics [114]. Rifampicin loaded microspheres of P (3HB) were investigated as chemoembolization agents [224]. The microspheres were prepared by solvent evaporation technique. Polyvinyl alcohol was used as an emulsifier, distilled water as the dispersion medium, and methylene chloride as the solvent. Upon evaporation of the solvent, granules in the range of 5–100 μm was formed. The pore size of microspheres plays an important role in controlling the drug release. The gravity field fractionation technique was used to classify the microspheres according to various sizes [224]. The rate of drug release was very rapid with almost 90% of the drug released within 24 h. It was shown that it is possible to control drug release with a precise particle pore size. So far, many experiments have demonstrated the controlled release of drugs from microspheres using SCL-PHA as the delivery agent [148]. Recently, Shishatskaya et al. [163] evaluated the biocompatibility of P(3HB) microspheres in Wistar rats for a period of 3 months. The intramuscular implantation resulted in mild inflammation, pronounced macrophage infiltration, no formation of fibrous capsules, and no adverse morphological changes, and tissue transformation was observed. These results suggest that P(3HB) is indeed a good material for fabricating prolonged-action drug release systems.

Cardiovascular Applications.

Artery Augmentation. In cardiovascular applications, P(3HB) has been evaluated for uses such as artery augmentation, pericardial patches, cardiovascular stents, and atrial septal defect repair. The evaluation of arterial tissue regeneration of the pulmonary artery using P(3HB) nonwoven patches and Darcon (polyethylene terepthalate) patches as scaffolds was reported by Malm and coworkers [225]. The scaffolds were removed between 3 and 24 months and the generation of arterial tissues was noticed. Interestingly, no aneurysms were observed either in P(3HB) or Darcon patches. The pore size in the nonwoven P(3HB) patch is small enough to prevent bleeding. Successful regeneration of

arterial tissue was observed on the P(3HB) patches. No inflammatory reaction was observed from P(3HB) patches unlike with the Darcon patches. The efficiency of nonwoven P(3HB) was also studied in the repair of atrial septal defect in claves [226]. The evaluation lasted for a period between 3 and 12 months. Regeneration of endothelial layers facing both right and left atrium were observed, including a subendothelial layer of collagen and smooth muscle cells. After 12 months, the patch was found to be degraded by polynuclear marcophages into small particles, and these macrophages still remained even 24 months after operation.

Pericardial Patches. Development of P(3HB) pericardial patches can be considered as a significant achievement in the application of biodegradable material in cardiovascular research. The patches were used to cover the pericardium and prevent adhesion between the heart and sternum [225, 226]. An initial study was done in sheep models. Their native pericardium was replaced with P(3HB) nonwoven patches. The patches were harvested between 2 and 30 months after operation. Pericardial patches were found to degrade at a slower rate compared to arterial tissue patches. After 24 months of implantation, the polymers still remained as small particles and later totally degraded after 30 months, leaving behind some macrophages marking the implant region. Adhesion was noticed and the pericardium was regenerated in all sheep that had received the patches. Later the application of P(3HB) patches was studied in calves, and the formation of adhesion was investigated [227]. However, no significant difference in adhesion formation was observed among the test animals.

Cardiovascular Stents. Cardiovascular stents fabricated from P(3HB) and its blend with triethyl citrate was evaluated in rabbit iliac arteries [228–230]. Upon the implantation into the arteries, proliferation was observed and later followed by the degradation of the stent between 16 and 26 weeks after the implantation.

Nerve Regeneration. Peripheral nerve injuries incurred during clinical practice such as surgery often lead to permanent disabilities [231, 232]. The usage of autologous nerve grafts is still the common clinical technique for nerve reconstruction even though other methods have been evaluated. Biomaterials such as PGA [233], PLGA [234, 235], gelatin [236–238], collagen [232, 234, 239, 240], chitosan [233, 238, 241], and silk [242, 243] have been investigated as nerve conduits. P(3HB) conduits have already been evaluated for nerve regeneration due to their structural integrity and biocompatibility. Besides this, the piezoelectric characteristic of P(3HB) is an added advantage for nerve repair and regeneration applications. Previously, conduits for peripheral nerve regeneration were formed with nonwoven P(3HB) sheets with unidirectionally oriented fibers. The sheets were wrapped around a 2- to 3-mm gap of the transected superficial radial nerve in cats [244, 245]. The fibers were located in

longitude position to support the growing axons. P(3HB) is considered as the most suitable polymer for this application due to its low degradation rate compared to other polymers. This is important to support the nerve for long-period regenerative processes and minimize the risk of accumulation of acidic compounds from P(3HB). It was noticed that the conduits became softer or fragmented with a reduction in size after 12 months of implantation.

Mosahebi and co-workers found that P(3HB) conduits can be used to bridge a 10-mm gap in the rat sciatic nerve [246]. The conduits were filled with alginate hydrogel with/without cultured allogeneic or syngeneic genetically labeled SC. Allogenic SC exhibited increased axonal regeneration without any deleterious immune reponse. Novikova and co-workers fabricated conduits from P(3HB) fibers [247]. The conduits were coated with alginate hydrogel and fibronectin and preseeded with SC before implantation. Results showed a reduction in spinal cord cavitation and retrograde degeneration of injured spinal tract neurons.

Orthopedic Applications.

Bone Repair. Bone has become a target of interest for tissue engineering studies. Tissue engineering of bone requires suitable matrices to be used as scaffold. Scaffold must act as a substrate for cellular attachment, proliferation, and differentiation. Besides this, cellular components together with growth and differentiation are considered as important elements in this application [184–186]. Tissue-engineered bone of osteoblasts and appropriate matrix can be used for the restoration of skeletal continuity and bone stock [248]. Osteoconductive matrices are fabricated from biodegradable materials of natural origin such as collagen, gelatin, hyaluronic acid, or synthetic polymers such as PLA, PGA, and their copolymers [249].

Biomaterial with a slower rate of degradation is preferred for orthopedic applications. P(3HB) is at an advantage due to its nature of slow degradation. Normally, easily degradable polymers are coated with P(3HB) before use, especially in load-bearing fracture fixation devices to slow down its rate of degradation [250]. The piezoelectric characteristic of P(3HB) is considered to be a special yet advantageous feature that makes it similar to natural bone [251]. With regards to this feature, P(3HB) composites are believed to aid in bone growth and the healing process [78]. Studies were carried out in vivo in various organisms. In rabbits, P(3HB) implants were tested for connecting osteotomies in the tibia and positive observation was made after 12 weeks [252]. In a separate study, P(3HB) material was used as osteosynthesis plates to repair fractures of the visceral cranium [253]. However, a contradicting observation was made in a similar approach to repair rhinobasal skull defects in minipigs using a P(3HB)/at-P(3HB) patch [213]. No differences were observed in the healing of test organisms with/without the polymer patches. P(3HB) films was used as an occlusive membrane for guided bone regenerations in the mandibula of rats [254, 255].

P(3HB)/HA composites were examined by Doyle and co-workers for bone repair purposes [256]. Incorporation of HA forms a bioactive and biodegradable composite for use in hard tissue replacement and regeneration. These P(3HB)/HA composites were found to resemble the mechanical properties of cortical bone but had limited strength and ductility. The in vitro experiment in SBF showed the formation of a bonelike apatite layer on the composites within a short period of time. The properties of P(3HB)/HA composites have been altered by controlling the composition of HA [257, 258]. The toxicity of P(3HB) and HA has been evaluated with human osteoblast CRL-1543 cells and comparable results were obtained [P(3HB) = 176.75%, HA = 123%] [259]. Blends of P(3HB) and coral powder were known to produce good composite scaffold for bone tissue engineering [260]. This is because the incorporation of both materials resulted in a blend polymer with excellent porosity that facilitates bone growth, vascular invasion, and bone development.

8.5.2 Poly(3-Hydroxybutyrate-*co*-3-Hydroxyvalerate) [P(3HB-*co*-3HV)]

Wound Management and Tissue Regeneration. Compared to P(3HB) homopolymer, the P(3HB-*co*-3HV) copolymer has improved properties as described in Section 8.2.2. Its advantages were taken into consideration for medical applications. The P(3HB-*co*-3HV) copolymer has been tested for tissue regenerative studies. In a study done by Galgut and co-workers, P(3HB-*co*-3HV) membranes were evaluated for guided regeneration of periodontal ligament in rats [261]. This membrane aided in creating a barrier to avoid gingival connective tissue and epithelial tissues to come in contact with the healing tissues. The presence of valerate is known to cause some level of inflammation at the tissues surrounding the implants. In a study by Gogolewski and co-workers, the in vivo tissue reaction toward P(3HB-*co*-3HV) and P(3HB) was compared with PLA through intraperitoneal implants in mice [262]. It was found that the presence of 3HV increased the severity of inflammation at the beginning stages after surgery (3 months). The inflammation eventually reduced and no further unwanted tissue response due to 3HV was detected in later stages of surgery (3–6 months). Chaput et al. [180] also reported similar observation when P(3HB-*co*-3HV) with various 3HV composition was implanted intramuscularly in sheep for a period of approximately 3 months. In general, detection of macrophages and fibrous capsules indicated a typical tissue response of tissues surrounding PHA polymer implants. The biocompatibility of the polymers was also exhibited by the absence of unwanted complications such as abscess or necrosis formation at the area of implantation.

In beagle dogs, P(3HB-*co*-3HV) was used in treating palatal defects [263] and wound healing after von Langenbeck's procedure [264]. Nonporous P(3HB-*co*-3HV) films were used to separate the mucoperiosteum and bone until the transition of teeth was completed at about 24 weeks. At 2 weeks, the films displayed deformation and later were unimpaired and surrounded by fibrous capsules. The implantation of P(3HB-*co*-3HV) films after von

Langenbeck's repair resulted in complete wound closure after 7 weeks. The histological results showed attachment of local scar tissue by means of Sharpey's fibers due to the implantation of P(3HB-*co*-3HV) films.

Studies have been carried out by Shishatskaya and co-workers on the toxicology evaluation and alteration in the biological properties of P(3HB-*co*-3HV) and P(3HB) sutures [116, 265]. In a related study, Shishatskaya and co-workers tested intramuscularly implanted sutures of P(3HB-*co*-15 mol% 3HV) and P(3HB) along with traditional surgical materials of silk and catgut [116]. The sutures were found to provide sufficient strength throughout the healing of a muscular facial wound in Wistar rats. No differences were noticed in the tissue response toward either PHA polymers. However, the reaction of tissues toward these polymers was similar to silk but was less pronounced than the reaction to catgut. Implanted PHA sutures did not exhibit adverse effect in vivo on the rats and tissue. Evaluation of P(3HB-*co*-3HV) and CaP-Gelfix foams seeded with rat bone marrow stromal cells was done through implantation into defected rat femurs [266]. Formation of fibrous tissues in the CaP-Gelfix groups was more compared to P(3HB-*co*-3HV) even though the severity of inflammation was higher with CaP-Gelfix groups. Macroscopic and radiological studies demonstrated better healing with P(3HB-*co*-3HV) matrices than with CaP-Gelfix in 3 weeks.

Drug Delivery. Microspheres of P(3HB-*co*-3HV) were evaluated for drug delivery applications on a par with P(3HB). The characteristics of systems designed using this copolymer basically depended on 3HV composition. The composition of 3HV determines the rate of drug release and resistance of the polymer to physical damage. The rate of drug release depends on variables such as the amount of drug loaded, polymer composition and its crystallization rate, additives, and the size of the microspheres. An early study on drug delivery applications with this copolymer was done by Brophy and Deasy in 1986. They examined the release profile of sulfamethizole from P(3HB-*co*-3HV) microspheres [267]. Decrease in the release rate of the drug was observed, and it was assumed that this was caused by improved distribution of the drug. A later study by Gangrade and Price [268] revealed the usage of this copolymer on the release of progesterone. It was found that the surface of the microspheres was smoother when examined under scanning electron microscopy (SEM). A copolymer with a higher 3HV composition (24 mol%) exhibited a more porous surface, which correlated with the higher rate of drug release.

The effect on porosity of P(3HB-*co*-3HV) microsphere was studied by incorporating other compounds such as polycaprolactone and polyphosphate-Ca^{2+} with the copolymer [269–271]. The blends were found to be more porous as a significant increase in the rate of drug release was noticed. Tetracycline and its neutral salt derivatives were investigated for the treatment of periodontis [272]. The molecular weight of the copolymer was found to determine the rate of drug release. However, the salt form of tetracycline was found to be a better compound due to more efficient encapsulation (52–65%). Besides microsphere

delivery system, Yagmurlu and co-workers designed and incorporated sulbactam-cefoperazone into P(3HB-*co*-3HV) rods to study for efficient drug release in the tibia of infected rabbits [273]. The rabbits infected with *Staphylococcus aureus* showed almost complete healing after 30 days.

Orthopedic Applications.

Bone Repair. P(3HB-*co*-3HV) membranes have been evaluated for successful bone regeneration. Studies were done by Kostopoulos and Karring [254] using P(3HB-*co*-3HV) membranes to treat jaw bone defects in rats. In an experiment, the membranes were placed on mandibular defects in order to create space for bone fill. The membranes were found to enhance bone fill after 15–180 days. The control specimens showed ingrowth of other tissues and slower bone development. In a separate study, P(3HB-*co*-3HV) membranes were found to be capable of increasing the height of the rat mandible [255]. The space created by the membrane was completely filled with bone by 6 months. It was noted that the polymer had to be tailored to improve its physical property due to ruptures in some membranes. Gotfredsen and coworkers [274] evaluated P(3HB-*co*-3HV) membrane reinforced with polyglactin 910 fibers as an occlusive barrier over fresh extraction sockets. However, it was found that the material prevented bone healing due to increased inflammatory reaction.

P(3HB-*co*-3HV)/hydroxyapatite (HA) composites have been fabricated and considered as suitable material for use in fracture fixation [275, 276]. Bone implants of P(3HB-*co*-3HV)/HA composites were implanted in the tibia of rabbits. The composites were found to be morphologically, biologically, and chemically active throughout the experiment. Bone regeneration was confirmed by observation of osteoblasts and osteocytes at the interface after implantation. In a recent study, P(3HB-*co*-3HV)/HA nanocomposite scaffolds were fabricated through an emulsion freezing/freeze-drying technique, which was initially developed for making pure polymer scaffolds [277]. The scaffolds produced were highly porous and exhibited an interconnected porous structure. The incorporation of HA was found to lower crystallinity. This is anticipated to increase the in vitro or in vivo degradation rate which then makes it a suitable material for bone tissue engineering. P(3HB-*co*-3HV)/HA nanocomposites had been evaluated in vitro in SBF, and a formation of a new apatite layer after immediate immersion exhibited good bioactivity, which is a necessary condition for biomedical applications [278].

In other studies, composites of porous P(3HB-*co*-3HV) with other compounds such as sol–gel bioactive glass (SGBG) [201], wollastonite (a naturally occurring $CaSiO_3$) [279], and tricalcium phosphate (TCP) [280] have been evaluated as scaffold materials for bone repair. Composites of P(3HB-*co*-3HV) with SGBG were evaluated in SBF and changes in the ion concentration was observed due to the degradation of the composite. Composites of P(3HB-*co*-7 mol% 3HV)/TCP exhibited good biocompatibility in the femur of rabbits.

8.5.3 Poly(4-Hydroxybutyrate) [P(4HB)] and Poly(3-Hydroxybutyrate-*co*-4-Hydroxybutyrate) [P(3HB-*co*-4HB)]

Cardiovascular Applications.

Heart Valves. Natural heart valves have evolved to induce unidirectional flow of blood throughout circulatory systems. Valvular heart diseases, a form of valve malfunction, can be caused by the following: congenital defects, bacterial endocarditis, or rheumatic fever. Several methods have been devised to treat heart valve malfunctions including drug treatments and/or valve replacements. Mechanical valves are excellent in terms of durability, but after surgery patients are required to rely on lifelong treatment of anticoagulants, for example, warfarin, and perform regular monitoring of coagulant levels in the blood. In addition, the nonviability of mechanical heart valves is fast becoming an important concern among young patients. Since young patients often outgrow these replacement valves, subsequent surgeries are often needed to replace new valves.

On the other hand, biological tissue valves, derived from heterologous resources such as animals (pigs), do not require anticoagulant therapy. However, this kind of heart valve does not possess suitable strength and durability to support the pressures generated by the heart. Unlike their mechanical counterparts, biological heart valves are usually obtained from bovine and porcine. They are nonviable and have to undergo chemical modification to increase biocompatibility with the human heart. However, the development of tissue-engineered heart valves seemed to be a better solution to address the limitations mentioned above.

Early studies on the development of tissue-engineered heart valves had evaluated the suitability of polyglactin/PGA composite as scaffold biomaterials in the pulmonary circulation of the sheep model [281]. Heart valves constructed solely from P(4HB) have been described [192, 203, 282] by means of rapid prototyping or from highly porous surface films. P(4HB) polymer is known to possess good biocompatibility in vivo. In initial studies, tissue response and absorption levels of P(4HB) were studied by subcutaneous implantation in rats [125, 196]. Minimal foreign body reaction was reported and good absorption was noticed. The rate of degradation depended on the porosity of the material. Complete loss in polymer mass was detected over a period of 10 weeks. Most studies on the development of porous scaffold materials in the form of 4HB-based heart trileaflet valves are on the PGA/P(4HB) composite. The comparison on both PGA/P(4HB) composite and noncoated PGA as porous scaffold materials showed that the P(4HB)-coated material was more stable in vitro. This is useful as it prolonged the maturation of tissues prior to implantation.

Echocardiography showed that implantation of PGA/P(4HB) composite in the native pulmonary valve site functioned well without any signs of stenois, thrombus, and aneurysm. The composite completely degraded after 8 weeks of implantation and was replaced by native-like new tissue-engineered heart valve in a period of 20 weeks. The mechanical properties of the regenerated tissues

exhibited similar strength to the native counterparts. Also, the size of valve had shown an increase from 19 mm (initial) to 23 mm after the period of 20 weeks implantation, thus indicating the viability of valves made from P(4HB) as scaffold materials [82]. A combination of nondividing porcine heart valve coated with P(3HB), P(4HB), or P(3HB-*co*-4HB) had been investigated. The acellular heart valves consist of collagen, elastin, and proteoglycans and proteins ligands for cell attachments and receptor activation. Tissue-derived scaffold coated with biodegradable polyesters improved mechanical stability and increased hemocompatibility of the protein matrix. These findings are significant as they hint at the possibility of constructing viable heart valves for pediatric patients and eliminating the need to replace new valves. Biochemical analysis on the new tissue-engineered valve also revealed similar compositions to original valves [82].

Vascular Grafts. Vascular grafting is employed to replace damaged blood vessels. Currently, the two types of synthetic grafting materials that are widely used are Darcon and polytetrafluoroethylene. These synthetic grafting materials are commonly used to replace large-diameter blood vessels but not as smaller diameter grafts. PGA/P(4HB) composite was evaluated as vascular grafts recently [283]. Before this, composite of P(3HO) and PGA was tested for replacement of abdominal segments in lambs [284]. The PGA/P(4HB) composite was used to fabricate tubular scaffolds for tissue engineering in blood vessels. Significant results were obtained after the scaffolds were seeded with ovine vascular myofibroblasts (4 days) and endothelial cells (28 days) under static conditions and pulse duplicator system. The amount of cells and collagen production as well as the mechanical strength were higher with the pulse duplicator system. In order to increase the effectiveness of culturing ovine vascular myofibroblasts onto the PGA-coated scaffolds, a dynamic rotational seeding in a hybridization oven was carried out and good results were obtained. In a separate experiment, P(4HB) polymer was further used to fabricate porous tubes using a solution-casting/salt-leaching method [194]. These tubes were seeded with ovine vascular smooth muscle cells, which were derived by enzymatic dispersion in order to control the differentiated myofibroblast-like cells. At the end of incubation under dynamic conditions, the synthesis of ECM, DNA, and protein content were higher compared with static conditions. In addition, in order to evaluate the ability of the tissue-engineered scaffolds to withstand high-pressure conditions, the tubes were implanted at the descending aorta of the sheep model [193]. After 12 weeks of implantation, no sign of stenosis was observed and minimal thrombus formation was detected. However, after 24 weeks increased thrombus formation was observed. In comparison, the regenerated tissues contained significantly less elastin and lower mechanical stability in high-pressure circulation when compared to the native aorta. This has become a limiting factor for tissue engineering application in the high-pressure circulation systems.

Artery Augmentation. P(3HB) has already been evaluated as scaffolds for the regeneration of arterial tissue of the pulmonary artery. P(4HB) has also been evaluated for this purpose, whereby P(4HB) patches with pore sizes ranging from 180 to 240 μm were prepared through the salt-leaching process. These scaffolds were seeded with autologous cells before implantation for augmentation of the pulmonary artery in a sheep model [193]. Interestingly, observation from postoperative echocardiography showed that no stenosis was formed on the P(4HB) patches preseeded with autologous vascular cells and the formation of the functional tissue was seen after 24 weeks implantation. However, unseeded patches did not promote any tissue growth in the surrounding native pulmonary artery and no swelling was observed after 20 weeks. P(4HB) has been noted as a better patching material compared to conventional materials such as polytetrafluoroethylene (PTFE). Results from SEM images [82] have clearly shown that P(4HB) could potentially prevent blood leakage due to its self-seal nature. Conversely, bleeding (leaking) is often noticed when PTFE is used as the patching material. The suture leaves an apparent hole through the PTFE patches, thus increasing the risk of blood leakage.

8.5.4 Poly(3-Hydroxybutyrate-*co*-3-Hydroxyhexanoate) [P(3HB-*co*-3HHx)]

Wound Management and Tissue Regeneration. P(3HB-*co*-3HHx) copolymer has been evaluated as potential implant material. This copolymer is characterized as a more flexible material with higher elongation to break and with low tensile strength. P(3HB-*co*-3HHx) has also been known to blend well with compounds such as PLLA to produce a less crystalline material. Until recently, cell compatibility of P(3HB-*co*-3HHx) has been tested in numerous in vitro studies. In a recent study conducted by Dai and co-workers an in situ implant system of P(3HB-*co*-3HHx) for possible application in tissue adhesion prevention for postsurgical applications was observed [285]. Injectable in situ forming system is usually prepared by dissolving a polymer in a nonharmful organic solvent to form a liquid polymer solution. The polymer solvent then dissipates in the aqueous body fluid and adheres to its surrounding tissues under mechanical force [286, 287]. Biodegradable polymers have been evaluated for injectable in situ forming implant systems for use in tissue repair and sustainable drug delivery applications [288].

P(3HB-*co*-3HHx) was considered as replacement of the commonly used PLA [289] due to its better physical properties. P(3HB-*co*-12 mol% 3HHx) was concentrated in various organic solvents and injected at the intra-abdominal position of Sprague-Dawley (SD) rats using a syringe in order to detect solid film formation. A porous film formation was observed with all solutions after almost the same duration. Cell culture experiments revealed better cell growth on the surfaces of chloroform-cast P(3HB-*co*-3HHx) films but higher viability was observed on PLA films. It was concluded that a P(3HB-*co*-3HHx)-based injectable system has potential to be developed for adhesion prevention application.

Drug Delivery. P(3HB-*co*-3HHx) nanoparticles are now considered as a suitable vector for a targeted drug delivery approach. Recently, Yao and co-workers investigated the use of PHA granule binding protein PhaP in a receptor-mediated drug delivery system [290]. PhaP is able to bind to hydrophobic polymers via strong hydrophobic interaction, thus it is conceived to fuse with polypeptides or protein ligands. This ligand−PhaP−PHA nanoparticle system is designed to deliver drugs to specific targeted cells. Two ligands were used to achieve specific delivery, that is, mannosylated human α1-acid glycoprotein (hAGP) (binds to a mannose receptor of macrophages) and human epidermal growth factor (hEGF) (binds to carcinoma cells). The receptor-mediated system was found to be taken up by macrophages, hepatocellular carcinoma cell BEL7402 in vitro, and hepatocellular carcinoma cells in vivo by mice subcutaneously implanted with murine hepatoma22 (H22) tumor cells at the axillary space. The system was detected in targeted cells and organs (liver/tumor) under fluorescence microscopy when rhodamine B isothiocyanate (RBITC) was used as a model due to specific ligand and receptor binding.

Nerve Regeneration. In a recent study by Bian et al. [291], porous P(3HB-*co*-3HHx) nerve conduits with uniform wall porosity and nonuniform wall porosity were employed to bridge the 10 mm gap in the sciatic nerve of SD rats. The conduits were prepared using a particle leaching method. Autograft nerves and unrepaired nerve defects were taken as positive and negative controls. The regenerative nerve tissues were clearly observed after opening the conduits. One month upon implantation, successful generation of nerve tissues approximately 10 mm in length was found to bridge the gap. The histological assessment showed that the diameter of the regenerated nerve in conduits with nonuniform wall porosity was statistically higher than that in the uniformed type. It was found that during the nerve regeneration process, the structure of the P(3HB-*co*-3HHx) conduits remained intact. Molecular weight loses was monitored for up to 3 months after implantation, and a reduction was noticed in both uniform and nonuniform wall porosity conduits.

Orthopedic Applications.

Cartilage Repair. The tissue engineering approach is currently being evaluated for fabrication of cartilage constructs. Articular cartilage repair usually requires the use of an appropriate scaffold to support the formation of cartilage tissue. Cells are cultured on porous and resorbable scaffolds such as PGA [292] PLA [293, 294], PLGA [295, 296], chitosan [297, 298], and silk fibroin [299], and these scaffolds have been evaluated in vitro and in vivo. Previously, P(3HB-*co*-3HHx) and its composites have generated positive effects when used as scaffold material in vitro [300, 301]. Wang and co-workers have evaluated the use of three-dimensional scaffolds made from P(3HB-*co*-3HHx) copolymer in a rabbit articular cartilage defect model [302]. The P(3HB-*co*-3HHx) cartilage constructs were initially inoculated in vitro with rabbit chondrocytes and subsequently

implanted into rabbits using the press-fit method after 10 days. Cartilage defects were surgically created on the femoropatellar groove of the knee joints of rabbits. Formation of new white tissues were observed in the organisms implanted with P(3HB-*co*-3HHx) scaffolds as the implants were found to be embedded in the cavity. Scaffold with a pore size of 100 μm and a porosity of 90% proved as favorable material for cell proliferation, migration, and differentiation state maintenance in vitro and in vivo. Successful full thickness cartilage repair in the rabbit articular cartilage was observed over a period of 16 weeks.

8.5.5 Poly(3-Hydroxyoctanoate-*co*-3-Hydroxyhexanoate) [P(3HO-*co*-3HHx)]

Cardiovascular Applications.

Vascular Graft. Potential applications of P(3HO-*co*-3HHx) as vascular grafts and heart valves were evaluated in late 1990s. The elastomeric MCL_{PHA} P(3HO-*co*-3HHx) had initially been evaluated in vitro and in vivo to determine its suitability as a biomaterial for medical applications. The in vivo testing of P(3HO-*co*-3HHx) was carried out by Williams and co-workers in mice [1]. Subcutaneous implantation of P(3HO-*co*-3HHx) microspheres, tubes, and pellets revealed mild tissue response for the period of 4–40 weeks. The polymer was found to be inert and encapsulated by a thin layer of fibroin and could be readily removed. The molecular weight of the polymer decreased approximately around 50% after 40 weeks of implantation. The use of P(3HO-*co*-3HHx) as an impregnation substrate has been evaluated [303]. Implants impregnated with polymer, protein, and fluoropolymer were tested in rats. Tissue infiltration was not observed in polymer-impregnated grafts. After 6 months of implantation, 30% reduction in molecular weight was noticed.

The biocompatibility of this material was also tested in rats through subcutaneous implantation of P(3HO-*co*-3HHx) impregnated with Dacron prostheses [304]. Histological examination of the tissues exhibited the existence of an acute inflammatory phase that proceeded to a chronic inflammatory phase between 10 and 15 days of implantation but decreased to a mild reaction at 30 days and later to a more discrete response after 6 months. The molecular weight of the polymer decreased around 30% after 6 months of implantation.

P(3HO-*co*-3HHx) tubular conduits comprising of nonwoven PGA mesh on the inside and layers of P(3HO-*co*-3HHx) outside were fabricated by Shum-Tim et al. [284]. The PGA mesh promotes cell attachment and tissue formation, while the P(3HO-*co*-3HHx) layers provides mechanical support. The conduits were tested as vascular grafts by embedding with a mixture of endothelial cells, smooth muscle cells, and fibroblasts. The scaffolds were implanted in the aortic position in lambs for a period of up to 5 months. The preseeded scaffolds remained without aneurysm development during the period of study with increased cell density and collagen formation.

Heart Valve. Stock et al. [195] fabricated heart valve leaflets that had a multilayer structure of PGA–P(3HO-*co*-3HHx)–PGA with highly porous PGA mesh on the inside of the conduit wall connected to a porous P(3HO-*co*-3HHx) film on the outside. This conduit was embedded with autologous vascular cells and implanted to replace the pulmonary valve and main pulmonary artery in lambs for up to 6 months. A nonporous P(3HO-*co*-3HHx) film was used initially, but the leaflet failed due to thrombus formation and lack of good tissue formation. In this following modified structure, preseeded scaffolds showed formation of viable tissue without thrombus formation. On the other hand, the unseeded scaffold (control) developed thrombi on all leaflets after 4 weeks. Less than 30% decrease was observed in the molecular weight of the polymer after 6 weeks. However, due to slow resorption, P(3HO-*co*-3HHx) was found to be not suitable for heart valve scaffold.

In a separate study, the entire heart valve scaffolds were fabricated from P(3HO-*co*-3HHx) [204]. This model was previously tested in vitro under static and pulsatile flow cell culture conditions [192, 204]. The scaffold exposed to pulsatile flow culture condition exhibited good cell proliferation with the formation of a uniformed cell layer. In a following experiment, the P(3HO-*co*-3HHx) was embedded with vascular cells and implanted in the pulmonary position in a lamb model [203]. The preseeded scaffold exhibited increased levels of tolerance with good functionality and tissue growth from the 17-week experiment. The unseeded control showed no tissue formation and inappropriate cell number and collagen content, which was related to a confluent endothelial cell layer formation. The mechanical property of the polymer was found to decrease with time.

8.6 CLINICAL STUDY

The first reported clinical study of PHA was the usage of P(3HB) pericardial patches on human patients admitted for bypass surgery and/or valvular replacement [305]. The patients were examined for possibility of adhesion using computer tomography. Of the 39 patients examined, 19 who were implanted with P(3HB) patches showed a reduction on postoperative adhesion after 6–24 months. P(4HB) has been comprehensively investigated in vitro and in vivo for potential medical application. Tepha Inc. has announced the first human usage of TephaFLEX bioabsorbable suture products (Tepha Medical Devices). The clinical evaluations are being conducted in both the United States and Europe.

8.7 ELECTROSPINNING

Biomaterials with high surface area and porous structures are desired to enhance the adhesion of cells and subsequently increase in-cell growth. For this, electrospinning offers a promising means in preparing biomaterials with high surface-area-to-volume ratio. In addition, nanofibrous scaffolds prepared

from the electrospinning process are nonwoven with the morphological structures similar to that of ECM. Therefore, it is suggested to be able to enhance cellular response and suitable for seeded cell growth [306–308]. Previous study has also mentioned that electrospun nanofibrous material consisting of smaller diameter (less than 500 nm) [309] demonstrated better mechanical properties than those produced from conventional techniques (with larger diameter) [310].

The interest in using electrospun nanofibrous materials for scaffold fabrication is gaining ground because the mechanical and biological properties of the resulting scaffold could be conveniently controlled by changing the composition of the polymer solution, the types of solvents, and the processing parameters [311]. Electrospun nonwoven nanofiber biomaterials have found a place in various medical applications, namely as biomimicking of collagen structures to produce scaffold for tissue engineering, biological membranes for immobilized enzymes and catalysts, artificial blood vessels, antiseptic wound dressing materials, and drug delivery system [312–319].

To date, many biodegradable and biocompatible materials have been fabricated into nanoscale fibrous materials for tissue engineering applications, for example, PLA, PGA, PLGA, poly(ε-caprolactone), poly(ethylene-*co*-vinyl alcohol) and including natural polymers, such as collagen, protein and fibrinogen [306, 312–325]. Recently, electrospun fibers of several constituents of PHA, for example, P(3HB-*co*-3HV), P(3HB-*co*-4HB), P(3HB), and P(3HB-*co*-3HHx), have been evaluated as scaffolds in vivo and in vitro [327–329]. Nonwoven electrospun P(3HB-*co*-3HV) nanofibers with a small diameter of around 1–4 μm, were prepared from chloroform polymer solution. The average diameters of the nanofibers were further reduced by the addition of benzyl triakylammonium chloride into the chloroform polymer solution and the resulting nanofibers were in linearized form [326]. Figure 8.4 shows the morphology of electrospun nanofibers of P(3HB). The diameter of the fibers normally ranges between 500 and 700 nm.

In 2008, Cheng and co-workers evaluated the effect of electrospinning parameters, that is, polymer concentration, solvents, and voltage on the formation of P(3HB-*co*-3HHx) nanofibers. Smooth, ultrafine electrospun P(3HB-*co*-3HHx) fibers with an average diameter of 340 nm was obtained with the addition of dimethylformamide (DMF) as co-solvent with chloroform [327]. Meanwhile, Ying and co-workers fabricated porous electrospun P(3HB), P(3HB-*co*-3HHx), P(3HB-*co*-7 mol% 4HB), and P(3HB-*co*-97 mol% 4HB) nanofibers prepared from 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as the solvent [328].

8.7.1 Biocompatibility

In vitro biocompatibility of electrospun P(3HB) and P(3HB-*co*-3HV) nanofibers was evaluated using mouse fibroblasts (L929) and Schwann cells (RT4-D6P2T) [329]. Indirect cytotoxicity assay revealed that these electrospun fibers were not toxic during the first 24 h of incubation. In terms of cell attachment

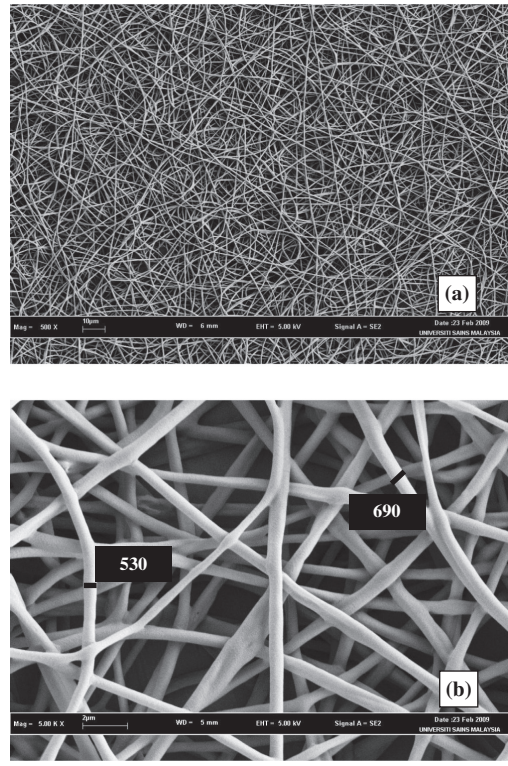

FIGURE 8.4 Morphology of electrospun nanofibres of P(3HB) showing the characteristics of high surface area and highly porous nature. a) Viewed under 500X magnification, b) Viewed under 5000X magnification

and cell proliferation, it was found that fibrous scaffolds were superior to support the growth of L929 but was inferior for RT4-D6P2T. SEM micrographs further proved that attached L929 cells exhibited spindle shape, which is typical in active fibroblast cells. However, in a separate study, SEM images of electrospun PHA scaffolds showed no degradation after 4 weeks of immersion in phosphate buffered saline [328]. Ito and co-workers had studied the biodegradability of electrospun P(3HB-*co*-3HV) nanofibers, which were dissolved in 2,2,2-trifluoroethanol (TFE) polymer solution and then composited with HA by soaking in SBF [330]. Image analysis of the elctrospun nanofibers showed uniformed distribution with an average diameter of 185 nm, while the incorporation of HA enhanced the degradability of the nanofibers.

Ying et al. [328] investigated tissue tolerances and bioabsorption behavior of various electrospun PHA scaffolds in vivo by subcutaneous implantation in rats. Periodic histological observation of implanted electrospun PHA nanofibers showed that the scaffolds of P(3HB-*co*-3HHx), P(3HB-*co*-7 mol% 4HB),

TABLE 8.5 Recent Patents on PHA Application in Medical Field

Patent Number	Assignee	Inventor(s)	Title	Date of Publications
U.S. 6,207,856	BTG International, Ltd., London	R. L. Veech,	Therapeutic compositions	March 27, 2001
U.S. 6,232,345	Shimizu Pharmaceutical Co., Ltd. Japan	A. Hiraide, S. Dohi, M. Suzuki, and Y. Shiba	Cerebral function improving agents	May 15, 2001
U.S. 6,245,537	Metabolic, Inc (Cambridge, USA)	S. F Williams, D. P. Martin, T. Gerngross, D. M. Horowitz,	Removing endotoxin with an oxidizing agent from polyhydroxyalkanoates produced by fermentation	June 12, 2001
U.S. 6,454,811	Massachusetts Institute of Technology (Cambridge, USA), Therics, Inc. (Princeton, NJ)	J. K. Sherwood, L. G. Griffith, S. Brown,	Composites for tissue regeneration and methods of manufacture thereof	September 24, 2002
U.S. 6,514,515	Tepha, Inc (Cambridge, USA)	Williams, S.F.	Bioabsorbable, biocompatible polymers for tissue engineering	February 4, 2003
U.S. 6,548,569	Metabolic, Inc (Cambridge, USA)	Williams, S.F, Martin, D.P, Skraly, F.A.	Medical devices and applications of polyhydroxyalkanoate polymer	April 15, 2003
U.S. 6,555,123	Tepha, Inc (Cambridge, USA)	Williams, S.F, Martin, D.P.	Polyhydroxyalkanoate compositions for soft tissue repair, augmentation, and viscosupplementation	April 29, 2003
U.S. 6,585,994	Tepha, Inc (Cambridge, USA)	Williams, S.F, Martin, D.P.	Polyhydroxyalkanoate compositions for soft tissue	July 1, 2003

			repair, augementation, and viscosupplementation	
U.S. 6,592,892	Tepha, Inc (Cambridge, USA)	Williams, S.F.	Flushable disposable polymeric products	July 15, 2003
U.S. 6,610,764	Metabolic, Inc. (Cambridge, USA)	G. W. Huisman, F. Skraly, D. P. Martin, O. P. Peoples,	Polyhydroxyalkanoate compositions having controlled degradation rates	August 26, 2003
U.S. 6,623,730	Tepha, Inc., andMetabolix, Inc. (Cambridge, USA)	S. F. Williams, D. P. Martin,	Therapeutic uses of polymers and oligomers comprising gamma-hydroxybutyrate	September 23, 2003
U.S. 6,623,749	Metabolic, Inc (Cambridge, USA)	S. F. Williams, D. P Martin, T. Gerngross, D. M. Horowitz,	Medical device containing polyhydroxyalkanoate treated with oxidizing agent to remove endotoxin	September 23, 2003
U.S. 6,746,685	Tepha, Inc. (Cambridge, USA)	S. F. Williams,	Bioabsorbable, biocompatible polymers for tissue engineering	June 8, 2004
U.S. 6,828,357	Metabolic, Inc. (Cambridge, USA)	D. P. Martin, F. Skraly, S. F. Williams,	Polyhydroxyalkanoate compositions having controlled degradation rates	December 7, 2004
U.S. 6,838,493	Metabolic, Inc. (Cambridge, USA)	D. P. Martin, S. F. Williams, F. A. Skraly,	Medicals devices and applications of polyhydroxyalkanoate polymers	January 4, 2005
U.S. 6,867,247	Metabolic, Inc. (Cambridge, USA)	S. F. Williams, D. P. Martin, F. A. Skraly,	Medical devices and applications of polyhydroxyalkanoate polymers	March 15, 2005
U.S. 6,867,248	Metabolic, Inc. (Cambridge, USA)	D. P. Martin, F. Skraly, S. F. Williams,	Polyhydroxyalkanoate compositions having controlled degradation rates	March 15, 2005

(*Continued*)

TABLE 8.5 (Continued)

Patent Number	Assignee	Inventor(s)	Title	Date of Publications
U.S. 6,878,758	Metabolic, Inc. (Cambridge, USA)	D. P. Martin, F. Skraly, S. F. Williams,	Polyhydroxyalkanoate compositions having controlled degradation rates	April 12, 2005
U.S. 7,202,325	Advanced Cardiovascular Systems, Inc. (Santa Clara, CA)	S. D. Pacetti, S. F. A. Hossainy,	Poly(hydroxyalkanoate-*co*-ester amides) and agents for use with medical articles	April 10, 2007
U.S. 7,229,804	Metabolic, Inc. (Cambridge, USA)	D. P. Martin, F. Skraly, S. F. Williams,	Biological systems for manufacture of polyhydroxyalkanoate polymers containing 4-hydroxyacids	June 12, 2007
U.S. 7,244,442	Metabolic, Inc. (Cambridge, USA)	S. F. Williams, D. P. Martin, T. Gerngross, D. M. Horowitz,	Method for making devices using polyhydroxyalkanoate having pyrogen removed	July 17, 2007
U.S. 7,262,219	Orphan Medical, Inc. (Palo Alto, CA)	H. Cook, M. Hamilton, D. Danielson, C. Goderstad, D. Reardan,	Microbiologically sound and stable solutions of γ-hydroxybutyrate salt for the treatment of narcolepsy	August 28, 2007
U.S. 7,268,205	Metabolic, Inc. (Cambridge, USA)	S. F. Williams, D. P. Martin, F. A. Skraly,	Medical devices and applications of polyhydroxyalkanoate polymers	September 11, 2007
U.S. 7,485,743	BTG International Limited (London)	R. A. Gross,	Oligomeric ketone compounds	February 3, 2009

and P(3HB-*co*-97 mol% 4HB) elicited mild tissue response. On the other hand, the bioabsorption and degradation rate was the fastest for the electrospun P(3HB-*co*-4HB) scaffold containing higher 4HB fractions.

8.8 PATENTS

Patents on the potential applications of PHA as medical devices and related research findings have been previously described [146]. The recent patents on this subject matter are described in Table 8.5.

8.9 OUTLOOK

Continuous development in PHA will ensure new and greater discoveries. Application of PHA as a biomaterial has gained much attention over the last decade. Various approaches are currently undertaken to develop cheaper processes of producing high yields of PHA. With global sustainability in mind, fellow researchers, industrialists, and policymakers are seriously considering the carbon footprint and its effects on our near future. Advancements in biomedical research is of utmost important with regard to current health-related problems due to our modern and hectic lifestyles. The recent breakthrough in biological synthesis of PHA–PLA copolymers [331–334] and especially copolymers consisting of 4HB and PLA has further expended the possibilities of discovering novel materials for medical applications. Since PHA has exhibited full potential as a biocompatible material, the future of PHA in medical and pharmaceutical fields is indeed bright.

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REFERENCES

1. Williams, S. F., D. P. Martin, D. M. Horowitz, and O. P. Peoples. PHA applications: Addressing the price performance issue I. tissue engineering. *Int. J. Biol. Macromol.* 1999;**25**:111–121.
2. Anderson, A. J. and E. A. Dawes. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. *Microbiol. Rev.* 1990;**54**: 450–472.

3. Doi, Y. *Microbial Polyesters*. VCH: New York, 1990.
4. Steinbüchel, A. and T. Lütke-Eversloh. Metabolic engineering and pathway construction for biotechnological production of relevant polyhydroxyalkanoates in microorganisms. *Biochem. Eng. J.* 2003;**16**:81–96.
5. Lemoigne, M. Produit de deÂshydratation et de polymeÂrisation de l'acide b-oxybutyrique. *Bull. Soc. Chim. Biol.* 1926;**8**:770–782.
6. Williamson, D. H. and J. F. Wilkinson. The isolation and estimation of the poly-b-hydroxybutyrate inclusions of *Bacillus* species. *J. Gen. Microbiol.* 1958;**19**:198–209.
7. Ostle, A. G. and J. G. Holt. Nile blue A as a fluorescent stain for poly-beta-hydroxybutyrate. *Appl. Environ. Microbiol.* 1982;**44**:238–241.
8. Kitamura, S. and Y. Doi. Staining method of poly(3-hydroxyalkanoic acids) producing bacteria by Nile blue. *Biotechnol. Tech.* 1994;**8**:345–350.
9. Gorenflo, V., A. Steinbüchel, S. Marose, M. Rieseberg, and T. Scheper. Quantification of bacterial polyhydroxyalkanoic acids by Nile red staining. *Appl. Microbiol. Biotechnol.* 1999;**51**:765–772.
10. Spiekermann, P., B. H. A. Rehm, R. Kalscheuer, D. Baumeister, and A. Steinbüchel. A sensitive, viable-colony staining method using Nile red for direct screening of bacteria that accumulate polyhydroxyalkanoic acids and other lipid storage compounds. *Arch. Microbiol.* 1999;**171**:73–80.
11. Findlay, R. H. and D. C. White. Polymeric beta-hydroxyalkanoates from environmental samples and *Bacillus megaterium*. *Appl. Environ. Microbiol.* 1983;**45**:71–78.
12. Witholt, B. and B. Kessler. Perspectives of medium chain length poly(hydroxyalkanoates), a versatile set of bacterial bioplastics. *Curr. Opin. Biotechnol.* 1999;**10**:279–285.
13. Sudesh, K. and T. Iwata. Sustainability of biobased and biodegradable plastics. *Clean* 2008;**36**:433–442.
14. Kessler, B., R. Weusthuis, B. Witholt, and G. Eggink. *Production of microbial polyesters: Fermentation and downstream processes. Polyesters from microorganisms*. In *Biopolyesters*. W. A. Babel, A. Steinbuchel, and J. U. Akermann (Eds.). Springer: New York, 2001, pp. 159–182.
15. Kim, Y. and R. Lenz. *Polyesters from microorganisms*. In *Biopolyesters*. W. A. Babel, A. Steinbuchel, J. U. Akermann(Ed.). Springer: New York, 2001, pp. 51–79.
16. Taguchi, K., S. Taguchi, K. Sudesh, A. Maehara, T. Takeharu, and Y. Doi. *Metabolic pathways and engineering of PHA biosynthesis*. In *Biopolymers: Polyesters I Biological Systems and Biotechnological Production*. Y. Doi, and A. Steinbüchel (Eds.). Wiley-VCH: Germany, 2002, pp. 217–372.
17. Tsuge, T. Metabolic improvements and use of inexpensive carbon sources in microbial production of polyhydroxyalkanoates. *J. Biosci. Bioeng.* 2002;**94**:579–584.
18. Senior, P. J. and E. A. Dawes. Poly-β-hydroxybutyrate biosynthesis and the regulation of glucose metabolism in *Azotobacter beijerinckii*. *Biochem. J.* 1971;**125**:55–66.
19. Senior, P. J. and E. A. Dawes. The regulation of poly-β-hydroxybutyrate metabolism in *Azotobacter beijerinckii*. *Biochem. J.* 1973;**134**:225–238.
20. Rehm, B. H. A. Polyester synthases: Natural catalysts for plastics. *Biochem. J.* 2003;**376**:15–33.
21. Nomura, C. T. and S. Taguchi. PHA synthase engineering toward superbiocatalysts for custom-made biopolymers. *Appl. Environ. Microbiol.* 2007;**73**;969–979.

22. Fukui, T. and Y. Doi. Cloning and analysis of the poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) biosynthesis genes of *Aeromonas caviae*. *J. Bacteriol.* 1997;**179**:4821–4830.
23. Fukui, T., N. Shiomi, and Y. Doi. Expression and characterization of (R)-specific enoyl coenzyme A hydratase involved in polyhydroxyalkanoate biosynthesis by *Aeromonas caviae*. *J. Bacteriol.* 1998;**180**:667–673.
24. Rehm, B. H. A., N. Kruger, and A. Steinbüchel. A new metabolic link between fatty acid de novo synthesis and polyhydroxyalkanoic acid synthesis. The PHAG gene from *Pseudomonas putida* KT2440 encodes a 3-hydroxyacyl-acyl carrier protein-coenzyme A transferase. *J. Biol. Chem.* 1998;**273**:24044–24051.
25. Matsusaki, H., H. Abe, and Y. Doi. Biosynthesis and properties of poly(3-hydroxybutyrate-*co*-3-hydroxyalkanoates) by recombinant strains of *Pseudomonas* sp. 61-3. *Biomacromolecules* 2000;**1**:17–22.
26. Tsuge, T., F. Toshiaki, H. Matsusaki, S. Taguchi, G. Kobayashi, A. Ishizaki, and Y. Doi. Molecular cloning of two (*R*)-specific enoyl-CoA hydratase genes from *Pseudomonas aeruginosa* and their use for polyhydroxyalkanoate synthesis. *FEMS Microbiol. Lett.* 2000;**184**:193–198.
27. Matsumoto, K., S. Nakae, K. Taguchi, H. Matsusaki, M. Seki, and Y. Doi. Biosynthesis of poly(3-hydroxybutyrate-*co*-3-hydroxyalkanoates) copolymer from sugars by recombinant *Ralstonia eutropha* harboring the phaC1Ps and the phaGPs genes of *Pseudomonas* sp. 61-3. *Biomacromolecules* 2001;**2**:934–939.
28. Poirier, Y., D. Dennis, K. Klomparens, C. Nawrath, and C. Sommerville. Perspectives on the production of polyhydroxyalkanoates in plants. *FEMS Microbiol. Rev.* 1992;**103**:237–246.
29. Poirier, Y., D. E. Dennis, K. Klomparens, and C. Somerville. Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants. *Science* 1992;**256**:520–523.
30. Matsumoto, K., R. Nagao, T. Murata, Y. Arai, T. Kichise, H. Nakashita, S. Taguchi, H. Shimada, and Y. Doi. Enhancement of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production in the transgenic *Arabidopsis thaliana* by the in vitro evolved highly active mutants of polyhydroxyalkanoate (PHA) synthase from *Aeromonas caviae*. *Biomacromolecules* 2005;**6**:2126–2130.
31. Matsumoto, K., Y. Arai, R. Nagao, T. Murata, K. Takase, H. Nakashita, S. Taguchi, H. Shimada, and Y. Doi. Synthesis of short-chain-length/medium-chain-length polyhydroxyalkanoate (PHA) copolymers in peroxisome of the transgenic *Arabidopsis thaliana* harboring the PHA synthase gene from *Pseudomonas* sp. 61-3. *J. Polym Environ.* 2006;**14**:369–374.
32. Nielsen, L. Polyhydroxyalkanoate production in sugarcane — recognizing tempor-ospatial complexity. *J. Biotechnol.* 2007;**131**:S28–S29.
33. Sudesh, K., H. Abe, and Y. Doi. Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. *Prog. Polym. Sci.* 2000;**25**:1503–1555.
34. Kahar, P., T. Tsuge, K. Taguchi, and Y. Doi. High yield production of polyhydroxyalkanoates from soybean oil by *Ralstonia eutropha* and its recombinant strain. *Polym. Degrad. Stabil.* 2004;**83**:79–86.
35. Chen, G. Q., G. Zhang, S. J. Park, and S. Y. Lee. Industrial scale production of poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate), *Appl. Microbiol. Biotechnol.* 2001;**57**: 50–55.

36. Lee, S. Y. and J. I. Choi. Effect of fermentation performance on the economics of poly(3-hydroxybutyrate) production by *Alcaligenes latus*. *Polym. Degrad. Stabil.* 1998;**59**:387–393.

37. Kellerhals, M. B., W. Hazenberg, and B. Witholt. High cell density fermentations of *Pseudomonas oleovorans* for the production of mcl-PHAs in two-liquid phase media. *Enzyme Microb. Tech.* 1999;**24**:111–116.

38. Kellerhals, M. B., B. Kessler, and B. Witholt. Closed-loop control of bacterial high-cell-density fed-batch cultures: Production of mcl-PHAs by *Pseudomonas putida* KT2442 under single-substrate and co-feeding conditions. *Biotechnol. Bioeng.* 1999;**65**:306–315.

39. Choi, J. I. and S. Y. Lee. High-level production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) by fed-batch culture of recombinant *Escherichia coli*. *Appl. Environ. Microb.* 1999;**65**:4363–4368.

40. Ren, Q., K. Ruth, L. Thony-Meyer, and M. Zinn. Process engineering for production of chiral hydroxycarboxylic acids from bacterial polyhydroxyalkanoates. *Macromol. Rapid Commun.* 2007;**28**:2131–2136.

41. Akiyama, M., T. Tsuge, and Y. Doi. Environmental life cycle comparison of polyhydroxyalkanoates produced from renewable carbon resources by bacterial fermentation. *Polym. Degrad. Stabil.* 2003;**80**:183–194.

42. Gagnon, K. D., R. C. Fuller, R. W. Lenz, and R. J. Farris. The mechanical properties of a thermoplastic elastomer produced by *Pseudomonas oleovorans* *Macromolecules*. 1992;**25**:3723–3731.

43. Du, G. C., J. Chen, J. Yu, and S. Lun. Feeding strategy of propionic acid for production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) with *Ralstonia eutropha*. *Biochem. Eng. J.* 2001;**8**:103–110.

44. Lee, W. H., C. Y. Loo, C. T. Nomura, and K. Sudesh. Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3-hydroxyvalerate precursors. *Bioresource Technol.* 2008;**99**:6844–6851.

45. Kim, J. S., B. H. Lee, and B. S. Kim. Production of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) by *Ralstonia eutropha*. *Biochem. Eng. J.* 2005;**23**:169–174.

46. Chanprateep, S. and S. Kulpreecha. Production and characterization of biodegradable terpolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxybutyrate) by *Alcaligenes* sp. A-04. *J. Biosci. Bioeng.* 2006;**101**:51–56.

47. Madden, L. A., A. J. Anderson, J. Asrar, and P. Berger. Production and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxybutyrate) synthesized by *Ralstonia eutropha* in fed-batch cultures. *Polymer* 2000;**41**:3499–3505.

48. Amirul, A. A., A. R. M. Yahya, K. Sudesh, M. N. M. Azizan, and M. I. A. Majid. Biosynthesis of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) copolymer by *Cupriavidus* sp. USMAA1020 isolated from Lake Kulim, Malaysia. *Bioresource Technol.* 2008;**99**:4903–4909.

49. Zheng, Z., F. F. Bei, H. L. Tian, and G. Q. Chen. Effects of crystallization of polyhydroxyalkanoate blend on surface physicochemical properties and interactions with rabbit articular cartilage chondrocytes. *Biomaterials* 2005;**26**: 3537–3548.

50. Amirul, A. A., A. R. M. Yahya, K. Sudesh, M. N. M. Azizan, and M. I. A. Majid. Isolation of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) producer from

Malaysian environment using γ-butyrolactone as carbon source. *World J. Microb. Biotechnol.* 2009;**25**:1199–1206.

51. Loo, C. Y., W. H. Lee, T. Tsuge, Y. Doi, and K. Sudesh. Biosynthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) from palm oil products in a *Wautersia eutropha* mutant. *Biotechnol. Lett.* 2005;**27**:1405–1410.
52. Bhubalan, K., W. H. Lee, C. Y. Loo, T. Yamamoto, T. Tsuge, Y. Doi, and K. Sudesh. Controlled biosynthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) from mixtures of palm kernel oil and 3HV-precursors. *Polym. Degrad. Stabil.* 2008;**93**:17–23.
53. Fukui, T., T. Kichise, Y. Yoshida, and Y. Doi. Biosynthesis of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyheptanoate) terpolymers by recombinant *Alcaligenes eutrophus*. *Biotechnol. Lett.* 1997;**19**:1093–1097.
54. Kichise, T., T. Fukui, Y. Yoshida, and Y. Doi. Biosynthesis of polyhydroxyalkanoates (PHA) by recombinant *Ralstonia eutropha* and effects of PHA synthase activity on in vivo PHA biosynthesis *Int. J. Biol. Macromol.* 1999;**25**:69–77.
55. Saito, Y. and Y. Doi. Microbial synthesis and properties of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) in *Comamonas acidovorans*. *Int. J. Biol. Macromol.* 1994;**16**:99–104.
56. Lee, W. H., M. N. M. Azizan, and K. Sudesh. Effects of culture conditions on the composition of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) synthesized by *Comamonas acidovorans*. *Polym. Degrad. Stabil.* 2004;**84**:129–134.
57. Sudesh, K., T. Fukui, K. Taguchi, T. Iwata, and Y. Doi. Improved production of poly(4-hydroxybutyrate) by *Comamonas acidovorans* and its freeze-fracture morphology. *Int. J. Biol. Macromol.* 1999;**25**:79–85.
58. Doi, Y., S. Kitamura, and H. Abe. Microbial synthesis and characterization of poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) *Macromolecules*. 1995;**28**:4822–4828.
59. Shimamura, E., M. Scandola, and Y. Doi. Microbial synthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxypropionate). *Macromolecules* 1994;**27**: 4429–4435.
60. Annuar, M. S. M., I. K. P. Tan, S. Ibrahim, and K. B. Ramachandran. Production of medium-chain-length poly(3-hydroxyalkanoates) from crude fatty acids mixture by *Pseudomonas putida*. *Food. Bioprod. Process.* 2007;**85**:104–119.
61. Haba, E., J. Vidal-Mas, M. Bassas, M. J. Espuny, J. Llorens, and A. Manresa. Poly 3-(hydroxyalkanoates) produced from oily substrates by *Pseudomonas aeruginosa* 47T2 (NCBIM 40044): Effect of nutrients and incubation temperature on polymer composition. *Biochem. Eng. J.* 2007;**35**:99–106.
62. Valappil, S. P., A. R. Boccaccini, C. Bucke, and I. Roy. Polyhydroxyalkanoates in Gram-positive bacteria: Insights from the genera *Bacillus* and *Streptomyces*. *Antonie van Leeuwen. Int. J. Gen. Mol. Microbiol.* 2007;**91**:1–17.
63. de Andrade Rodrigues, M. F., E. J. Vicente, and A. Steinbüchel. Studies on polyhydroxyalkanoate (PHA) accumulation in a PHA synthase I-negative mutant of *Burkholderia cepacia* generated by homogenotization, *FEMS Microbiol. Lett.* 2000;**193**:179–185.
64. Steinbüchel, A., E. M. Debzi, R. H. Marchessault, and A. Timm. Synthesis and production of poly(3-hydroxyvaleric acid) homopolyester by *Chromobacterium violaceum*. *Appl. Microbiol. Biotechnol.* 1993;**39**:443–449.

65. Choi, J. I. and S. Y. Lee. Production of poly(3-hydroxybutyrate) [P(3HB)] with high P(3HB) content by recombinant *Escherichia coli* harboring the *Alcaligenes latus* P(3HB) biosynthesis genes and the *E. coli* fts Z gene. *J. Microbiol. Biotechnol.* 1999;**9**:722–725.

66. Park, S. J., W. S. Ahn, P. R. Green, and S. Y. Lee. Biosynthesis of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) by metabolically engineered *Escherichia coli* strains. *Biotechnol. Bioeng.* 2001;**74**:81–86.

67. Ahn, W. S., S. J. Park, and S. Y. Lee. Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli*. *Biotechnol. Lett.* 2001;**23**:235–240.

68. Solaiman, D. K. Y., R. D. Ashby, A. T. Hotchkiss, and T. A. Foglia. Biosynthesis of medium-chain-length poly(hydroxyalkanoates) from soy molasses. *Biotechnol. Lett.* 2006;**28**:157–162.

69. Yu, J. Production of PHA from starchy wastewater via organic acids. *J. Biotechnol.* 2001;**86**:105–112.

70. Solaiman, D. K., R. D. Ashby, and T. A. Foglia. Physiological characterization and genetic engineering of *Pseudomonas corrugata* for medium-chain-length polyhydroxyalkanoates synthesis from triacylglycerols. *Curr. Microbiol.* 2002;**44**:189–195.

71. Hu, P. H., H. Chua, A. L. Huang, and P. K. Ho. Conversion of industrial food wastes by *Alcaligenes latus* into polyhydroxyalkanoates. *Appl. Biochem. Biotechnol.* 1999;**77**:445–454.

72. Lee, K. M. and D. F. Gilmore. Formulation and process modeling of biopolymer (polyhydroxyalkanoates: PHAs) production from industrial wastes by novel crossed experimental design. *Process Biochem.* 2005;**40**:229–246.

73. Kek, Y. K., W. H. Lee, and K. Sudesh. Efficient bioconversion of palm acid oil and palm kernel acid oil to poly(3-hydroxybutyrate) by *Cupriavidus necator*. *Can. J. Chem.* 2008;**86**:533–539.

74. Tsuge, T., K. Tanaka, and A. Ishizaki. Development of a novel method for feeding a mixture of lactic acid and acetic acid in fed-batch culture of *Ralstonia eutropha* for poly(3-hydroxybutyrate) production. *J. Biosci. Bioeng.* 2001;**91**: 545–550.

75. Tsuge, T., K. Tanaka, M. Shimoda, and A. Ishizaki. Optimization of L-lactic acid feeding for the production of poly-D-3-hydroxybutyric acid by *Alcaligenes eutrophus* in fed-batch culture. *J. Biosci. Bioeng.* 1999;**88**:404–409.

76. Kobayashi, G., K. Tanaka, H. Itoh, T. Tsuge, K. Sonomoto, and A. Ishizaki. Fermentative production of P(3HB-*co*-3HV) from propionic acid by *Alcaligenes eutrophus* in fed-batch culture with pH-stat continuous substrate feeding method. *Biotechnol. Lett.* 2000;**22**:1067–1069.

77. Hassan, M. A., Y. Shirai, N. Kusubayashi, I. A. Karim, K. Nakanisihi, and K. Hashimoto. The production of polyhydroxyalkanoate from anaerobically treated palm oil mill effluent by *Rhodobacter sphaeroides*. *J. Ferment. Bioeng.* 1997;**83**:485–488.

78. Holmes, P. A. Applications of PHB — a microbially produced biodegradable thermoplastic. *Phys. Technol.* 1985;**16**:32–36.

79. Kusaka, S., T. Iwata, and Y. Doi. Microbial synthesis and physical properties of ultra-high-molecular-weight poly[(*R*)-3-hydroxybutyrate]. *J. Macromol. Sci–Pure. Appl. Chem.* 1998;**35**:319–335.

80. Ji, Y., X. T. Li, and G. Q. Chen. Interactions between a poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) terpolyester and human keratinocytes. *Biomaterials*. 2008;**29**:3807–3814.
81. Hu, Y. J., X. Wei, W. Zhao, Y. S. Liu, and G. Q. Chen. Biocompatibility of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) with bone marrow mesenchymal stem cells. *Acta Biomater*. 2009;**5**:1115–1125.
82. Martin, D. P. and S. F. Williams. Medical applications of poly-4-hydroxybutyrate: A strong flexible absorbable biomaterial. *Biochem. Eng. J*. 2003;**16**:97–105.
83. Pederson, E. N., C. W. J. McChalicher, and F. Srienc. Bacterial synthesis of PHA block copolymers. *Biomacromolecules* 2006;**7**:1904–1911.
84. Bates, F. S. and G. H. Fredrickson. Block copolymer thermodynamics—theory and experiment. *Annu. Rev. Phys. Chem*. 1990;**41**:525–557.
85. Bates, F. S. Polymer-polymer phase behavior. *Science* 1991;**251**:898–905.
86. Doi, Y., M. Kunioka, Y. Nakamura, and K. Soga. Nuclear-magnetic-resonance studies on poly(beta-hydroxybutyrate) and a copolyester of beta-hydroxybutyrate and beta-hydroxyvalerate isolated from *Alcaligenes eutrophus* H16. *Macromolecules* 1986;**19**:2860–2864.
87. Yoshie, N., Y. Azuma, M. Sakurai, and Y. Inoue. Crystallization and compatibility of poly(vinyl alcohol)/poly(3-hydroxybutyrate) blends: Influence of blend composition and tacticity of poly(vinyl alcohol). *J. Appl. Polym. Sci*. 1995;**56**:17–24.
88. Žagar, E., A. Kržan, G. Adamus, and M. Kowalczuk. Sequence distribution in microbial poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) co-polyesters determined by NMR and MS. *Biomacromolecules* 2006;**7**:2210–2216.
89. Valentin, H. E. and D. Dennis. Production of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) in recombinant *Escherichia coli* grown on glucose. *J. Biotechnol*. 1997;**58**:33–38.
90. Lütke-Eversloh, T. and A. Steinbüchel. Novel precursor substrates for polythioesters (PTE) and limits of PTE biosynthesis in *Ralstonia eutropha*. *FEMS Microbiol. Lett*. 2003;**221**:191–196.
91. Doi, Y., A. Segawa, and M. Kunioka. Biosynthesis and characterization of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) in *Alcaligenes eutrophus*. *Int. J. Biol. Macromol*. 1990;**12**:106–111.
92. Vigneswari, S., S. Vijaya, M. Majid, K. Sudesh, C. Sipaut, M. Azizan, and A. Amirul. Enhanced production of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) copolymer with manipulated variables and its properties. *J. Ind. Microbiol. Biotechnol*. 2009;**36**:547–556.
93. Madison, L. L. and G. W. Huisman. Metabolic engineering of poly(3-hydroxyalkanoates): From DNA to plastic, *Microbiol. Mol. Biol. Rev*. 1999;**63**:21–53.
94. Luengo, J. M., B. Garcí, A. Sandoval, G. Naharro, and E. R. Olivera. Bioplastics from microorganisms. *Curr. Opin. Microbiol*. 2003;**6**:251–260.
95. Kobayashi, T., K. Uchino, T. Abe, Y. Yamazaki, and T. Saito. Novel intracellular 3-hydroxybutyrate-oligomer hydrolase in *Wautersia eutropha* H16. *J. Bacteriol*. 2005;**187**:5129–5135.
96. Doi, Y., Y. Kawaguchi, N. Koyama, S. Nakamura, M. Hiramitsu, Y. Yoshida, and H. Kimura. Synthesis and degradation of polyhydroxyalkanoates in *Alcaligenes eutrophus*. *FEMS Microbiol. Rev*. 1992;**103**:103–108.

97. Khanna, S. and A. K. Srivastava. Production of poly(3-hydroxybutyric-*co*-3-hydroxyvaleric acid) having a high hydroxyvalerate content with valeric acid feeding. *J. Ind. Microbiol. Biotechnol.* 2005;**34**:457–461.

98. Mergaert, J., A. Webb, C. Anderson, A. Wouters, and J. Swings. Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) in soils. *Appl. Environ. Microbiol.* 1993;**59**:3233–3238.

99. Wang, Y. W., W. Mo, H. Yao, Q. Wu, J. Chen, and G. Q. Chen. Biodegradation studies of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate). *Polym. Degrad. Stabil.* 2004;**85**:815–821.

100. Yew, S. P., H. Y. Tang, and K. Sudesh. Photocatalytic activity and biodegradation of polyhydroxybutyrate films containing titanium dioxide. *Polym. Degrad. Stabil.* 2006;**91**:1800–1807.

101. Sridewi, N., K. Bhubalan, and K. Sudesh. Degradation of commercially important polyhydroxyalkanoates in tropical mangrove ecosystem. *Polym. Degrad. Stabil.* 2006;**91**:2931–2940.

102. Doi, Y., Y. Kanesawa, Y. Kawaguchi, and M. Kunioka. Hydrolytic degradation of microbial poly(hydroxyalkanoates). *Macromol. Chem. Rapid Commun.* 1989;**10**:227–230.

103. Doi, Y., Y. Kanesawa, N. Tanahashi, and Y. Kumagai. Biodegradation of microbial polyesters in the marine environment. *Polym. Degrad. Stabil.* 1992:**36**:173–177.

104. Kasuya, K., Y. Doi, and T. Yao. Enzymatic degradation of poly[(*R*)-3-hydroxybutyrate] by *Comamonas testosteroni* ATSU of soil bacterium. *Polym. Degrad. Stabil.* 1994;**45**:379–386.

105. Mergaert, J., A. Wouters, C. Anderson, and J. Swings. In situ biodegradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) in natural waters. *Can. J. Microbiol.* 1995;**41**:154–159.

106. Jendrossek, D., A. Schirmer, and H. G. Schlegel. Biodegradation polyhydroxyalkanoic acids. *Appl. Microbiol. Biotechnol.* 1996;**46**:451–463.

107. Adams, J. H., G. Irving, and J. H. Koeslag. β-Adrenergic blockade restores glucose's antiketogenic activity after exercise in carbohydrate-depleted athletes. *J. Physiol.* 1987;**386**:439–454.

108. Bessman, S. P. and W. N. Fishbein. Gamma-hydroxybutyrate, a normal brain metabolite, *Nature* 1963;**200**:1207–1208.

109. Wiggam, M. I., M. J. Okane, R. Harper, A. B. Atkinson, D. R. Hadden, E. R. Trimble, and P. M. Bell. Treatment of diabetic ketoacidosis using normalization of blood 3-hydroxybutyrate concentration as the endpoint of emergency management—a randomized controlled study. *Diabetes Care* 1997;**20**:1347–1352.

110. Yajnik, C. S., B. S. Sardesai, D. S. Bhat, S. S. Naik, K. N. Raut, K. M. Shelgikar, H. Orskov, K. G. M. M. Alberti, and T. D. R. Hockaday. Ketosis resistance in fibrocalculous pancreatic diabetes: II. Hepatic ketogenesis after oral medium-chain triglycerides. *Metabol. Clin. Exp.* 1997;**46**:1–4.

111. Robinson, A. M. and D. H. Williamson. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol. Rev.* 1980;**60**:143–187.

112. Massieu, L., M. L. Haces, T. Montiel, and K. Hernandez-Fonseca. Acetoacetate protects hippocampal neurons against glutamate mediated neuronal damage during glycolysis inhibition. *Neuroscience* 2003;**120**:365–378.

113. Hocking, P. J. and R. H. Marchessault. *Biopolyesters*. In *Chemistry and Technology of Biodegradable Polymers*. G. J. L. Griffin (Ed.). Blackie: Glasgow, 1994, pp. 48–96.

114. Pouton, C. W. and S. Akhtar. Biosynthetic polyhydroxyalkanoates and their potential in drug delivery. *Adv. Drug. Deliv. Rev.* 1996;**18**:133–162.

115. Borkenhagen, M., R. C. Stoll, P. Neuenschwander, U. W. Suter, and P. Aebischer. *In vivo* performance of a new biodegradable polyester urethane system used as a nerve guidance channel. *Biomaterials* 1998;**19**:2155–2165.

116. Shishatskaya, E. I., T. G. Volova, A. P. Puzyr, O. A. Mogilnaya, and S. N. Efremov. Tissue response to the implantation of biodegradable polyhydroxyalkanoate sutures. *J. Mater. Sci. Mater. Med.* 2004;**15**:719–728.

117. Saito, Y., S. Nakamura, M. Hiramitsu, and Y. Doi. Microbial synthesis and properties of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate). *Polym. Int.* 1996;**39**: 169–174.

118. Yang, X., K. Zhao, and G. Q. Chen. Effect of surface treatment on the biocompatibility of microbial polyhydroxyalkanoates. *Biomaterials* 2002;**23**:1391–1397.

119. Chee, J. W., A. A. Amirul, T. S. Tengku Muhammad, M. I. A. Majid, and S. M. Mansor. The influence of copolymer ratio and drug loading level on the biocompatibility of P(3HB-*co*-4HB) synthesized by *Cupriavidus* sp. (USMAA2-4). *Biochem. Eng. J.* 2008;**38**:314–318.

120. Mukai, K., K. Yamada, and Y. Doi. Efficient hydrolysis of polyhydroxyalkanoates by *Pseudomonas stutzeri* YM1414 isolated from lake water. *Polym. Degrad. Stabil.* 1994;**43**:319–327.

121. Reusch, R. N. Biological complexes of poly-beta-hydroxybutyrate. *FEMS Microbiol. Rev.* 1992;**103**:119–129.

122. Seebach, D. and M. G. Fritz. Detection, synthesis, structure, and function of oligo (3-hydroxyalkanoates): Contributions by synthetic organic chemist. *Int. J. Biol. Macromol.* 1999;**25**:217–236.

123. Reusch, R. N. Low molecular weight complexed poly(3-hydroxybutyrate): A dynamic and versatile molecule in vivo. *Can. J. Microbiol.* 1995;**41**:50–54.

124. Seebach, D., C. Hak-Fun, and R. F. W. Jackson. (+)-11,11'-Di-O-methylelaiophylidine — Preparation from elaiophylin and total synthesis from (*R*)-3-hydroxybutyrate and (*S*)-malate. *Liebigs Ann. Chem.* 1986:1281–1308.

125. Martin, D. P., O. P. Peoples, and S. F. Williams. Nutritional and therapeutic uses of 3-hydroxy-alkanoate oligomers. PCT Patent Application No. WO 00/04895, 2000.

126. Martin, D. P., F. A. Skraly, and S. F. Williams. Polyhydroxyalkanoate compositions having controlled degradation rates. PCT Patent Application No. WO 99/32536, 1999.

127. Hiraide, A. and M. Katayama. Use of 3-hydroxybutyric acid as an energy source. European Patent Application No. 355,453 A2, 1990.

128. Tetrick, M. A., S. H. Adams, J. Odle, and N. J. Benevenga. Contribution of D-(−)-3-hydroxybutyrate to the energy expenditure of neonatal pigs. *J. Nutr.* 1995;**125**:264–272.

129. Chen, C. H. and S. C. Chen. Effective ophthalmic irrigation solution. U.S. Patent No. 5,116,868, 1992.

130. Kashiwaya, Y., T. Takeshima, N. Mori, K. Nakashima, K. Clarke, and R. L. Veech. D-Beta-hydorxybutyrate protects neurons in models of Alzheimer's and Parkinson's Disease. *Proc. Natl. Acad. USA* 2000;**97**:5440–5444.

131. Tieu, K., C. Perier, C. Caspersen, P. Tiesmann, D. C. Wu, S. D. Yan, A. Naini, M. Vila, V. Jackson-Lewis, R. Ramasamy, and S. Przedborski. D-β-Hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J. Clin. Invest*. 2003;**112**:892–901.

132. Chung, H. J., J. P. Steinberg, R. L. Huganir, and D. J. Linden. Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. *Science* 2003;**14**:1751–1755.

133. Chu, Y., W. Le, K. Kompiliti, J. Jankovic, E. J. Mufson, and J. H. Kordower. Nurr1 in Parkinson's disease and related disorders. *J. Comp. Neurol.* 2006;**494**:495–514.

134. Xiao, X. Q., Y. Zhao, and G. Q. Chen. The effect of 3-hydroxybutyrate and its derivatives on the growth of glial cells. *Biomaterials* 2007;**28**:3608–3616.

135. Zao, X. H., H. M. Li, S. Wang, M. Leski, Y. C. Yao, X. D. Yang, Q. J. Huang, and G. Q. Chen. The effect of 3-hydroxybutyrate methyl ester on learning and memory in mice. *Biomaterials* 2009;**30**:1532–1541.

136. Laborit, H. Sodium 4-hydroxybutyrate. *Int. J. Neuropharmacol.* 1964;**43**:433–452.

137. Hunter, A. S., W. J. Long, and C. C. Ryrie. An evaluation of gamma hydroxybutyric acid in paediatric practice. *Br. J. Anaesth.* 1971;**43**:620–627.

138. Mamelak, M., M. B. Scharf, and M. Woods. Treatment of narcolepsy with γ-hydroxybutyrate. A review of clinical and sleep laboratory findings. *Sleep* 1986;**9**:285–289.

139. Gessa, G. L., L. Vargiu, F. Crabai, G. C. Boero, F. Caboni, and R. Camba. Selective increase of brain dopamine induced by gamma-hydroxybutyrate. *Life. Sci.* 1966;**5**:1921–1930.

140. Gallimberti, L., M. Ferri, S. D. Ferrara, F. Fadda, and G. L. Gessa. Gamma-hydroxybutyric acid in the treatment of alcohol dependence: a double-blind study. *Alcohol Clin. Exp. Res.* 1992;**16**:673–676.

141. Addolorato, G., G. Balducci, E. Capristo, M. L. Attilia, F. Taggi, G. Gasbarrini, and M. Ceccanti. Gamma-hydroxybutyric acid (GHB) in the treatment of alcohol withdrawal syndrome: A randomized comparative study versus benzodiazepine, *Alcohol Clin. Exp. Res.* 1999;**23**:1596–1604.

142. Mamelak, M. Gammahydroxybutyrate: An endogenous regulator of energy metabolism. *Neurosci. Biobehav. Rev.* 1989;**13**:187–198.

143. Cash, D. Gammahydroxybutyrate: An overview of the pros and cons for it being a neurotransmitter and/or a useful therapeutic agent. *Neurosci. Biobehav. Rev.* 1994;**18**:291–304.

144. Nelson, T., E. Kaufman, J. Kline, and L. Sokoloff. The extraneural distribution of γ-hydroxybutyrate. *J. Neurochem.* 1981;**37**:1345–1348.

145. Williams, S. F. Bioabsorbable, biocompatible polymers for tissue engineering, PCT Patent Application No. WO 00/51662, 2000.

146. Williams, S. F. and D. P. Martin. *Applications of PHAs in medicine and pharmacy*. In *Biopolymers: Polyesters III Applications and Commercial Products*. Y. Doi, and A. Steinbüchel (Eds.). Wiley-VCH: Germany, 2002, pp. 91–127.

147. American Heart Association (AHA). *Heart And Stroke Statistical Update*. AHA: Dallas, 2002.

148. Zinn, M., B. Witholt, and T. Egli. Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. *Adv. Drug Deliver. Rev.* 2001;**53**:5–21.

149. Shishatskaya, E. I., A. V. Eremeev, I. I. Gitelson, N. A. Setkov, and T. G. Volova. Cytotoxicity of polyhydroxyalkanoates in animal cell cultures. *Doklady Biol. Sci.* 2000;**374**:539–542.

150. Shishatskaya, E. I. and T. G. Volova. A comparative investigation of biodegradable polyhydroxyalkanoate films as matrices for in vitro cell cultures. *J. Mater. Sci. Mater. Med.* 2004;**15**:915–923.

151. Shishatskaya, E. I., T. G. Volova, S. A. Gordeev, and A. P. Puzyr. Degradation of P(3HB) and P(3HB-*co*-3HV) in biological media. *J. Biomater. Sci. Polym. Edn.* 2005;**16**:643–657.

152. Garrido, L. Non-destructive evaluation of synthetic tissue scaffolds with NMR. *Mat. Res. Soc. Symp. – Proc.* 1999;**550**:171–176.

153. Rouxhet, L., F. Duhoux, O. Borecky, R. Legras, and Y. J. Schneider. Adsorption of albumin, collagen, and fibronectin on the surface of poly (hydroxybutyrate-hydroxyvalerate) (PHB/HV) and of poly(β-caprolactone) (PCL) films modified by an alkaline hydrolysis and of poly(ethylene terephtalate) (PET) track-etched membranes. *J. Biomater. Sci. Polym. Edn.* 1998;**9**:1279–1304.

154. Valappil, S. P., S. K. Misra, A. Boccaccini, and I. Roy. Biomedical applications of polyhydroxyalkanoates, an overview of animal testing and in vivo responses. *Expert Rev. Med. Devic.* 2006;**3**:853–868.

155. Valappil, S. P., D. Peiris, G. J. Langley, J. M. Herniman, A. R. Boccaccini, C. Bucke, and I. Roy. Polyhydroxyalkanoate (PHA) biosynthesis from structurally unrelated carbon sources by a newly characterized *Bacillus* spp. *J. Biotechnol.* 2007;**127**:475–487.

156. Lee, S. Y., J. I. Choi, K. Han, and J. Y. Song. Removal of endotoxin during purification of poly(3-hydroxybutyrate) from gram-negative bacteria. *Appl. Environ. Microbiol.* 1999;**65**:2762–2764.

157. Marois, Y., Z. Zhang, M. Vert, X. Deng, R. Lenz, and R. Guidoin. Effect of sterilization on the physical and structural characterization of polyhydroxyoctanoate. *J. Biomater. Sci. Polym. Edn.* 1999;**10**:469–482.

158. Miller, N. D. and D. F. Williams. On the biodegradation of poly-β-hydroxybutyrate (PHB) homopolymer and poly-β-hydroxybutyrate-hydroxyvalerate copolymers. *Biomaterials* 1987;**8**:129–137.

159. Luo, S. and A. N. Netravali. Effect of ^{60}Co γ-radiation on the properties of poly(hydroxybutyrate-*co*-hydroxyvalerate). *Appl. Polym. Sci.* 1999;**73**: 1059–1067.

160. Siew, E. L., N. F. Rajab, A. B. Osman, K. Sudesh, and S. H. Inayat-Hussain. Mutagenic and clastogenic characterization of poststerilized poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) copolymer biosynthesized by *Delftia acidovorans*. *J. Biomed. Mater. Res. A*, 2009;**91**:786–794.

161. Lee, S. Y. Bacterial polyhydroxyalkanoates, *Biotechnol. Bioeng.* 1996;**49**:1–14.

162. Freier, T. Biopolyesters in tissue engineering applications. *Adv. Polym. Sci.* 2006;**203**:1–61.

163. Shishatskaya, E. I., O. N. Voinova, A. V. Goreva, O. A. Mogilnaya, and T. G. Volova. Biocompatibility of polyhydroxybutyrate microspheres: In vitro and in vivo evaluation. *J. Mater. Sci. Mater. Med.* 2008;**19**:2493–2502.

164. Ali, A. Q., T. P. Kannan, A. Ahmad, and A. R. Samsudin. *In vitro* genotoxicity tests for polyhydroxybutyrate - A synthetic biomaterial. *Toxicol. In Vitro* 2008;**22**:57–67.

165. Sun, J., Z. Dai, Y. Zhao, and G. Q. Chen. In vitro effect of oligo-hydroxyalkanoates on the growth of mouse fibroblast cell line L929. *Biomaterials* 2007;**28**:3896–3903.

166. Piddubnyak, V., P. Kurcok, A. Matuszowicz, M. Glowala, A. Fiszer-Kierzkowska, Z. Jedlinski, M. Juzwa, and Z. Krawczyk. Oligo-3-hydroxybutyrates as potential carriers for drug delivery. *Biomaterials* 2004;**25**:5271–5279.

167. Wang, Y. W., F. Yang, Q. Wu, Y. C. Cheng, P. H. F. Yu, J. Chen, and G. Q. Chen. Effect of composition of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) on growth of fibroblast and osteoblast. *Biomaterials* 2005;**26**:755–761.

168. Tabesh, H., G. H. Amoabediny, N. Salehi Nik, M. Heydari, M. Yosefifard, S. O. Ranaei Siadat, and K. Mottaghy. The role of biodegradable engineered scaffolds seeded with Schwann cells for spinal cord regeneration. *Neurochem. Int.* 2009; **54**:73–83.

169. Zhang, D. M., F. Z. Cui, Z. S. Luo, Y. B. Lin, K. Zhao, and G. Q. Chen. Wettability improvement of bacterial polyhydroxyalkanoates via ion implantation. *Surf. Coat. Tech.* 2000;**131**:350–354.

170. Hasirci, V., A. Tezcaner, N. Hasirci, and S. Süzer. Oxygen plasma modification of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) film surfaces for tissue engineering purposes. *J. Appl. Polym. Sci.* 2002;**87**:1285–1289.

171. Nitschke, M., G. Schmack, A. Janke, F. Simon, D. Pleul, and C. Werner. Low pressure plasma treatment of poly(3-hydroxybutyrate): Toward tailored polymer surfaces for tissue engineering scaffolds. *J. Biomed. Mater. Res.* 2002;**59**:632–638.

172. Etzrodt, D., M. Rybka, C. Röpke, I. Michalik, D. Behrend, and K. P. Schmitz. Effect of molecular weight and protein coating of surface properties of poly(beta-hydroxybutyric acid) and the effect on polymer-cell interaction. *Biomed. Tech.* 1997;**42**:445–446.

173. Zhao, K., X. Yang, G. Q. Chen, and J. C. Chen. Effect of lipase treatment on the biocompatibility of microbial polyhydroxyalkanoates. *J. Mater. Sci. Mater. Med.* 2002;**13**:849–854.

174. Zhao, K., Y. Deng, and G. Q. Chen. Effects of surface morphology on the biocompatibility of polyhydroxyalkanoates. *Biochem. Eng. J.* 2003;**16**:115–123.

175. Wang, Y. W., Q. Wu, and G. Q. Chen. Reduced mouse fibroblast cell growth by increased hydrophilicity of microbial polyhydroxyalkanoates via hyaluronan coating. *Biomaterials* 2004;**24**:4621–4629.

176. Davies, S. M. and B. J. Tighe. Cell attachment to gel-spun polyhydroxybutyrate fibers. *Polym. Prepr.* (*Am. Chem. Soc. Div. Polym. Chem.*) 1995;**36**:103–104.

177. Foster, L. J. R., S. M. Davies, and B. J. Tighe. Centrifugally-spun polyhydroxybutyrate fibres: Effect of process solvent on structure, morphology and cell response. *J. Biomat. Sci. Polym. E.* 2001;**12**:317–336.

178. Wang, Y. W., Q. Wu, J. C. Chen, and G. Q. Chen. Evaluation of three-dimensional scaffolds made of blends of hydroxyapatite and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) for bone reconstruction. *Biomaterials* 2005;**26**:899–904.

179. Peschel, G., H. Dahse, A. Konrad, G. D. Wieland, P. Mueller, D. P. Martin, and M. Roth. Growth of keratinocytes on porous films of poly(3-hydroxybutyrate) and poly(4-hydroxybutyrate) blended with hyaluronic and chitosan. *J. Biomed. Mater. Res. A* 2007;**85A**:1072–1081.

180. Chaput, C., L. H. Yahia, A. Selmani, and C. H. Rivard. *Natural poly(hydroxybutyrate-hydroxyvalerate) polymers as degradable biomaterials*. In *Polymers in Medicine and Pharmacy*. A. G. Mikos, K. W. Leong, M. J. Yaszemski, J. A. Tamada, and M. L. Radomsky (Eds.). MRS Symposium Proceedings, Vol. 394, Materials Research Society. Pittsburgh, 1995, p. 111–116.

181. Chaput, C., M. Assad, L. H. Yahia, C. H. Rivard, and A. Selmani. Cytotoxicity and hemolysis testing of microbial poly(hydroxybutyrate)-based copolymers in-vitro. *Biomater. Living Syst*. 1995;**3**:29–38.

182. Taylor, M. S., A. U. Daniels, K. P. Andriano, and J. Heller. Six bioabsorbable polymers: In vitro acute toxicity of accumulated degradation products. *J. Appl. Biomater*. 1994;**5**:151–157.

183. Giavaresi, G., M. Tschon, J. H. Daly, J. J. Liggat, M. Fini, P. Torricelli, and R. Giardino. Natural and synthetic polyesters for musculoskeletal tissue repair: Experimental in vitro and in vivo evaluations. *Int. J. Artif. Organs* 2004;**27**:796–805.

184. Köse, G. T., S. Ber, F. Korkusuz, and V. Hasirci. Poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) based tissue engineering matrices. *J. Mater. Sci. Mater. M*. Med 2003;**14**:121–126.

185. Köse, G. T., H. Kenar, N. Hasirci, and V. Hasirci. Macroporous poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) matrices for bone tissue engineering. *Biomaterials* 2003;**24**:1949–1958.

186. Köse, G. T., F. Korkusuz, P. Korkusuz, N. Purali, A. Özkul, and V. Hasirci. Bone generation on PHBV matrices: An in vitro study. *Biomaterials* 2003; **24**:4999–5007.

187. Lee, S. J., Y. M. Lee, G. Khang, U. Y. Kim, B. Lee, and H. B. Lee. Effect of poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) surface with different wettability on fibroblast behavior. *Macromol. Res*. 2002;**10**:150–157.

188. Tezcaner, A., K. Bugra, and V. Hasirci. Retinal pigment epithelium cell culture on surface modified poly(hydroxybutyrate-*co*-hydroxyvalerate) thin films. *Biomaterials* 2003;**24**:4573–4583.

189. Dang, M. H., F. Birchler, and E. Wintermantel. Toxicity screening of biodegradable polymers. 2. Evaluation of cell culture test with medium extract. *J. Environ. Polym. Degrad*. 1997;**5**:49–56.

190. Hu, S. G., C. H. Jou, and M. C. Yang. Protein adsorption, fibroblast activity and antibacterial properties of poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) grafted with chitosan and chitooligosaccharide after immobilized with hyaluronic acid. *Biomaterials* 2003;**24**:2685–2693.

191. Tesema, Y., J. Raghavan, and J. Stubbs III. Bone cell viability on collagen immobilized poly(3-hydroxybutrate-*co*-3-hydroxyvalerate) membrane: Effect of surface chemistry. *J. Appl. Polym. Sci*. 2004;**93**:2445–2453.

192. Sodian, R., S. P. Hoerstrup, J. S. Sperling, S. Daebritz, D. P. Martin, A. M. Moran, B. S. Kim, F. J. Schoen, J. P. Vacanti, and J. E. Mayer. Early in vivo experience with tissue-engineered trileaflet heart valves. *Circulation* 2000;**102**(Suppl.):22–29.

193. Opitz, F., K. Schenke-Layland, T. U. Cohnert, B. Starcher, K. J. Halbhuber, D. P. Martin, and U. A. Stock. Tissue engineering of aortic tissue: Dire consequence of suboptimal elastic fiber synthesis in vivo. *Cardiovasc. Res.* 2004;**63**:719–730.

194. Opitz, F., K. Schenke-Layland, W. Richter, D. P. Martin, I. Degenkolbe, T. Wahlers, and U. A. Stock. Tissue engineering of ovine aortic blood vessel substitutes using applied shear stress and enzymatically derived vascular smooth muscle cells. *Ann. Biomed. Eng.* 2004;**32**:212–222.

195. Stock, U. A., T. Sakamoto, S. Hatsuoka, D. P. Martin, M. Nagashima, A. M. Moran, M. A. Moses, P. N. Khalil, F. J. Schoen, J. P. Vacanti, and J. E. Mayer. Patch augmentation of the pulmonary artery with bioabsorbable polymers and autologous cell seeding. *J. Thorac. Cardiovasc. Surg.* 2000;**120**:1158–1167.

196. Hoerstrup, S. P., R. Sodian, S. Daebritz, J. Wang, E. A. Bacha, D. P. Martin, A. M. Moran, K. J. Guleserian, J. S. Sperling, S. Kaushal, J. P. Vacanti, F. J. Schoen, and J. E. Mayer. Functional living trileaflet heart valves grown in vitro. *Circulation* 2000;**102**:III-44–III-49.

197. Sendelbeck, S. L. and C. L. Girdis. Disposition of a ^{14}C-labeled bioerodible polyorthoester and its hydrolysis products, 4-hydroxybutyrate and cis,trans-1,4-bis(hydroxymethyl)cyclohexane in rats, *Drug Metab. Dispos.* 1985;**13**:291–295.

198. Doi, Y., Y. Kanesawa, M. Kunioka, and T. Saito. Biodegradation of microbial copolyesters: Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate). *Macromolecules.* 1990;**23**:26–31.

199. Siew, E. L., N. F. Rajab, A. B. Osman, K. Sudesh, S. H. Inayat-Hussain. In vitro biocompatibility evaluation of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) copolymer in fibroblast cells, J. Biomed. Mater. Res. A. 2007;**81**: 317–325.

200. Wang, Y. W., Q. Wu, and G. Q. chen. In vitro biocompatibility evaluation of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) copolymer in fibroblast cells, *J. Biomed. Mater. Res. A Biomaterials.* 2004;**25**:669–675.

201. Zheng, Y. D., Y. J. Wang, X. F. Chen, Y. B. Ren, and G. Wu. Chemical reaction of PHBV/sol-gel bioglass foams for born tissue engineering in simulated body fluid. *Chem. J. Chin. U.* 2003;**24**:1325–1328.

202. Deng, Y., K. Zhao, X. F. Zhang P. Hu, and G. Q. Chen. Study on the three-dimensional proliferation of rabbit articular cartilage-derived chondrocytes on polyhydroxyalkanoate scaffolds. *Biomaterials* 2002;**23**:4049–4056.

203. Sodian, R., S. P. Hoerstrup, J. S. Sperling, D. P. Martin, S. Daebritz, J. E. Mayer, and J. P. Vacanti. Evaluation of biodegradable, three-dimensional matrices for tissue engineering of heart valves. *ASAIO J.* 2000;**46**:107–110.

204. Sodian, R., J. S. Sperling, D. P. Martin, A. Egozy, U. Stock, J. E. Mayer, J. P. Vacanti. Fabrication of a trileaflet heart valve scaffold from a polyhydroxyalkanoate biopolyester for use in tissue engineering. *Tissue Eng.* 2000;**6**:183–188.

205. Marois, Y., Z. Zhang, M. Vert, X. Deng, R. Lenz, and R. Guidoin. Hydrolytic and enzymatic incubation of polyhydroxyoctanoate (PHO): A short-term in vitro study of a degradable bacterial polyester. *J. Biomater. Sci. Polym. Edn.* 1999;**10**:483–489.

206. Marois, Y., Z. Zhang, M. Vert, X. Deng, R. Lenz, and R. Guidoin. Mechanism and rate of degradation of polyhydroxyoctanoate films in aqueous media: A long-term in vitro study. *J. Biomed. Mater. Res.* 2000;**49**:216–224.

207. Behrend, D., C. Nischan, C. Kunze, M. Sass, and K. P. Schmitz. Resorbable scaffolds for tissue engineering. Proc. European Medical and Biological Engineering Conference. *Med. Biol. Eng. Comput*. 1999;**37**:1510–1511.

208. Schmack, C., D. Jehnichen, R. Vogel, and B. Tandler. Biodegradable fibers of poly (3-hydroxybutyrate) produced by high-speed melt spinning and spin drawing. *J. Polym. Sci. Polym. Phys*. 2000;**38**:2841–2850.

209. Schmack, G., S. Kramer, H. Dorschner, and K. Gliesche. Effect of electron irradiation on the properties of poly(3-hydroxybutyric acid) fibres and their in vitro degradation. *Polym. Degrad. Stabil*. 2004;**83**:467–472.

210. Cheng, G. X., Z. J. Cai, and L. Wang. Biocompatibility and biodegradation of poly(hydroxybutyrate)/poly(ethylene glycol) blend films. *J. Mater. Sci. Mater. Med*. 2003;**14**:1073–1078.

211. Freier, T., C. Kunze, C. Nischan, S. Kramer, K. Sternberg, M. Saß, U. T. Hopt, and K. P. Schmitz. In vitro and in vivo degradation studies for development of a biodegradable patch based on poly(3-hydroxybutyrate). *Biomaterials* 2002;**23**: 2649–2657.

212. Löbler, M., M. Saß, C. Kunze, K. P. Schmitz, and U. T. Hopt. Biomaterial patches sutured onto the rat stomach induce a set of genes encoding pancreatic enzymes. *Biomaterials* 2002;**23**:577–583.

213. Kunze, C., H. E. Bernd, R. Androsch, C. Nischan, T. Freier, S. Kramer, B. Kramp, K. P. Schmitz. In vitro and in vivo studies on blends of isotactic and atactic poly (3-hydroxybutyrate) for development of a dura substitute material. *Biomaterials* 2006;**27**:192–201.

214. Korsatko, W., B. Wabnegg, G. Braunegg, R. M. Lafferty, and F. Strempfl. Poly-D(−)-3-hydroxybutyric acid (PHBA) — a biodegradable carrier for long term medication dosage. I. Development of parenteral matrix tablets for long term application of pharmaceuticals. *Pharm. Ind*. 1983;**45**:525–527.

215. Korsatko, W., B. Wabnegg, H. M. Tillian, G. Braunegg, and R. M. Lafferty. Poly-D(−)-3-hydroxybutyric acid - a biodegradable carrier for long term medication dosage. 2. The biodegradation in animals and in vitro – in vivo correlation of the liberation of pharmaceuticals from parenteral matrix retard tablets. *Pharm. Ind*. 1983;**45**:1004–1007.

216. Korsatko, W., B. Korsatko, R. M. Lafferty, and V. Weidmann.The influence of the molecular weight of poly-D(−)-3-hydroxybutyric acid on its use as a retard matrix for sustained drug release. Proceedings, Third European Congress of Biopharmacology and Pharmakinetics. Vol. I. 1987, pp. 234–242.

217. Kharenko, A. V. and A. L. Iordanskii. Diltiazem release from matrices based on polyhydroxybutyrate. *Proc. Int. Symp. Control. Release Bioact. Mater*. 1999;**26**:919–920.

218. Juni, K., M. Nakano, and M. Kubota. Controlled release of aclarubicin, an anticancer antibiotic, from poly-β-hydroxybutyric acid microspheres. *J. Control. Release* 1986;**4**:25–32.

219. Juni, K. and M. Nakano. Poly(hydroxy acids) in drug delivery. *CRC Crit. Rev. Ther. Drug Carrier Syst*. 1987;**3**:209–232.

220. Collins, A. E. M., P. B. Deasy, D. MacCarthy, and D. B. Shanley. Evaluation of a controlled-release compact containing tetracycline hydrochloride bonded to tooth for treatment of periodontal disease. *Int. J. Pharm*. 1989;**5**:103–114.

221. Deasy, P. B., A. E. M. Collins, D. J. MacCarthy, and R. J. Russell. Use of strips containing tetracycline hydrochloride or metronidazole for the treatment of advanced periodontal disease. *J. Pharm. Pharmacol.* 1989;**41**:694–699.

222. Cargill, R., K. Engle, C. R. Gardner, P. Porter, R. V. Sparer, and J. A. Fix. Controlled gastric emptying. II. In vitro erosion and gastric residence times of an erodible device in Beagle dogs. *Pharm. Res.* 1989;**6**:506–509.

223. McLeod, B. J., W. Haresign, A. R. Peters, R. Humke, and G. E. Lamming. The development of subcutaneous-delivery preparations of GnRH for the induction of ovulation in acyclic sheep and cattle. *Anim. Reprod. Sci.* 1988;**17**:33–50.

224. Kassab, A. C., K. Xu, E. B. Denkbas, Y. Dou, S. Zhao, and E. Piskin. Rifampicin carrying polyhydroxybutyrate microspheres as a potential chemoembolization agent. *J. Biomater. Sci-Polm. E.* 1997;**8**:947–961.

225. Malm, T., S. Bowald, A. Bylock, C. Busch, and T. Saldeen. Enlargement of the right-ventricular outflow tract and the pulmonary-artery with a new biodegradable patch in transannular position. *Eur. Surg. Res.* 1994;**26**:298–308.

226. Malm, T., S. Bowald, S. Karacagil, A. Bylock, and C. Busch. A new biodegradable patch for closure of atrial septal-defect — an experimental study. *Scand. J. Thorac. Cardiovasc. Surg.* 1992;**26**:9–14.

227. Nkere, U. U., S. A. Whawell, C. E. Sarraf, J. B. Schofield, and P. A. O'Keefe. Pericardial substitution after cardiopulmonary bypass surgery: A trial of an absorbable patch. *Thorac. Cardiovas. Surg.* 1998;**46**:77–83.

228. Schmitz, K. P. and D. Behrend. Method of manufacturing intraluminal stents made of polymer material. European Patent Application No. 0,770,401 A2, 1997.

229. Unverdorben, M., M. Schywalsky, D. Labahn, S. Hartwig, F. Laenger, D. Lootz, D. Behrend, K. P. Schmitz, M. Schaldach, and C. Vallbracht. Polyhydroxybutyrate (PHB) biodegradable stent-experience in the rabbit. *Am. J. Cardiol., Tenth Annual Symposium Transcatheter Cardiovascular Therapeutics, Abstracts TCT-8* 1998;46.

230. Behrend, D., D. Lootz, K. P. Schmitz, M. Schywalsky, D. Labahn, S. Hartwig, M. Schaldach, M. Unverdorben, C. Vallbracht, and F. Laenger. PHB as a bioresorbable material for intravascular stents. *Am. J. Cardiol., Tenth Annual Symposium Transcatheter Cardiovascular Therapeutics*, *Abstracts TCT-8* 1998;45.

231. Nakayama, K., K. Takakuda, Y. Koyama, S. Itoh, W. Wang, T. Mukai, and N. Shirahama. Enhancement of peripheral nerve regeneration using bioabsorbable polymer tubes packed with fibrin gel. *Brain* 2007;**31**:500–508.

232. Schnell, E., K. Klinkhammer, S. Balzer, G. Brook, D. Klee, P. Dalton, and J. Mey. Guidance of cell migration and axonal growth on electrospun nanofibers of poly-epsilon-caprolactone and a collagen/poly-epsilon-caprolactone blend. *Biomaterials* 2007;**28**:3012–3025.

233. Wang, X., W. Hu, Y. Cao, J. Yao, J. Wu, and X. Gu. Dog sciatic nerve regeneration across a 30-mm defect bridged by a chitosan/PGA artificial nerve graft. *Brain* 2007;**128**:1897–1910.

234. Liu, B., S. X. Cai, K. W. Ma, Z. L. Xu, X. Z. Dai, L. Yang, C. Lin, X. B. Fu, K. L. Sung, and X. K. Li. Fabrication of a PLGA-collagen peripheral nerve scaffold and investigation of its sustained release property in vitro. *J. Mater. Sci. Mater. Med.* 2008;**19**:1127–1132.

235. Oh, S. H., J. H. Kim, K. S. Song, B. H. Jeon, J. H. Yoon, T. B. Seo, U. Namgung, I. W. Lee and J. H. Lee. Peripheral nerve regeneration within an asymmetrically porous PLGA/Pluronic F127 nerve guide conduit. *Biomaterials* 2008;**29**: 1601–1609.

236. Chen, Y. S., J. Y. Chang, C. Y. Cheng, F. J. Tsai, C. H. Yao, and B. S. Liu. An in vivo evaluation of a biodegradable genipin-cross-linked gelatin peripheral nerve guide conduit material. *Biomaterials* 2005;**26**:3911–3918.

237. Chen, M. H., P. R. Chen, M. H. Chen, S. T. Hsieh, J. S. Huang, and F. H. Lin. An in vivo study of tricalcium phosphate and glutaraldehyde crosslinking gelatin conduits in peripheral nerve repair. *J. Biomed. Mater. Res. Part. B. Appl. Biomater.* 2006;**77B**:89–97.

238. Lu, G., L. Kong, B. Sheng, G. Wang, Y. Gong, and X. Zhang. Degradation of covalently cross-linked carboxymethyl chitosan and its potential application for peripheral nerve regeneration. *Eur. Polym. J.* 2007;**43**:3807–3818.

239. Kim, S. W., H. K. Bae, H. S. Nam, D. J. Chung, and P. H. Choung. Peripheral nerve regeneration through nerve conduit composed of alginate–collagen–chitosan. *Macromol. Res.* 2006;**14**:94–100.

240. Li, W., Y. Guo, H. Wang, D. Shi, C. Liang, Z. Ye, F. Qing, and J. Gong. Electrospun nanofibers immobilized with collagen for neural stem cells culture. *J. Mater. Sci. Mater. Med.* 2008;**19**:847–854.

241. Wang, A., Q. Ao, Y. Wei, K. Gong, X. Liu, N. Zhao, Y. Gong, and X. Zhang. Physical properties and biocompatibility of a porous chitosan-based fiber-reinforced conduit for nerve regeneration. *Biotechnol. Lett.* 2007;**29**:1697–1702.

242. Uebersax, L., M. Mattotti, M. Papaloizos, H. P. Merkle, B. Gander, and L. Meinel. Silk fibroin matrices for the controlled release of nerve growth factor (NGF). *Biomaterials* 2007;**28**:4449–4460.

243. Yang, Y., F. Ding, J. Wu, W. Hu, W. Liu, J. Liu, and X. Gu. Development and evaluation of silk fibroin-based nerve grafts used for peripheral nerve regeneration. *Biomaterials* 2007;**28**, 5526–5535.

244. Hazari, A., M. Wiberg, G. Johansson-Rudén, C. Green, and G. Terenghi. A resorbable nerve conduit as an alternative to nerve autograft in nerve gap repair. *Br. J. Plast. Surg.* 1999;**52**:653–657.

245. Ljungberg, C., G. Johansson-Ruden, K. J. Bostrom, L. Novikov, and M. Wiberg. Neuronal survival using a resorbable synthetic conduit as an alternative to primary nerve repair. *Microsurgery* 1999;**19**:259–264.

246. Mosahebi, A., P. Fuller, M. Wiberg, and G. Terenghi. Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. *Exp. Neurol.* 2002;**173**:213–223.

247. Novikov, L. N., L. N. Novicova, A. Mosahebi, M. Wiberg, G. Terenghi, and J. Kellerth. A novel biodegradable implant for neuronal rescue and regeneration after spinal cord injury. *Biomaterials* 2002;**23**:3369–3376.

248. Fleming, J. E., C. N. Cornell, and G. F. Muschler. Bone cells and matrices in orthopedic tissue engineering. *Orthop. Clin. North. Am.* 2000;**31**:357–374.

249. Thomson, R. C., M. C. Wake, M. J. Yaszemski, and A. G. Mikos. Biodegradable polymer scaffolds to regenerate organs. *Adv. Polym. Sci.* 1995;**122**:245–274.

250. Knowles, J. C. and G. W. Hastings. In vitro degradation of a polyhydroxybutyrate/polyhydroxyvalerate copolymer. *J. Mater. Sci. Mater. Med.* 1992;**3**:352–358.

251. Fukada, E. and Y. Ando. Bending piezoelectricity in a microbially produced poly-beta-hydroxybutyrate. *Biorheology* 1988;**25**:297–302.

252. Vainionpää, S., K. Vihtonen, M. Mero, H. Pätiälä, P. Rokkanen, J. Kilpikari, P. Törmälä. Biodegradable fixation of rabbit osteotomies. *Acta Orthopaed. Scand.* 1986;**57**:237–239.

253. Kramp, B., H. E. Bernd, W. A. Schumacher, M. Blynow, W. Schmidt, C. Kunze, D. Behrend, and K. P. Schmitz. Poly-beta-hydroxybutyric acid (PHB) films and plates in defect covering of the osseus skull in a rabbit model. *Laryngo-Rhino-Otol* 2002;**81**:351–356.

254. Kostopoulos, L. and T. Karring. Guided bone regeneration in mandibular defects in rats using a bioresorbable polymer. *Clin. Oral Impl. Res.* 1994;**5**:66–74.

255. Kostopoulos, L. and T. Karring. Augmentation of the rat mandible using guided tissue regeneration. *Clin. Oral Impl. Res.* 1994;**5**:75–82.

256. Doyle, C., E. T. Tanner, and W. Bonfield. In vitro and in vivo evaluation of polyhydroxybutyrate and of polyhydroxybutyrate reinforced with hydroxyapatite. *Biomaterials* 1991;**12**:841–847.

257. Luklinska, Z. B. and W. Bonfield. Morphology and ultrastructure of the interface between hydroxyapatite-polyhydroxybutyrate composite implant and bone. *J. Mater. Sci. Mater. M* 1997;**8**:379–383.

258. Ni, J. and M. Wang. In vitro evaluation of hydroxyapatite reinforced polyhydroxybutyrate composite. *Mater. Sci. Eng. C-Bio. S.* 2002;**20**:101–109.

259. Shamsuria, O., A. S. Fadilah, A. B. Asiah, M. R. Rodiah, A. H. Suzina, and A. R. Samsudin. In vitro cytotoxicity evaluation of biomaterials on human osteoblast cells CRL-1543; hydroxyapatite, natural coral and polyhydroxybutarate. *Med. J. Mal.* 2004;**59**:174–175.

260. Al-Salihi, K. A. and A. R. Samsudin. Coral-polyhydroxybutrate composite scaffold for tissue engineering: Prefabrication properties. *Med. J. Mal.* 2004;**59**: 202–203.

261. Galgut, P., R. Pitrola, I. Waite, C. Doyle, and R. Smith. Histological evaluation of biodegradable and non-degradable membranes placed transcutaneously in rats. *J. Clin. Periodontol.* 1991;**18**:581–586.

262. Gogolewski, S., M. Jovanovic, S. M. Perren, J. G. Dillon, and M. K. Hughes. Tissue response and in vivo degradation of selected polyhydroxyacids: Polylactides (PLA), poly(3-hydroxybutyrate) (PHB), and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB/VA). *J. Biomed. Mater. Res.* 1993;**27**:1135–1148.

263. Leenstra, T. S., J. C. Maltha, and A. M. Kuipers-Jagtman. Biodegradation of non porous films after submucoperiosteal implantation on the palate of Beagle dogs. *J. Mater. Sci. Mater. Med.* 1995;**6**:445–450.

264. Leenstra, T. S., A. M. Kuijpers-Jagtman, and J. C. Maltha. The healing process of palatal tissues after palatal surgery with and without implantation of membranes: an experimental study in dogs. *J. Mater. Sci. Mater. Med.* 1998;**9**: 249–255.

265. Shishatskaya, E. I., T. G. Volova, and I. I. Gitelson. In vivo toxicological evaluation of polyhydroxyalkanoates. *Doklady Biol. Sci.* 2002;**383**:565–567.

266. Köse, G. T., F. Korkusuz, P. Korkusuz, and V. Hasirci. In vivo tissue engineering of bone using poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) and collagen scaffolds. *Tissue. Eng*. 2004;**10**:1234–1250.

267. Brophy, M. R. and P. B. Deasy. In vitro and in vivo studies on biodegradable polyester microparticles containing sulphamethizole. *Int. J. Pharm*. 1986;**29**: 223–231.

268. Gangrade, N. and J. C. Price. Poly(hydroxybutyrate-hydroxyvalerate) microspheres containing progesterone: Preparation, morphology and release properties. *J. Microencapsul*. 1991;**8**:185–202.

269. Embleton, J. K. and B. J. Tighe. Regulation of polyester microcapsule morphology. *Drug Target. Deliv*. 1992;**1**:45–54.

270. Gürsel, I. and V. Hasirci. Properties and drug release behavior of poly(3-hydroxybutyric acid) and various poly(3-hydroxybutyrate-*co*-hydroxyvalerate) copolymer microcapsules. *J. Microencapsul*. 1995;**12**:185–193.

271. Atkins, T. W. and S. J. Peacock. The incorporation and release of bovine serum albumin from poly-hydroxybutyrate-hydroxyvalerate microcapsules, *J. Microencapsulation* 1996;**13**:709–717.

272. Sendil, D., I. Gursel, D. L. Wise, and V. Hasirci. Antibiotic release from biodegradable PHBV microparticles. *J. Control. Release* 1999;**59**:207–217.

273. Yagmurlu, M. F., F. Korkusuz, I. Gursel, P. Kokusuz, U. Ors, and V. Hasirci. Sulbactam-cefoperazone polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV) local antibiotic delivery system: In vivo effectiveness and biocompatibility in the treatment of implant-related experimental osteomyelitis. *J. Biomed. Mater. Res*. 1999;**46**:494–503.

274. Gotfredsen, K., L. Nimb, and E. Hjørting-Hansen. Immediate implant placement using a biodegradable barrier, polyhydroxybutyrate-hydroxyvalerate reinforced with polyglactin 910. An experimental study in dogs. *Clin. Oral Imp. Res*. 1994;**5**:83–91.

275. Galego, N., C. Rozsa, R. Sánchez, J. Fung, A. Vázquez, and J. S. Tomás. Characterization and application of poly(β-hydroxyalkanoates) family as composite biomaterials. *Polym. Test* 2000;**19**:485–492.

276. Luklinska, Z. B. and H. Schluckwerder. In vivo response to HA-polyhydroxybutyrate/polyhydroxyvalerate composite. *J. Microsc. Oxford* 2003;**211**:121–129.

277. Sultana, N. and M. Wang. Fabrication of HA/PHBV composite scaffolds through the emulsion freezing/freeze-drying process and charaterisation of the scaffolds. *J. Mater. Sci: Mater. Med*. 2008;**19**:2535–2561.

278. Chen, D. Z., C. Y. Tang, K. C. Chan, C. P. Tsui, P. H. F. Yu, M. C. P. Leung, and P. S. Uskokovic. Dynamic mechanical properties and in vitro bioactivity of PHBHV/HA nanocomposite. *Compos. Sci. Technol*. 2007;**67**:1617–1626.

279. Li, H. and J. Chang. Fabrication and characterization of bioactive wollastonite/PHBV composite scaffolds. *Biomaterials* 2004;**25**:5473–5480.

280. Chen, L. J. and M. Wang. Production and evaluation of biodegradable composites based on PHB–PHV copolymer. *Biomaterials* 2002;**23**:2631–2639.

281. Shinoka, T., C. K. Breuer, R. E. Tanel, G. Zund, T. Miura, P. X. Ma, R. Langer, J. P. Vacanti, J. E. Jr. Mayer. Tissue engineering heart valves: Valve leaflet replacement study in a lamb model. *Ann. Thor. Surg*. 1995;**60**:S513–S516.

282. Sodian, R., M. Loebe, A. Hein, D. P. Martin, S. P. Hoerstrup, E. V. Potapov, H. Hausmann, T. Lueth, and R. Hetzer. Application of stereolithography for scaffold fabrication for tissue engineered heart valves. *ASAIO J*. 2002;**48**: 12–16.

283. Hoerstrup, S. P., G. Zünd, R. Sodian, A. M. Schnell, J. Grünenfelder, and M. I. Turina. Tissue engineering of small caliber vascular grafts. *Eur. J. Cardiothorac. Surg*. 2001;**20**:164–169.

284. Shum-Tim, D., U. Stock, J. Stock, T. Shinoka, J. Lien, M. A. Moses, A. Stamp, G. Taylor, A. M. Moran, W. Landis, R. Langer, J. P. Vacanti, and J. E. Mayer. Tissue engineering of autologous aorta using a new biodegradable polymer. *Ann. Thorac. Surg*. 1999;**68**:2298–2305.

285. Dai, Z. W., X. H. Zou, and G. Q. Chen. Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) as an injectable implant system for prevention of post-surgical tissue adhesion. *Biomaterials* 2009;**30**:3075–3083.

286. Dunn, R. L., J. P. English, D. R. Cowsar, and D. P. Vanderbilt. Biodegradable in situ forming implants and methods of producing the same. U.S. Patent Application No. 4,938,763, 1990.

287. Ruel-Gariépy, E. and J. C. Leroux. In situ-forming hydrogels – review of temperature-sensitive systems. *Eur. J. Pharm. Biopharm*. 2004;**58**:409–426.

288. Hatefi, A. and B. Amsden. Biodegradable injectable in situ forming drug delivery systems. *J. Control. Release* 2002;**80**:9–28.

289. Rothen-Weinhold, A., K. Besseghir, E. Vuaridel, E. Sublet, N. Oudry, F. Kubel, and R. Gurny. Injection-molding versus extrusion as manufacturing technique for the preparation of biodegradable implants. *Eur. J. Pharm. Biopharm*. 1999;**48**:113–121.

290. Yao, Y. C., X. Y. Zhan, J. Zhang, X. H. Zou, Z. H. Wang, Y. C. Xiong, J. Chen, and G. Q. Chen. A specific drug targeting system based on polyhydroxyalkanoate granule binding protein PhaP fused with targeted cell ligands. *Biomaterials* 2008;**29**:4823–4830.

291. Bian, Y. Z., Y. Wang, G. Aibaidoula, G. Q. Chen, and Q. Wu. Evaluation of poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) conduits for peripheral nerve regeneration. *Biomaterials* 2009;**30**:217–225.

292. Moutos, F. T., L. E. Freed, and F. Guilak. A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. *Nature Mater*. 2007;**6**:162–167.

293. Chen, R., S. J. Curran, J. M. Curran, and J. A. Hunt. The use of poly(L-lactide) and RGD modified microspheres as cell carriers in a flow intermittency bioreactor for tissue engineering cartilage. *Biomaterials* 2006;**27**:4453–4460.

294. Gong, Y., L. He, J. Li, Q. Zhou, Z. Ma, C. Gao, and J. Shen. Hydrogel-filled polylactide porous scaffolds for cartilage tissue engineering. *J. Biomed. Mater. Res. B* 2007;**82**:92–204.

295. Fan, H., Y. Hu, C. Zhang, X. Li, R. Lv, L. Qin, and R. Zhu. Cartilage regeneration using mesenchymal stem cells and a PLGA gelatin/chondroitin/hyaluronate hybrid scaffold. *Biomaterials* 2006;**27**:4573–4580.

296. Xin, X. J., M. Hussain, and J. J. Mao. Continuing differentiation of human mesenchymal stem cells and induced chondrogenic and osteogenic lineages in electrospun PLGA nanofiber scaffold. *Biomaterials* 2007;**28**:316–325.

297. Xia, W., W. Liu, L. Cui, Y. Liu, W. Zhong, D. Liu, J. Wu, K. Chua, and Y. Cao. Tissue engineering of cartilage with the use of chitosan–gelatin complex scaffolds. *J. Biomed. Mater. Res. B* 2004;**71**:373–380.

298. Yamane, S., N. Iwasaki, Y. Kasahara, K. Harada, T. Majima, K. Monde, S. Nishimura, and A. Minami. Effect of pore size on in vitro cartilage formation using chitosan-based hyaluronic acid hybrid polymer fibers. *J. Biomed. Mater. Res. A* 2007;**81**:586–593.

299. Wang, Y., D. J. Blasioli, H. J. Kim, H. S. Kim, and D. L. Kaplan. Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. *Biomaterials* 2006;**27**:4434–4442.

300. Deng, Y., X. S. Lin, Z. Zheng, J. G. Deng, J. C. Chen, H. Ma, and G. Q. Chen. Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) promoted production of extracellular matrix of articular cartilage chondrocytes in vitro. *Biomaterials* 2003; **24**:4273–4281.

301. Jing, X., L. Zhang, Z. A. Zheng, G. Q. Chen, Y. Gong, N. Zhao, and X. Zhang. Preparation and evaluation of porous poly(3-hydroxybutyrate-*co*-3-hydroxyhexanaote) hydroxyapatite composite scaffolds. *J. Biomater. Appl.* 2008;**22**:293–307.

302. Wang, Y., Y. Z. Bian, Q. Wu, and G. Q. Chen. Evaluation of three-dimensional scaffolds prepared from poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) for growth of allogeneic chondrocytes for cartilage repair in rabbits. *Biomaterials* 2008;**29**:2858–2868.

303. Marois, Y., Z. Zhang, M. Vert, L. Beaulieu, R. W. Lenz, and R. Guidoin. In vivo biocompatibility and degradation studies of polyhydroxyoctanoate in the rat: a new sealant for the polyester arterial prosthesis. *Tissue Eng.*1999;**5**:369–386.

304. Marois, Y., Z. Zhang, M. Vert, X. Deng, R. W. Lenz, and R. Guidoin. *Bacterial polyesters for biomedical applications: In vitro and in vivo assessments of sterilization, degradation rate and biocompatibility of poly(β-hydroxyoctanoate) (PHO)*. In: *Synthetic Bioabsorbable Polymers for Implants*. C. M. Agrawal, J. E. Parr, and S. T. Lin (Eds.). ASTM: Scranton, PA, 2000, pp. 12–38.

305. Duvernoy, O., T. Malm, J. Ramström, and S. Bowald. A biodegradable patch used as a pericardial substitute after cardiac surgery: 6- and 24- month evaluation with CT. *Thorac. Cardiovas. Surg.* 1995;**43**:271–274.

306. Li, W. J., C. T. Laurencin, E. J. Caterson, R. S. Tuan, and F. K. Ko. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. *J. Biomed. Mater. Res.* 2002;**60**:613–621.

307. Li, W. J., R. Tuli, X. Huang, P. Laquerriere, and R. S. Tuan. Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold. *Biomaterials* 2005;**26**:5158–5166.

308. Li, W. J., Y. Guo, Y. Wei, A. G. MacDiarmid, and P. I. Lelkes. Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. *Biomaterials* 2006;**27**:2705–2715.

309. Huang, Z. M., Y. Z. Zhang, M. Kotaki, and S. Ramakrishna. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Compos. Sci. Technol.* 2003;**63**:2223–2253.

310. Kim, J. and D. H. Reneker. Mechanical properties of composites using ultrafine electrospun fibers. *Polym. Composite*. 1999;**20**:124–131.

311. Chong, E. J., T. T. Phan, I. J. Lim, Y. Z. Zhang, B. H. Bay, S. Ramakrishna, and C. T. Lim. Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta. Biomater*. 2007;**3**: 321–330.

312. Boland, E. D., G. L. Bowlin, D. G. Simpson, and G. E. Wnek. Electrospinning of tissue engineering scaffolds. *Abstr. Papers. Am. Chem. Soc*. 2001;**222**, 31-PMSE.

313. Boland, E. D., G. E. Wnek, D. G. Simpson, K. J. Pawlowski, and G. L. Bowlin. Tailoring tissue engineering scaffolds using electrostatic processing techniques: A study of poly(glycolic acid) electrospinning. *J. Macromol. Sci. Pure. Appl. Chem*. 2001;**38**:1231–1243.

314. Jia, H., G. Zhu, B. Vugrinovich, W. Kataphinan, D. H. Reneker, and P. Wang. Enzyme-carrying polymeric nanofibers prepared via electrospinning for use as unique biocatalysts. *Biotechnol. Progr*. 2002;**18**:1027–1032.

315. Kim, K., M. Yu, X. Zong, J. Chiu, D. Fang, and Y. S. Seo. Control of degradation rate and hydrophilicity in electrospun non-woven poly(D,L-lactide) nanofiber scaffolds for biomedical applications. *Biomaterials* 2003;**24**:4977–4985.

316. Khil, M. S., D. I. Cha, H. Y. Kim, I. S. Kim, and N. Bhattarai. Electrospun nanofibrous polyurethane membrane as wound dressing. *J. Biomed. Mater. Res*. 2003;**67B**:675–679.

317. Min, B. M., G. Lee, S. H. Kim, Y. S. Nam, T. S. Lee, and W. H. Park. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials* 2004;**25**:1289–1297.

318. Xu, C. Y., R. Inai, M. Kotaki, and S. Ramakrishna. Aligned biodegradable nanofibrous structure: A potential scaffold for blood vessel engineering. *Biomaterials* 2004;**25**:877–886.

319. Zong, X. H., S. Li, E. Chen, B. Garlick, K. S. Kim, and D. F. Fang. Prevention of postsurgery-induced abdominal adhesions by electrospun bioabsorbable nanofibrous poly(lactide-*co*-glycolide)-based membranes. *Ann. Surg*. 2004;**240**:910–915.

320. Zong, X., S. Ran, K. S. Kim, D. Fang, B. S. Hsiao, and B. Chu. Structure and morphology changes during in vitro degradation of electrospun poly(glycolide-*co*-lactide) nanofiber membrane. *Biomacromolecules* 2003;**4**:416–423.

321. Zong, X. H., S. F. Ran, D. F. Fang, B. S. Hsiao, and B. Chu. Control of structure, morphology and property in electrospun poly(glycolide-*co*-lactide) non-woven membranes via post-draw treatments. *Polymer* 2003;**44**:4959–4967.

322. Huang, L., R. P. Apkarian, and E. L. Chaikof. High-resolution analysis of engineered type I collagen nanofibers by electron microscopy. *Scanning* 2001;**23**:372–375.

323. Matthews, J. A., G. E. Wnek, D. G. Simpson, and G. L. Bowlin. Electrospinning of collagen nanofibers. *Biomacromolecules* 2002;**3**:232–238.

324. Zong, X. H., K. Kim, D. F. Fang, S. F. Ran, B. S. Hsiao, and B. Chu. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *Polymer* 2002;**43**:4403–4412.

325. Luu, Y. K., K. Kim, B. S. Hsiao, B. Chu, and M. Hadjiargyrou. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA–PEG block copolymers. *J. Control. Release* 2003;**89**:341–353.

326. Choi, J. S., S. W. Lee, L. Jeong, S. H. Bae, B. C. Min, J. H. Youk, and W. H. Park. Effect of organosoluble salts on the nanofibrous structure of electrospun poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate). *Int. J. Biol. Macromol.* 2004;**34**:249–256.

327. Cheng, M. L., C. C. Lin, H. L. Su, P. Y. Chen, and Y. M. Sun. Sun. Processing and characterization of electrospun poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) nanofibrous membranes. *Polymer* 2008;**49**:546–553.

328. Ying, T. H., D. Ishii, A. Mahara, S. Murakami, T. Yamaoka, K. Sudesh, R. Samian, M. Fujita, M. Maeda, and T. Iwata. Scafflods from electrospun polyhydroxyalkanoate copolymer: Fabrication, characterization, biosbsorption and tissue response. *Biomaterials* 2008;**29**:1307–1317.

329. Suwantong, O., S. Waleetorncheepsawat, N. Sanchavanakit, P. Pavasant, P. Cheepsunthorn, T. Bunaprasert, and P. Supaphol. In vitro biocompatibility of electrospun poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) fiber mats. *Int. J. Biol. Macromol.* 2007;**40**:217–223.

330. Ito, Y., H. Hasuda, M. Kamitakahara, C. Ohtsuki, M. Tanihara, I. K. Kang, and O. H. Kwon. A composite of hydroxyapatite with electrospun biodegradable nanofibers as a tissue engineering material. *J. Biosci. Bioeng*. 2005;**100**:43–49.

331. Park, S. J., S. H. Lee, E. J. Lee, H. O. Kang, T. W. Kim, T. H. Yang, and S. Y. Lee. Copolymer comprising 4-hydroxybutyrate unit and lactate unit and its manufacturing method. European Patent Publication No. WO2008062995 (A1), 2008.

332. Park, S. J., S. H. Lee, E. J. Lee, H. O. Kang, T. W. Kim, T. H. Yang, and S. Y. Lee. Copolymer containing 3-hydroxyalkanoate unit and lactate unit, and its manufacturing method, European Patent Publication No. WO2008062996 (A1), 2008.

333. Park, S. J., T. H. Yang, H. O. Kang, S. H. Lee, E. J. Lee, T. W. Kim, and S. Y. Lee. Mutants of PHA synthase from *Pseudomonas* sp. 6-19 and method for preparing lactate homopolymer or copolymer using the same, European Patent Publication No. WO2008062999 (A1), 2008.

334. Taguchi, S., M. Yamada, K. Matsumoto, K. Tajima, Y. Satoh, M. Munekata, K. Ohno, K. Kohda, T. Shimamura, H. Kambe, and S. Obata. A microbial factory for lactate-based polyesters using a lactate-polymerizing enzyme. *Proc. Natl. Acad. Sci. USA*. 2008;**105**:17323–17327.

PART V

SYNTHETIC BIODEGRADABLE POLYMERS

CHAPTER 9

LACTIDE AND GLYCOLIDE POLYMERS

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9.1 INTRODUCTION

Polymers derived from lactic acid, glycolic acid, or combinations thereof have been developed for medical applications for almost half a century [1]. Applications have included devices for bone fracture fixation [2], suture materials [3], sheets for preventing adhesion [4], blood vessel prostheses [5], and drug delivery [6]. Examples of commercially available medical-grade polyesters, their product names, and manufacturers are summarized in Table 9.1 [7].

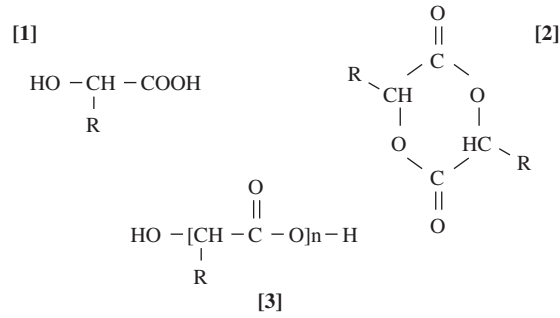
9.2 SYNTHESIS, PROCESSING, AND PROPERTIES

9.2.1 Chemistry, Synthesis, and Characterization of Polymers from Lactide and Glycolide

Lactides and Glycolides—Origin, Structure, and Properties. Lactic acid and glycolic acid are naturally occurring hydroxyacids. The difunctional structure of α-hydroxyacids makes it possible for the molecule to undergo self-condensation reactions and form oligomers and/or polymers (Fig. 9.1). Due to the reversibility of these reactions, the number of repeating units remains fairly low and the polycondensation is generally difficult to control. The most convenient and well-controlled route for preparing polymers with repeating lactoyl or glycoyl units is to use the ring-formed and dehydrated dimer of the corresponding hydroxyacid as the starting point and perform ring-opening polymerization (ROP). Polymers prepared by ROP are the most commonly studied since polymer chemistry and hence properties can be accurately controlled. Preparation of the dimer includes polycondensation of the hydroxyacid followed by a depolymerization, which is conventionally done by increasing the polycondensation temperature and lowering the pressure. The repeating units of the poly(hydroxy acid) are reorganized into the dehydrated cyclic dimer by unzipping depolymerization at high temperatures. The dimer is generally separated by fractional distillation under reduced pressure and the formed glycolide (1,4-dioxane-2,5-dione) and lactide (3,6-dimethyl-1,4-dioxane-2,5-dione) can be further purified if desired. Due to the two stereoforms of lactic

TABLE 9.1 Commercially Available Medical-Grade Aliphatic Polyesters Derived from Lactic Acid and/or Glycolic Acid

Polymer	Short Name	Product Name	Manufacturer	Reference
Polyglycolide	PGA	Purasorb PG	Purac	8
Poly(L-lactide)	PLLA	Purasorb PL	Purac	8
Poly(D,L-lactide)	PDLA	Purasorb PDL	Purac	8
Poly(L-lactide-*co*-D,L-lactide)	PLDLA	Purasorb PLDL	Purac	8
Poly(D,L-lactide-*co*-glycolide)	PDLLGA	Purasorb PDLG	Purac	8
Poly(L-lactide-*co*-glycolide)	PLLGA	Purasorb PLG	Purac	8
Poly(L-lactide)	PLLA	Lactel	Durect	8
Poly(L-*co*-D,L-lactide)	PDLA	Lactel	Durect	9
Poly(D,L-lactide-*co*-glycolide)	PDLGA	Lactel	Durect	9
Poly(L-lactide)	PLLA	Resomer L	Boeringer Ingelheim	9
Poly(L-lactide-*co*-glycolide)	PLLGA	Resomer LG	Boeringer Ingelheim	10
Poly(D,L-lactide-*co*-glycolide)	PDLGA	Resomer RG	Boeringer Ingelheim	10
Poly(L-*co*-DL-lactide)	PLDLA	Resomer LR	Boeringer Ingelheim	10
Poly(D,L-lactide)	PDLA	N/A	Polyscience	10
Poly(D,L-lactide-*co*-glycolide)	PDLLGA	N/A	Polyscience	11
Polyglycolide	PGA	N/A	Polyscience	11
Poly(L-lactide)	PLLA	N/A	Polyscience	11
Poly(L-lactide-*co*-glycolide)	PLLGA	N/A	Polyscience	11
Poly(D,L-lactide)	PDLA	Lactel	Birmingham Polymers	11
Poly(D,L-lactide-*co*-glycolide)	PDLLGA	Lactel	Birmingham Polymers	12
Poly(L-lactide)	PLLA	Lactel	Birmingham Polymers	12



R = H for glycolic acid [1], glycolide [2], and poly(glycolic acid) [3]

R = CH_3 for lactic acid [1], lactide [2], and poly(lactic acid) [3]

FIGURE 9.1 Structures of monomers, intermediates, and polymers of glycolic and lactic acid.

TABLE 9.2 Properties of Glycolic Acid, Lactic Acid, and Related Derivatives *(Measured for 88% D,L-lactic acid at 25°C)

Compound	CAS Number	Molar Mass (g/mol)	Density $(g/cm)^3$	Melting Point (°C)	Reference
Glycolic acid	79-14-1	76.05	1.27	75	12
Glycolide	502-97-6	116.1		83–86	8
D,L-lactic acid	50-21-5	90.08	1.20*	16.8	13
L,L-lactide/ D,D-lactide	95-96-5	90.08		95	14
rac-lactide		90.08		125	8
meso-lactide		90.08		43–46	15
Lactoyllactic acid	517-52-2	162.14			16

acid, the corresponding optically active lactide can be found in two different versions (i.e., D,D-lactide and L,L-lactide). In addition, lactide can be formed from one D- and one L-lactic acid molecule, yielding D,L-lactide (*meso*-lactide). The term *rac*-lactide is used for a 50/50 mixture of D,D-lactide and L,L-lactide. The properties of glycolic acid, lactic acid, and related derivatives are listed in Table 9.2.

Polymerization. Although ROP of cyclic diesters into high molar mass polymers has been known since the1930s, such reactions were not studied in detail until the late 1950s [17]. The simplest types of polyester are the homopolymers of one type of repeating unit. Polymers with two or more types of repeating unit are copolymers

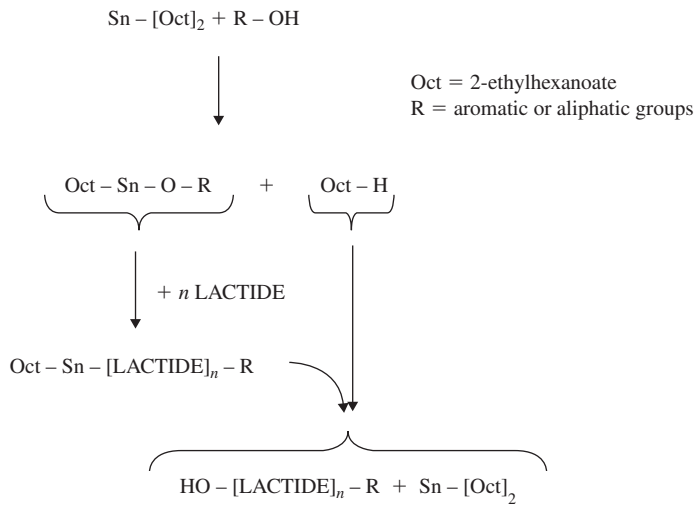


FIGURE 9.2 Schematic description of tin octanoate-catalyzed ring-opening polymerization of lactide.

of lactides with different stereoforms or copolymers of lactide with glycolide. The monomer combinations and length of the sequences have a large influence on polymer properties [18, 19]. The preparation of lactic-acid-based polymers by ROP has been carried out successfully using solution polymerization, bulk polymerization, melt polymerization, and suspension polymerization [20]. The polymerization mechanism involved in ROP can be of ionic, coordination, or free radical type depending on the catalyst system used [21, 22]. The ROP of lactide is reported to be catalyzed by transition metal compounds. Derivatives with a potentially low toxicity are, for instance, organometallic compounds of tin [23, 24], aluminum [25], zinc [26], iron [27], and yttrium [28]. However, tin(II)-2-ethylhexanoate (tin octanoate) is the most frequently used catalyst, which is largely related to the Food and Drug Administration (FDA) acceptance of the compound [29]. It has been suggested that the polymerization mechanisms for this catalyst involve an initiation step by hydroxyl groups (Fig. 9.2) [30].

Several other ring-formed monomers can be incorporated into the lactic-acid-based polymer by ring-opening copolymerization [31]. The most commonly used comonomer is glycolide, and the comonomer units can be organized in block sequences or can be distributed in a random manner. The conversion and distribution of the comonomers depends on the ratio of the comonomers and the polymerization conditions [32]. A large difference in the reactivity of the monomers generally results in a block copolymer. Glycolide shows a reactivity similar to that of lactide [33] and therefore can occur either randomly in copolymers or in blocks depending on the applied polymerization conditions. Lactic acid-based polymers can also be prepared by using a molecule with a

specific structure as an initiating compound (e.g., pentaerythritol). Polymers of this type generally have a complex structure and the properties are accordingly unique [34].

Characterization Techniques. Polymer characterization is of importance in terms of product development, production quality control, and changes occurring during degradation. The following paragraphs provide a brief description of characterization methods that are appropriate for glycolide and lactide polymers.

Size Exclusion Chromatography (SEC). SEC, also referred to as gel permeation chromatography (GPC), is used to determine the molar mass distribution of polymers. Polymers elute from the GPC column at retention times that are related inversely to their hydrodynamic volume, which is in turn related to molar mass. In principle, use of a triple detector system allows information to be obtained on polymer branching through Mark–Houwink plots [35]. Chloroform is the most commonly used mobile phase for poly(lactic acid) (PLA) and lactide–glycolide copolymers in GPC experiments. Fluorinated solvents are the only option for poly(glycolide) (PGA) because of its generally poor solubility. An SEC system consists of a degassing unit, a solvent pump, column(s), and a detector system (e.g., refractive index, viscometry, and light-scattering detectors) and the column(s) are generally calibrated against narrow distribution polystyrene standards. When preparing samples for GPC measurements, the material, typically a few milligrams, is dissolved in 10 mL of $CHCl_3$ to yield a solution of a known concentration. Absolute molecular weights can potentially be determined when a multi-angle light-scattering detector is in place.

Differential Scanning Calorimetry (DSC). DSC measures changes in enthalpy as the temperature is varied in a controlled way and allows the determination of melting or crystallization temperatures (T_m, T_c), corresponding enthalpies (ΔH_m, ΔH_c) and glass transition temperatures (T_g). Polymer samples are typically weighed into aluminum pans that are then sealed and placed in the measuring cell. A standard DSC method includes a first heating cycle in which the enthalpy changes give information about the material as received. The first heating cycle erases any previous thermal history and a second heating cycle can be used for comparative analysis between different samples. DSC allows the simulation of conditions during injection molding or other processing methods.

Thermogravimetric Analysis (TGA). TGA is used to evaluate the thermal stability of polymers. The thermal decomposition temperature and the amount of inorganic residue in a sample can, for example, be determined. Measurements are usually conducted under N_2 atmosphere and samples are placed in an aluminum pan after which exact weighing is performed by the TGA unit.

When measuring the thermal decomposition temperature of standard samples, the heating cycle is typically set from ambient temperature to 500°C at 10°C/min. However, by using TGA it is also possible to conduct isothermal measurements or more complicated heating programs in order to, for example, simulate processing or storage conditions.

Proton Nuclear Magnetic Resonance (^{1}H-NMR). ^{1}H-NMR can be used as an identification and quantification tool in the characterization of polyesters. NMR can, for example, be used to estimate the molar masses or to determine the amount of residual lactide or glycolide. NMR also enables the monomer ratios in the final product to be calculated, which provides information on monomer reactivity. As an example, the amount of residual lactide in PLA can be calculated by relating the peak integrals of lactide at a chemical shift of ~5 ppm to the peak integrals of the CH proton in the repeating unit of PLA at a chemical shift of ~5.2 ppm. The typical solvent used is *d*-$CHCl_3$, although, depending on the sample and its solubility, other deuterated solvents may be used.

9.2.2 Manufacturing Processes

Melt Processing. Lactide and glycolide polymers can be melt processed by extrusion, extrusion coating, thermoforming, injection molding, fiber drawing, and film blowing. Product properties depend on the conditions during processing (e.g., shear rate, temperature, processing time). As an example, melt processing by injection molding and fiber spinning has been reported in manufacturing of fracture fixation devices and sutures [36]. The most important parameters during melt processing will be (i) temperature, (ii) residence time, (iii) moisture content, and (iv) atmosphere, regardless of the type of secondary process applied [37]. A major problem in melt processing of aliphatic polyesters is their limited thermal stability since these polymers tend to degrade into smaller fractions when exposed to heat even for short periods.

Fiber Spinning. Fibers made from lactide and/or glycolide polyesters are commercially important in applications such as sutures, clips, staples, and as reinforcement in orthopedic devices [38, 39]. One of the first commercially available bioresorbable products for medical use was poly(glycolide) fibers (Dexon), developed in the 1960s for use in sutures. Other fiber-based bioresorbable medical products use copolymers of glycolide and L-lactide (Vicryl) [36]. Fibers can be manufactured either by solvent- or melt-spinning processes [40, 41] and drawn in order to orient the polymer chains [42]. Fibers prepared by solvent spinning usually have better mechanical properties because this method avoids the thermal degradation that can occur during melt spinning [43].

Preparation of Micro- and Nanoparticles. Micro- and nanoparticles are important delivery systems used in medicine, and aliphatic polyesters are of interest due to their tunable hydrolytic degradability and low toxicity. Micro- and

nanoparticles can be prepared directly by ROP [44] or by postpolymerization processing as described by Benoit et al. [45]. The preparation of micro- and nanoparticles from aliphatic polyesters by solvent evaporation and solvent extraction methods has been reviewed by Arshady [46]. The most important properties of the micro- and nanoparticles are the release rate of the drug and the degradation profile of the matrix. These properties are affected by the particle design and especially by the polymer design. The particle size, porosity, and drug loading are mainly related to the type of drug and parameters in the preparation (i.e., solvent/nonsolvent system, stirring rate, and temperature). Polymer properties related to the drug release rate and degradation pattern are strongly dependent on solubility, morphology, and stability, which in turn can be related to the structure. Considering the number of reported studies, it appears that copolymers of glycolide and *rac*-lactide have the most suitable combination of properties for use as a drug delivery matrix [1].

Fabrication of Porous Scaffolds. Porous biodegradable polymer scaffolds are potential devices for reconstruction of damaged tissues and organs, and several techniques are available for their manufacture. Many of the fabrication methods involve polymer dissolving and salt leaching [47], but porous aliphatic polyesters have also been prepared by use of emulsion freeze-drying [48], gas-foam forming agents [49], high-pressure gas saturation techniques [50], phase inversion by immersion precipitation [51], thermally induced phase separation (TIPS) [52], and polymer blending followed by extraction [53].

9.2.3 Properties of Glycolide-Lactide Homo- and Copolymers

The properties of PLA depend significantly upon its enantiomeric form and can be modified by polymerization, processing, or manufacturing techniques. Properties are also modified by varying the polymer structure through cross-linking, branching, introduction of other comonomers, or by altering the ratio of the stereoisomers.

Thermophysical Properties. Homopolymers of glycolide have a T_g and T_m of 35–40°C and 225–230°C respectively. PGA is a semicrystalline polymer with a maximum degree of crystallinity (X_c) of about 50% [53]. Enantiomerically pure poly(L-lactide) (PLLA) or poly(D-lactide) (PDDLA) are also semicrystalline polymers, with T_g in the range of 55–60°C and T_m of about 180°C. Polymers prepared from *meso*- or *rac*-lactide are usually amorphous with a T_g of ~50°C. The melt enthalpy estimated for an enantiopure PLLA with a 100% degree of crystallinity (ΔH_m°) is 93 J/g [54, 55], and this value is most often used to calculate the X_c for semicrystalline polylactides. The melting behavior and degree of crystallinity are dependent on the molar mass, thermal history, and purity of the polymers [56, 57]. It has been observed for polylactide that an optical purity of at least 72–75%, corresponding to about 30 isotactic repeating units, is required for the crystallization to take place [58], which

means that copolymers of lactide and glycolide with a random distribution are generally amorphous polymers. Copolymers of L-lactide and glycolide show a T_g ranging from 35 to 60°C depending on composition. Stereocomplexes are formed if polylactides of the opposite stereoforms are mixed together, as first reported by Ikada et al. [59]. These authors discovered that the melting point of blended PLLA and PDDLA is about 50°C higher than that of the pure enantiomeric polymers. Stereocomplexes have since been further investigated both for PLA oligomers and high molar mass polymers [60]. Stereocomplexation of enantiomeric copolymers of lactide and glycolide has also been reported, where the melting point and enthalpy of fusion decreased as the glycolide content in the copolymers increased [61].

Solubility. PGA is insoluble in most organic solvents with the exception of fluorinated solvents (e.g., hexafluoroisopropanol). The solubility of PLA is highly dependent on the molar mass, degree of crystallinity, and the presence of other comonomer units in the polymer. Good solvents for enantiomerically pure poly(lactide) are, for example, chlorinated or fluorinated organic solvents, dioxane, and dioxolane. Poly(*rac*-lactide) and poly(*meso*-lactide) are, in addition to the previously mentioned solvents, soluble in organic solvents such as acetone, pyridine, ethyl lactate, tetrahydrofuran, xylene, ethyl acetate, dimethylsulfoxide, *N*,*N*-dimethylformamide, and methyl ethyl ketone. Typical nonsolvents for lactic-acid-based polymers are water, alcohols (e.g., methanol, ethanol, propylene glycol), and unsubstituted hydrocarbons (e.g., hexane, heptane). Copolymers of lactide and glycolide are soluble in chlorinated solvents and insoluble in, for instance, acetone and cyclohexane [62].

Mechanical Properties. The molar mass and degree of crystallinity significantly influence the mechanical properties of polymers. This is well demonstrated for aliphatic polyesters, for which a wide range of mechanical properties has been reported for lactide and glycolide polymers [62]. PGA is a tough polymer, with a reported tensile modulus of 7 GPa [63]. The semicrystalline PLA is generally preferred to the amorphous polymer when higher mechanical properties are desired. Semicrystalline PLA has an approximate tensile modulus of 3 GPa, tensile strength of 50–70 MPa, flexural modulus of 5 GPa, flexural strength of 100 MPa, and an elongation at break of about 4% [62]. Superior mechanical properties (e.g., tensile strength up to 920 MPa) can be achieved by stereocomplexation of enantiomeric polylactides, which results from the formation of stereocomplex crystallites giving intermolecular crosslinks [64]. Processes in which the polymer chains become oriented can lead to increased mechanical strength, as can happen in fiber preparation by melt or solution spinning from aliphatic polyesters [65, 66]. The resulting fiber mechanical properties are largely dependent on the spinning method and draw ratios [62]. The melt spinning of lactic acid-based polymers is associated with thermal degradation, with decreases in molar mass during the melt spinning reported to be as high as 85% for copolymers of *rac*- and L-lactide

of high initial molar mass (600 kDa) [65]. PLLA of high molar mass has sufficient strength for use as load-bearing materials in medical applications; however, the crystalline domains tend to degrade slowly [67]. The crystallinity can be reduced by copolymerizing with D-lactide or glycolide, leading to amorphous polymers with faster degradation profiles [68]. However, this process also affects polymer toughness and the impact strength of PLA has been shown to decrease threefold (to 5 kJ/m^2) when copolymerizing with 5 mol % D-lactide [69].

Thermal Stability. The thermal stability of glycolic-acid- [70] and/or lactic-acid-based polymers is poor at elevated temperatures, especially at temperatures above the melting point. Reactions involved in the thermal degradation of lactide and glycolide polymers include thermohydrolysis [71], zipper-like depolymerization [72], thermooxidative degradation [73], and transesterification [74]. Rheological measurements have shown that the thermal degradation of poly(L-lactide) is accelerated when the moisture content of the polymer is increased [75]; however, optimal drying conditions can reduce degradation during extrusion. The presence of catalyst and especially the catalyst concentration are of great importance for the thermal stability of poly(lactide) [76]. Polymers used for medical applications are generally purified in order to remove unbound catalyst, residual monomer, and other impurities, which are reported to influence thermal stability [77].

Radiation Stability. Medical devices need to be sterilized and common sterilization methods use γ-or β-radiation. Radiation can cause either crosslinking or chain scission reactions. For most polymers both reactions occur simultaneously. The irradiation effects on aliphatic polyesters have been thoroughly studied by D'Alelio et al. [78], and these authors concluded that the crosslinking-to-scission ratio for aliphatic polyesters increased on irradiation as a function of increasing -CH_2- to -COO- ratio in the main chain. Further studies on radiation stability have been reported for polyglycolide, poly(L-lactide) [79], poly(D,L-lactide) [80], and for copolymers of lactide and glycolide [79]. Polylactides mainly undergo chain scissions at radiation doses below 250 kGy. For higher doses, crosslinking reactions increase as a function of the irradiation dose both in air and in inert atmosphere [81]. It has been suggested that irradiation causes reactions in the amorphous phase of the polymer and a difference in the radiation effects may therefore be expected for poly(*rac*-lactide) and poly(L-lactide) [79]. The irradiation of poly(glycolide) can cause a decrease in molar mass, and it has been suggested that this involves about the same amount of crosslinking as chain scission [82]. Radiation has been reported to reduce tensile strength and to accelerate the start of hydrolytic degradation in PGA fibers [83]. Copolymers of lactide and glycolide, P(LA-*co*-GA), have been reported to undergo dose-dependent degradation upon irradiation [84]. The irradiation of P(LA-*co*-GA) results in either formation of radicals at the carbon

atom adjacent to the ester group or by loss of the ester linkage. Electron spin resonance studies have suggested that the radicals formed at the lactide units are more stable than those formed at the glycolide units.

Hydrolytic Stability. Hydrolysis of polymers leads to molecular fragmentation, which is determined by various factors such as chemical structure, molar mass and its distribution, purity, morphology, shape of the specimen and history of the polymer, as well as the conditions under which the hydrolysis is conducted [85]. The hydrolytic degradation of glycolide and/or lactide polymers is undesirable during processing or material storage but is ultimately essential for most intended applications. The hydrolysis of aliphatic polyesters starts with a water uptake phase followed by hydrolytic splitting of the ester bonds in a random manner. The initial degree of crystallinity of the polyesters affects the rate of hydrolytic degradation because the crystalline regions reduce water permeation in the matrix. The hydrolytic degradation process therefore begins with degradation of the amorphous regions in which the molecular fragments, which tie the crystalline segments together by entanglement, are hydrolyzed. As a result, the remaining chain segments occupy more space and have greater mobility, leading to reorganization of the polymer chains and inducing crystallization [86]. These phenomena generally result in whitening of specimens and a simultaneous decrease in mechanical strength and molar mass. The temperature during hydrolysis is a key influence on the degradation rate, not only because the rate of hydrolysis increases at elevated temperatures but also because of increased polymer chain flexibility and permeability at temperatures above the T_g. The hydrolysis of aliphatic polyesters has been studied for various compositions including polyglycolide [87], poly(L-lactide) [88], poly(*rac*-lactide) [89], poly(L-lactide-*co*-glycolide) [83], and poly(*rac*-lactide-*co*-glycolide) [90]. The hydrolytic degradation of glycolide/lactide homo- and copolymers is homogeneous (i.e., the number-average molar mass has significantly decreased before any weight loss is noticed). In the second phase of hydrolysis the degradation of the crystalline regions of the polyester leads to an increased rate of mass loss and finally to complete resorption. The degradation of poly(lactide) in aqueous media is reported to proceed more rapidly in the bulk of a specimen. The explanation for this behavior is an autocatalytic effect due to the increasing presence of compounds containing carboxylic end groups. These low molar mass compounds cannot permeate the outer crystalline shell [91]. The resorption time of L-lactide homopolymers varies from a few months for a low molar mass poly(L-lactic acid) film to an estimated 50–60 years for oriented fibers [88]. The reasons for the differences in stability can be found in the purity of the polymer, molar mass and its distribution, crystallinity, and orientation. Since poly(*rac*-lactide) lacks crystalline regions, water uptake and hence hydrolysis occur more rapidly. The hydrolytic degradation rate for copolymers of *rac*-lactide and glycolide is significantly higher than that for *rac*-lactide homopolymers [92].

9.3 TOXICITY AND SAFETY

9.3.1 Introduction

PLA, PGA, and their copolymers have been investigated for a very wide range of biomedical uses, including resorbable medical implants, bone and tissue engineering, drug delivery systems, and films for wound treatment. In these applications the chosen polymer must not only meet physical requirements, such as the need for high mechanical strength in orthopedic devices, but must also be nontoxic and safe to use. The degradation of these polymers during use can occur by simple hydrolysis of the ester bond and does not require the presence of catalytic enzymes. Degradation of PLA or poly(L-lactide-*co*-glycolide) (PLGA) occurs by autocatalytic cleavage of the ester bonds through spontaneous hydrolysis into oligomers and D,L-lactic and glycolic acid monomers [93]. Lactate is converted into pyruvate and glycolate and then enters the Krebs cycle to be degraded into CO_2 and H_2O. Degradation products of PLA are generally considered nontoxic to living organisms since lactic acid, one of the primary breakdown products, occurs naturally through metabolic activity in the human body [94]. A general description of the degradation pathways of PLA is included in a recent review by Gupta and Kumar [95].

For acceptable biocompatibility and safety in medical use, the polymers should also not contain polymerization intiators, stabilizers, solvents, or emulsifiers. General indications are that PLA, PGA, and PLA-*co*-PGA show good biocompatibility and the absence of significant toxicity, although some reduction in cell proliferation has been reported for PLA in vitro [96, 97], which may be associated with the presence of acidic degradation products [98]. The resorbability of α-polyesters such as PLA, PGA, and PLA-*co*-PGA has also been discussed by other authors [99, 100]. Toxicity, biocompatibility, and clinical applications of PLA/PGA copolymers were reviewed in the 1990s, and the authors concluded that these copolymers were suitable materials for controlled release of bioactive agents and for orthopedic applications [101].

9.3.2 Drug or Vaccine Delivery

PLA-*co*-PGA copolymers are the most common type used at present in the form of biodegradable microspheres for drug delivery or vaccine development and are approved for use in drug delivery by the U.S. Food and Drug Administration (FDA) [102]. As a result of their biodegradability, these polymers form biocompatible products that are removed from the body at a slow rate but are reported not to affect normal cell function [103]. Jostel et al. [104] examined the safety profile of a sustained-release formulation of human growth hormone. The formulation was based upon a preparation of recombinant human growth hormone contained in amylopectin microspheres coated with PLA-*co*-PGA. The treatments based on these microspheres were well tolerated and suitable for longer-term trials. Injectable PLGA microspheres have been widely investigated for delivery of antigens and have a proven safety record with established use in

marketed products for controlled delivery of a number of peptide drugs and proteins [105, 106]. There is also significant interest in this copolymer for use in the rapidly developing field of DNA (deoxyribonucleic acid) delivery [107]. PLA-*co*-PGA generally has a history of safe use in humans and good manufacturing practices (GMP) grade material is available from commercial sources.

Olivier discussed safety issues when reviewing the use of PLA nanoparticles to deliver bioactive molecules across the blood–brain barrier [108]. This author observed that degradation rate depends on four basic parameters: hydrolysis rate constant (depending on the molecular weight, the lactic/glycolic ratio, and the morphology), amount of water absorbed, the diffusion coefficient of polymer fragments through the polymer matrix, and the solubility of the degradation products in the surrounding aqueous medium [94, 95]. These parameters are in turn influenced by such factors as temperature, additives (including drug molecules), pH, ionic strength, buffering capacity, nanoparticle size, and processing history. Generally considered as biocompatible [95], PLA or PLGA microspheres are also reported to have good central nervous system (CNS) biocompatibility [88, 109]. No mortality was reported with albumin-coated nanoparticles in mice with up to a 2000 mg/kg dose [110]. However, nanoparticles based on PLA with molar mass of 60,000 stabilized with sodium cholate were much more toxic with two of five deaths at a 220 mg/kg dose and five of five at a 440 mg/kg dose associated with marked clinical signs such as alteration of hematological and biochemical parameters and lung hemorrhage. This toxicity was attributed to intravascular coagulation and associated events related to the physical surface properties of the nanoparticles rather than to the inherent chemical toxicity of cholate or PLA. In contrast, mPEG-PLA nanoparticles based on poly (ethylene glycol) (PEG) with a molar mass of 2000 and PLA with a molar mass of 30,000 were shown to have a good safety profile, with no apparent signs of toxicity at the highest studied dose of 440 mg/kg in mice [111].

Drug-eluting stents based on PLA are under development and have been the focus of pilot studies related to biocompatibility, toxicity, and safety. Uurto et al. [112]. investigated poly-D/L-lactic acid vascular stents loaded with dexamethasone or simvastatin and two different coatings based on PLA plus PDLA and PLA plus polycaprolactone (PCL). Comparison was made with a self-expanding stainless steel stent. The biodegradable polymer stents were found to be biocompatible and reliable and therefore useful for local drug delivery. However, the authors concluded that further study was needed in order to prove the safety and efficacy of the biodegradable polymer drug-eluting stents.

9.3.3 Implants

The α-polyesters are among more than 40 different polymer types that have been used as implants and are generally considered to be biocompatible with no indications of a foreign body response. Applications have ranged from

arthroscopy to tissue engineering [113]. Early reports [114–116] indicated that up to 22% of patients receiving degradable PLA or related polymer implants developed irritation at the implant site; however, negative bacterial culture testing suggested that the response was a consequence of chemical irritation arising from acidic breakdown products [117]. Other implant studies [118–120] have pointed to PLA as a more suitable material for applications such as intervertebral cages than copolymers of PGA, not only in regards to degradation time but also in terms of biocompatibility. Iera et al. [121] reviewed bioabsorbable fixation devices and noted that these materials have been investigated since the 1960s with much research focused on how to overcome any problems associated with such devices. Over time there has been a move toward self-reinforced materials and newer generations containing bioactive substances. Bioabsorbable fixation devices have been in use in craniomaxillofacial (CMF) surgery for several decades with clinical operations starting in the early 1990s [122]. At present, most CMF surgery of this type utilizes L-polylactide combined with either D-polylactide or polyglycolide. These are polymer combinations that have reduced crystallinity, which may explain the lack of clinically adverse reactions. Holmes et al. [123] also pointed out the excellent safety profile of PLA and PGA in craniofacial surgery. The clinical characteristics of PLA, PGA, and related polymers have been reviewed by Suuronen et al. and their safety in use described [124]. In another study, reaction to the use of PLA- or PGA-based implants, such as pins, bolts, rods, and screws, in more than 2500 patients was monitored, and clinically significant adverse reactions were found in only 4.3% of the cases. All but one of these cases involved PGA implants and so the likelihood of nonspecific foreign body reaction to PGA may be estimated from this study. The authors suggested that PLA biocompatibility could be thoroughly confirmed by longer-term studies [125]. This discussion is not to imply that adverse reactions with implants never occur, and in fact there have been reports of foreign body reactions with most bioabsorbable implants. Despite this observation, there is little evidence for seriously adverse clinical symptoms, and the bioabsorbable implant category offers the advantage that properties and degradation characteristics can be modified by appropriate tuning of the polymer chemistry or adjustment of pH in the implant [126].

9.3.4 Electrospun Fiber Applications

Corey et al. investigated the use of electrospun PLGA nanofiber scaffolds for alignment of neuron growth as part of nervous system repair [127]. PLLA nanofibers were spun on to substrates precoated with PLGA. This approach was found to be less toxic to primary neurons than bandage and glue used in other studies. Murakami et al. examined tissue-adhesive hydrogels and stated that PLA is known to be biocompatible and noncytotoxic [128]. The potential of electrospun nanofibrous scaffolds composed of biodegradable polymers such as PLA, PGA, and PLA-*co*-PGA as safe and nontoxic substrates

for a wide variety of uses in drug release and tissue engineering has been recognized [129].

9.3.5 Bone Cements

The use of acrylic bone cement is supported by laboratory and clinical data, validating it as a depot for administration of antibiotics. However, the search continues for alternative materials that do not require secondary operations for removal. Many biodegradable polymers including PLA, PGA, and copolymers have been studied for this use and some are in use outside the United States for clinical applications. However, in the United States none have been approved so far and none are commercially available. McLaren [130] reviewed alternative bone cements and suggested that the total antibiotic load in such materials should not exceed the acceptable toxicity risk when administering the antibiotic intravenously over 24 h.

9.3.6 Other Applications

El-Beyrouty et al. [131] reviewed the clinical data for L-PLA in respect to its use as an intradermal injection for treatment of facial fat loss associated with the human immunodeficiency virus (HIV). PLA is approved by the FDA for correction of facial lipoatrophy in people with HIV. The results of six clinical trials showed an increase in cutaneous thickness as a result of such treatments, although adverse effects included nodule and hematoma formation as well as pain at the injection site. Researchers [122, 127] have discussed the use of resorbable devices for treatment of acquired and congenital craniofacial deformities. These reports note that PLA and PGA have demonstrated excellent safety profiles in multiple in vitro animal and clinical studies and are currently being used for a wide variety of such uses.

9.4 DRUG DELIVERY APPLICATIONS

9.4.1 Introduction

Polymeric drug delivery systems provide numerous advantages for the administration of bioactive agents, including protection against degradation, increased aqueous solubility, controlled release, retention of the drug at the site of action, and, in some cases, a method by which the drug can be targeted to the disease site. Several nondegradable polymeric drug delivery systems have reached the market, including contraceptive devices (Norplant), ocular inserts (Vitrasert), and coated medical devices (Taxus stent), demonstrating their superior ability to provide controlled release in chronic treatment. In some cases, the obvious drawback of these systems is the need to remove the device once the drug loading has been exhausted. This may have negative implications in circumstances where device retrieval could have deleterious effects or be

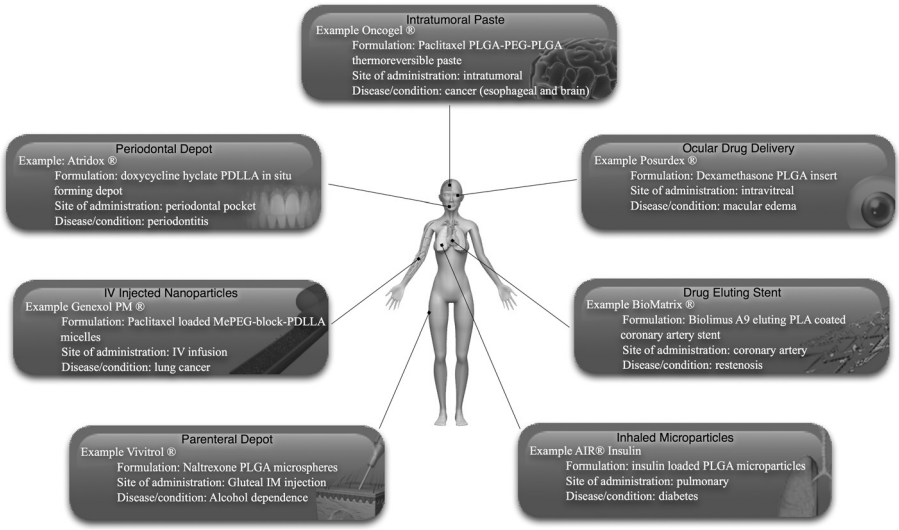


FIGURE 9.3 Polymeric drug delivery systems based on PLA and PLGA currently marketed or in clinical trials are administered to several sites for the treatment of a variety of disease states.

impossible to use as in ocular, pulmonary, and intravenous delivery. Accordingly, the investigation of biodegradable, biocompatible polymers has become the focus of polymeric drug delivery research in recent decades.

The utility of PGA for drug delivery is limited due to its high degree of crystallinity and lack of solubility in appropriate solvents for drug loading. PLLA is more hydrophobic, resulting in prolonged degradation and solubility in a variety of organic solvents. Since the L(+) form is metabolized in the body, this polymer is more commonly used for biomedical applications compared to PDLA [132]. PLGA copolymers are by far the most utilized aliphatic polyesters for drug delivery applications. These copolymers have been used, and are currently being investigated, for the delivery of therapeutic agents to a number of administration sites, whether intended for localized delivery, as in drug-eluting stents, or as a means of gaining access to the systemic circulation, as for parenterally administered depot formulations (Fig. 9.3).

9.4.2 Parenterally Administered Delivery Systems

In nondegradable polymeric drug delivery systems, controlled release is achieved by diffusion of the drug through the polymer matrix, often resulting in predictable zero-order release profiles. Drug release from a degradable system composed of aliphatic polyesters is achieved through a combination of diffusion and polymer degradation mechanisms. This commonly results in a triphasic

release curve characterized by an initial burst phase, followed by a lag period of decreased or no drug release and a final phase of increased release caused by the total disintegration of the device. Thus, by controlling the biodegradation rate of the polymer, the drug release rate can be adjusted. Parameters that influence polymer biodegradation include molecular weight, degree of crystallinity, and hydrophobicity and have been extensively reviewed elsewhere [132–134]. In the case of PLGA, changing the lactide content from 50 to 85%, and hence increasing the hydrophobicity of the polymer, results in prolonged biodegradation times of 2 to 5 months [134]. The proven biocompatibility, FDA approval, and the ability to tailor the release rate are no doubt responsible for the considerable number of parenterally administered PLGA depot formulations on the market (Table 9.3).

9.4.3 Microspheres

Polymeric microspheres may be defined as particles with diameters ranging from 1 to 1000 μm in which drugs are homogeneously dissolved or dispersed in a polymeric matrix [135]. In recent years, parenterally administered microspheres composed of PLA and PLGA have received attention for their tremendous ability to provide controlled release of bioactive agents, resulting in controlled therapeutic plasma levels for the treatment of diseases.

Protein and Peptide Therapeutics. Through advances in biotechnology, a number of protein and peptide therapies have been developed, providing the potential to treat previously untreatable conditions. Since the first FDA-approved peptide delivery system, Lupron Depot, there have been several PLGA microparticulate formulations investigated for the treatment of a variety of therapeutic applications, including cancer and endocrine disorders, with several reaching the market (Table 9.3).

Formulation Issues and Excipients. The formulation of proteins and peptides in polymeric microparticles presents several challenges. In order for these agents to remain active once released, they must retain their native conformation, a task not easily achieved because of the harsh conditions imposed during encapsulation and release. Due to the aqueous solubility of proteins and peptides, a water-in-oil-in-water (w/o/w) emulsification technique is often employed to ensure adequate loading within the hydrophobic polymer matrix. In this method, the protein is first solubilized in an aqueous phase, which is subsequently emulsified in a polymer solution in an organic solvent. This primary emulsion is then emulsified in a secondary aqueous phase, resulting in the extraction of the organic solvent, precipitation of the polymer, and formation of hardened microspheres with the drug entrapped in the matrix. Proteins and peptides are frequently amphipathic in nature, adsorbing at solid or water and organic solvent interfaces and possibly leading to protein denaturation, aggregation, and large burst release profiles. Several methods have been employed to combat this

TABLE 9.3 Parenterally Administered PLGA Depot Formulations

Drug	Trade Name	Polymer	Indication	Duration	Injection Site	Device	Company
Leuprolide acetate	Lupron Depot	PLGA	Prostate cancer Endometriosis Fibroids Central precocious puberty	12 or 16 weeks	Gluteal IM	Microspheres	TAP Pharmaceuticals
Leuprolide acetate	Eligard	PLGA	Prostate cancer	3–6 months	Abdominal SC	In situ depot	Sanofi–Aventis
Goserelin acetate	Zoladex	PLGA	Prostate cancer	4 or 12 weeks	Abdominal SC	Implant	AstraZeneca
Buserelin acetate	Suprefact Depot	PLGA	Prostate cancer	8 or 12 weeks	Abdominal SC	Implant	Sanofi–Aventis
Triptorelin pamoate	Decapeptyl	PLGA	Prostate cancer	4 weeks	Gluteal IM	Microspheres	Ferring Pharmaceuticals
Octreotide acetate	Sandostatin LAR	PLGA	Acromegaly	4 weeks	Gluteal IM	Microspheres	Novartis
Lanreotide	Somatuline LA	PLGA	Acromegaly	2 weeks	Gluteal IM	Microspheres	Ipsen
Triptorelin pamoate	Trelstar Depot	PLGA	Prostate cancer	4 weeks	Gluteal IM	Microspheres	Watson Pharma, Inc.
Triptorelin pamoate	Trelstar LA	PLGA	Prostate cancer	12 weeks	Gluteal IM	Microspheres	Watson Pharma, Inc.
Risperidone	Risperdal Consta	PLGA	Antipsychotic	2 weeks	Gluteal IM	Microspheres	Janssen
Naltrexone	Vivitrol	PLGA	Alcohol dependence	4 weeks	Gluteal IM	Microspheres	Alkermes, Inc.

potential instability. Excipients that preferentially accumulate at the phase interface and therefore take the place of the therapeutic molecule are added to the aqueous phase of the primary emulsion. Such excipients include albumin [136], phosphatidyl choline [137], polyethylene glycol [138], and gelatin [139]. Trehalose was demonstrated to be particularly effective at stabilizing proteins such as recombinant human growth hormone (rhGH) [140] and interferon-γ [141] by acting to shield the protein from the organic solvent. One way of solving instability problems associated with w/o/w emulsification is to avoid it altogether. This can be achieved by using a cryogenic method of protein encapsulation. In this technique, the protein, in a solid form prepared by lyophilization, is first suspended as a solid in the PLGA and organic solvent solution. This dispersion is then ultrasonically sprayed into liquid nitrogen, and frozen ethanol is subsequently added to extract the organic solvent. Since the process is completely anhydrous and the protein is insoluble in all components used, the protein retains its integrity and is encapsulated with a high efficiency. This process, termed ProLease technology, was developed by Alkermes, Inc. and was successfully used for the development of the controlled-release formulation of rhGH, Nutropin Depot [140].

Protein instability can also occur during the release from PLGA microparticles. Once in contact with the aqueous phase, PLGA begins to absorb water, which in turn may increase the mobility of loaded proteins, potentially causing aggregation and adsorption of protein to the PLGA. These effects may cause incomplete release of the protein or release of inactive (aggregated) proteins. Forming insoluble but reversible complexes with zinc can avoid this instability. This method was used in the formulation of rhGH resulting in the release of monomeric and bioactive protein [142]. Furthermore, during the degradation of PLGA the pH of the surrounding media has been shown to dramatically decrease due to the production of carboxylic acids during hydrolysis of the ester bonds. This acidic pH can lead to unfolding, hydrolysis, and aggregation of proteins and peptides. The addition of inorganic salts that act as antacids, such as magnesium hydroxide or sodium bicarbonate, can improve the stability of encapsulated proteins during their release [143].

PEGylation Strategies for Protein and Pepetide Delivery. A number of poly(ethylene glycol)-conjugated (PEGylated) proteins have been approved by the FDA [144]. PEGylation has been shown to improve the plasma half-life of these proteins; however, regular injection is still required. PEGylation of proteins may also hold the answer to many of the problems associated with the development of controlled microparticulate protein delivery previously mentioned. It has been shown in several instances that PEGylated proteins exhibit improved stability in organic solvents, such as methylene chloride, decreased aggregation, and reduced adsorption to PLGA [145, 146]. Furthermore, PEGylated proteins have demonstrated more favorable release profiles with a decreased burst phase and prolonged and often complete protein release [145–147]. This may be attributed to the reduced aggregation and more even distribution of the protein in the microsphere.

Clinical Trial Formulations. Amylin Pharmaceuticals Inc. in collaboration with Eli Lilly and Company have recently developed a once-weekly, long-acting release (LAR), subcutaneous injection of exenatide (exenatide LAR), an incretin mimetic or glucagon-like receptor agonist based on Alkermes' proprietary PLGA microspheres system, Medisorb, for the treatment of type II diabetes. Although available details on the formulation are limited, the results from clinical trials have recently been published. Compared to placebo, patients treated with exenatide LAR had reduced mean hemoglobin A_{1c} (HbA_{1c}) levels, an indicator of average plasma glucose concentration over extended periods of time, reduced fasting glucose levels, and significant body weight reductions [148]. In a recent noninferiority study, exenatide LAR was compared to twice-daily dosed exenatide. Patients receiving once-weekly injections of exenatide LAR had significantly lower HbA_{1c} levels with a greater proportion of patients reaching target HbA_{1c} levels of 7.0% or less [149]. This medication has the potential to provide superior glycemic control and weight loss for type II diabetic patients with convenient once-weekly treatment. Exenatide LAR is currently in phase III trials.

Small-Molecule Therapeutics. The use of parenterally administered microparticles is a common strategy for the controlled delivery of small-molecule drugs. Recently, a number of drugs that have previously been approved for oral administration have been reformulated as controlled-release parenterally administered PLGA microspheres. In many cases the advantage of these systems is improved patient compliance by eliminating the need for frequent dosing, maintaining prolonged therapeutic blood levels or, in some cases, delivering the drug directly to the site of action. In 2003 an intramuscularly (IM) injected controlled-release formulation for the antipsychotic agent risperidone was approved under the trade name Risperdal Consta. These microspheres are composed of PLGA and contain a 38% w/w loading of risperidone produced by a proprietary single oil-in-water emulsification process developed by Alkermes, Inc. The IM injection of Risperdal Consta resulted in an initial burst of drug in the first day followed by a lag of approximately 3 weeks and then a nearly 2-week period of approximately zero-order release kinetics. Therapeutic plasma levels of risperidone were obtained between 3 and 6 weeks after injection [150].

A controlled-release formulation of the nonselective opioid antagonist naltrexone has been approved for the treatment of alcohol dependence. Oral naltrexone was approved for opioid dependence in 1985, with approval for use in cases of alcohol dependence in 1994. Oral naltrexone is efficacious in improving some, but not all, drinking outcomes with a major limiting factor being the compliance of the patient. Therefore, the efficacy of oral naltrexone is highly dependent on the adherence of the patient to the drug regimen [151]. The use of extended-release naltrexone formulations has been shown to improve abstinence rates; however, many formulations are plagued by poor tolerability due to injection site reactions and subtherapeutic drug plasma concentrations [152]. The extended release intramuscular injection of naltrexone (Vivitrol) was approved in 2006. These microspheres, similar to those of Risperdal Consta, are

composed of PLGA with a drug loading of 34% w/w achieved by the Medisorb process. Administration of Vivitrol to alcohol-dependent adults, in combination with psychosocial therapy, was shown to effectively reduce the frequency of heavy drinking [153]. Vivitrol is currently approved as a once-monthly IM injection for the treatment of alcohol dependence along with psychosocial support.

9.4.4 In Situ Forming Depots

In situ forming solid depots have been shown to be valuable in the site-specific delivery of a number of therapeutics by providing the convenience of an injectable system with the benefit of controlled release from a solid implant. In particular, the Atrigel system (Fig. 9.4) has been developed into marketed products for the treatment of prostate cancer, periodontitis, and periodontal tissue regeneration [154]. This system, developed by Dunn and co-workers, is a liquid composed of PLGA or PDLLA dissolved in a hydrophilic, nontoxic solvent, *N*-methyl-2-pyrrolidone (NMP). Atrigel has been formulated with leuprolide acetate as the marketed product Eligard for the controlled delivery of the peptide for the treatment of prostate cancer. Four formulations have been approved by the FDA for repeated treatment every 1, 3, 4, or 6 months. As determined in preclinical studies, varying the drug loading and molecular weight can control the length of time of testosterone suppression [155]. In preclinical rat and dog animal models, the PLGA composition tested had a

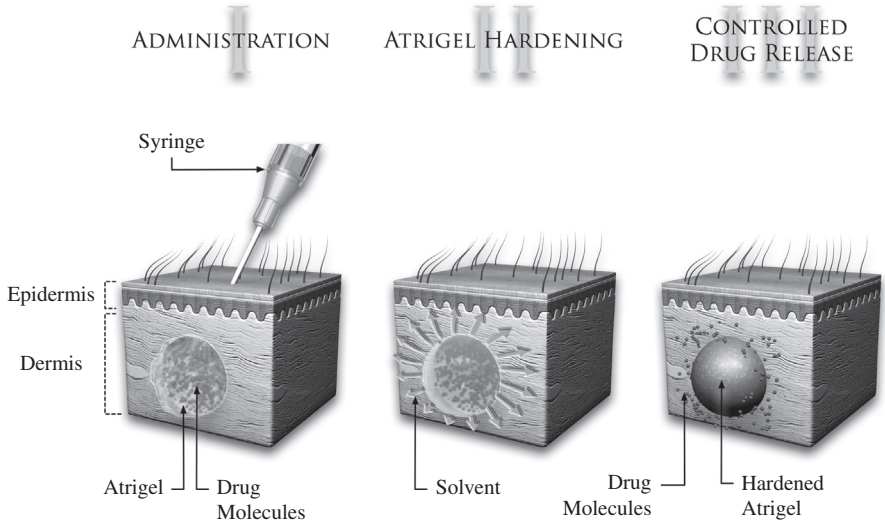


FIGURE 9.4 Atrigel delivery system is an injectable liquid composed of drug and polymer dissolved in *N*-methyl-2-pyrrolidone (I). After IM injection, the solvent diffuses into the surrounding tissue leaving a solid depot of polymer and drug (II). Controlled release of drug occurs via diffusion and polymer degradation (III).

ratio of lactide to glycolide of 75 : 25 with a 45 : 55 ratio of polymer to NMP [155, 156]. By 21 days postadministration in clinical trials, all four formulations reduced serum testosterone levels to $\leq$50 ng/dL, equivalent to those produced by orchidectomy, Furthermore, prostate serum antigen levels, a predictor of reoccurrence or metastasis, were found to decrease to normal levels in almost all of the patients. The formulations were well tolerated with no reports of severe injection site reactions and only one report of severe hot flushes with the monthly injection formulation [157–159].

9.4.5 Nanoparticles

For the purpose of drug delivery, nanoparticles may be defined as colloidal-sized particles, possessing diameters ranging between 1 and 1000 nm, into which drugs may be encapsulated, adsorbed, or dispersed. The use of amphiphilic block copolymers for drug delivery applications has been increasingly more popular over the past two decades. These materials are composed of two different monomers, one hydrophilic and the other hydrophobic, arranged so that the polymer has significant sections, or blocks, entirely composed of one type of monomer and are typically diblock or triblock configurations. The most common choice for the hydrophilic block is PEG due to its high degree of water solubility and biocompatibility. Numerous hydrophobic blocks have been investigated including polyethers and poly(amino acids); however, the aliphatic polyesters including PLA and PLGA are often selected due to their aforementioned biocompatibility and potential for controlling drug release through degradation.

Properties of Block Copolymers Forming Nanoparticles. At a specific and narrow concentration range of amphiphile in solution, termed the critical micelle concentration (CMC), several amphiphiles will self-assemble into colloidal-sized particles termed micelles. Micelles typically range from 10 to 50 nm in diameter and are characterized by a core consisting of hydrophobic blocks surrounded by a corona composed of highly water-bound hydrophilic blocks. Using light scattering, sedimentation velocity, and small-angle x-ray scattering, it has been demonstrated that a dynamic equilibrium exists between the aggregated individual molecules and the unimers in the bulk solution. Therefore, micelles obey what is termed a closed association model [160–162] and should not be considered solid particles but rather should be described as association colloids [163]. If the hydrophobic block is considerably larger than the hydrophilic block, the copolymer is no longer water soluble and therefore will not self-assemble through dissolution; however, it is possible to form nanoparticles by precipitation and emulsification techniques. These systems, termed nanospheres, possess a core–shell architecture similar to micelles; however, their constitutive unimers are not in a dynamic equilibrium with those in the bulk solution. These particles are more solid-like and are typically larger than micelles, often having diameters greater than 200 nm and being considerably more polydisperse [164].

The use of amphiphilic copolymer nanoparticles offers several advantages for drug delivery. These systems can be used to increase the aqueous solubility of several hydrophobic drugs, potentially alleviating the use of toxic cosolvents and low-molecular-weight surfactants [165, 166]. The highly water-bound corona of PEGylated nanoparticles confers "stealth-like" properties to nanoparticles, allowing them to circulate for prolonged periods by repelling plasma proteins including complement proteins, immunoglobulins, and apolipoproteins, which mediate the recognition and phagocytosis by macrophages [167, 168]. Prolonged circulation, combined with the nanoscopic diameter of these systems, has been attributed to their ability to accumulate in tissues possessing leaky vasculature and poor lymphatic drainage, such as solid tumors [169]. Nanoparticles also provide a platform for the conjugation of targeting moieties such as antibodies [170], peptides [171], and most recently aptamers [172]. These ligands are capable of recognizing specific antigens or receptors on the surface of cancer cells or in some cases the microvasculature of tumors, resulting in greater specificity and increased receptor-mediated cell internalization.

Clinical Trial Formulations. Despite significant research interest in the development of polymeric nanoparticulate drug delivery systems, no such formulations have yet reached the market; however, a polymeric micellar formulation of the anticancer drug paclitaxel, marketed under the name Genexol PM, is currently undergoing clinical trials in the United States for a variety of cancers. Due to the highly hydrophobic nature of paclitaxel, its commercial formulation, Taxol, consists of the drug at a concentration of 6 mg/mL solubilized in polyethoxylated castor oil (Cremophor EL) with 50% anhydrous alcohol. Administration of Cremophor EL is associated with several adverse effects, including anaphylactic reactions, hyperlipidemia, and modification of electrophoretic and density gradient behavior of lipoproteins [173]. The goal of developing Genexol PM was to produce a formulation that would adequately solubilize paclitaxel without the toxicity of Cremophor EL. Genexol PM is composed of the amphiphilic diblock copolymer methoxy poly(ethylene glycol)-block-poly(D,L-lactide) with molecular weights of 2000 and 1750 g/mol for the MePEG and PDLLA blocks, respectively [174]. Phase I clinical studies determined the maximum tolerated dose (MTD) to be 390 mg/m^2, twice that of Taxol, with no acute hypersensitivities attributed to the formulation reported [175]. In phase II trials, Genexol PM was determined to be more efficacious as a first-line therapy for metastatic breast cancer than Taxol, with an overall response rate of 58.5% as compared to 21–54% for Taxol [176].

9.4.6 Periodontal Delivery Systems

The first marketed implantable device for periodontal disease, one of the most prevalent diseases in the western world, was Actisite, a tetracycline-loaded ethylene vinyl acetate fiber providing sustained release for 10 days. Reports of

the extrusion of the device from the periodontal pocket and need for removal after treatment limited the usefulness of this system [177]. Atrigel has been used for the successful formulation of doxycycline hyclate for the treatment of periodontal disease as the product Atridox. This formulation contains 8.5% w/w doxycycline hyclate dissolved in 37% w/w PDLLA in NMP [178]. When administered, the product flows deep into the periodontal pocket, forming a wax-like depot when it comes in contact with the crevicular fluid. Atridox releases doxycycline over a period of 7 days. Clinical trials demonstrated that Atridox was equally as effective as scaling and root planing (SRP) in reducing periodontitis [178].

Minocycline HCl has also been formulated into a polymeric delivery system for the treatment of periodontitis as the marketed product Arestin. Arestin consists of minocycline HCL-loaded PLGA microspheres with a drug loading of 1 mg per 3 mg of polymer [179]. After scaling and root planing, the dry microspheres are deposited in the periodontal pocket via a syringelike applicator. Upon contact with the crevicular fluid, the drug is released in a controlled manner, obtaining local concentrations up to 340 μg/mL after 14 days [179]. In clinical trials, Arestin with SRP was more effective at reducing probing depth compared to SRP or SRP with blank microspheres [179].

9.4.7 Ocular Delivery Systems

Posterior segmental ocular diseases, including, age-related macular degeneration and diabetic retinopathy, are the leading cause of visual impairment in industrialized countries [180]. Treatments for these conditions may be met by advances in biotechnology; however, the delivery of these therapeutics remains a formidable hurdle to treating these diseases as the eye has several tissue barriers that limit effective drug doses from reaching their target tissues. Drugs formulated in topically applied delivery systems such as solutions, suspensions, and gels are effective in treating diseases of the anterior segment but do not reach the posterior segment of the eye in sufficient quantities. To date, the most effective strategy for treating diseases of the posterior segment is via local or systemic administration; however, these methods have their limitations. Due to the small blood flow to the eye and the protective mechanisms of the blood–retinal barrier, many systemically administered drugs are not capable of reaching the posterior segment. Those that are capable of passing through the blood–retinal barrier must be given in large doses in order to reach therapeutic concentrations in the eye, and therefore only drugs with a wide therapeutic index are acceptable candidates for this method. Local administration by intraocular and periocular injection are effective but invasive, and repeated administration in order to maintain therapeutic drug concentrations may lead to complications such as retinal detachment, hemorrhages, and endophthalmitis [181]. In order to minimize the number of injections required, it is necessary to achieve controlled release of the drug in the eye. Intravitreal or periocular injected polymeric implants and micro- or nanoparticles composed of PLGA have been used to achieve controlled, localized drug delivery (Fig. 9.5).

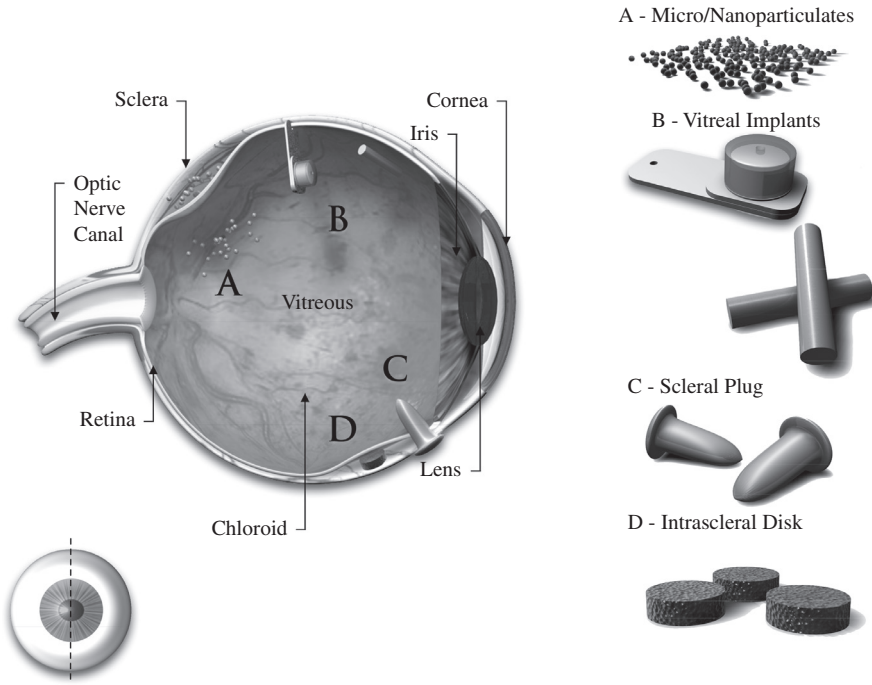


FIGURE 9.5 Intraocular and periocular polymeric drug delivery systems: (A) intraocular or periocular administered micro or nanoparticles, (B) vitreal implants, (C) scleral plug, and (D) intrascleral disks [187].

Implants. To date only one biodegradable ocular implant, Posurdex (Allergan USA, Inc.), is close to market approval. This implant, made of PLGA, can deliver dexamethasone for the treatment of macular edema due to retinal vein occlusion over a 1-month period. The device is implanted through a small incision or puncture in the pars plana. An initial clinical trial of Posurdex using two drug loadings, either 350 or 700 μg, has been completed [182]. It was found that after 90 days of implantation a greater proportion of treated patients achieved an improvement in their best-corrected visual acuity than for untreated patients. The implant was well tolerated with 11% of treated patients displaying increased intraocular pressure. Posurdex is currently in phase III trials for the treatment of retinal vein occlusion and diabetic macular edema.

Micro- and Nanoparticles. A common strategy for the delivery of therapeutics to the posterior segment is via intravitreal or periocular injection of micro- or nanoparticles. This procedure is less invasive than surgical implantation as the particles can be injected as a dispersion, typically via a 18- to 30-gauge needle [183]. Several polymers have been explored for use as intraocular drug delivery

systems; however, the use of PLA and PLGA remains the most popular due to their biocompatibility and lack of retinal cytotoxicity [184, 185]. The development of vitreal clouding and subsequent loss of vision is of concern with intravitreal injection of particulates. However, it has been reported that microspheres larger than 2 μm sink in vitreal fluid, alleviating the problem of clouding, and thus making intravitreal injections of microspheres clinically feasible [186].

Although a microparticle or nanoparticle system has not yet been approved for intraocular injection, a variety of preclinical studies have shown promise for an assortment of diseases including proliferative vitreoretinopathy (PVR), ocular neovascularization, and cytomegalovirus retinitis (CMV). Antiproliferative agents including 5-fluorouracil (5-FU), adriamycin, and retinoic acid have been used as treatments of PVR. Moritera and co-workers demonstrated that, depending on the molecular weight of the polymer, 5-FU could be released from PLA microspheres for up to 7 days and completely disappeared from the vitreous humor in 6 weeks [185]. In further investigations using the same PLA delivery system, a dose of 10 μg of adriamycin resulted in a significant decrease in the number of detached retinas as compared to controls in a rabbit model [187]. Similarly, Giordano et al. showed that retinoic acid could be delivered at a constant rate of 6 μg/day for 40 days from PLGA microspheres. In a PVR rabbit model, a single injection of this formulation was effective at reducing the incidence of retinal detachment after 2 months [188]. After the success of Vitrasert, ganciclovir-loaded PLGA microspheres for the treatment of CMV have been investigated to eliminate complications associated with the implantation and replacement of the device. Veloso et al. demonstrated that ganciclovir microspheres were capable of controlling disease progression in CMV-inoculated rabbit eyes [189]. There have been investigations into the use of intravitreally administered nanoparticles. Bourges et al. demonstrated that, after a single intravitreal injection of PLA nanoparticles, transretinal migration of the nanoparticles occurred with gradual uptake in the retinal pigment epithelium (RPE) cells. These nanoparticles were still present 4 months postinjection [190]. Follow-up studies by the same group demonstrated in vivo that these nanoparticles, loaded with red nuclear fluorescent protein plasmid, were specifically targeted to the RPE cells. Gene expression within the RPE cells occurred as early as 4 days and remained detectable for 14 days [191]. Although still in the early stages of development, this passive targeting mechanism shows potential in the treatment of diseases affecting the retina, such as macular degeneration.

9.4.8 Thermoreversible Gels for Intratumoral Injection

Thermoreversible gels composed of triblock copolymers demonstrate great potential as drug delivery systems. The first of these systems investigated were Pluronic gels used for the delivery of a variety of agents including anticancer drugs and peptides [192, 193]. The downfall of this approach is the lack of polymer biodegradability and potential toxicity [194] as well as the rapid dissolution of the gel, limiting the use as drug delivery systems for controlled

drug release [195]. Triblocks composed of PEG and PLGA with ABA or BAB architecture, in which A represents the PLGA block and B the PEG block, also display thermoreversible characteristics [196, 197]. The added advantage of biodegradability and the formation of solid, slowly dissolving implants make these materials good candidates for delivery systems.

A considerable amount of research on the synthesis, characterization, and use of PEG–PLGA–PEG copolymers as delivery systems has been done by Jeong and co-workers [198–199]. If the polymer is present as a solution above a concentration termed the critical gel concentration (CGC), the solution will exist as a freely flowing sol below a temperature termed the critical gel temperature (CGT). If the solution is heated above the CGT, the solution undergoes a phase transition to a nonflowing gel. At sufficiently high temperatures the gel will undergo another phase change back to a sol. The lower temperature phase transition (i.e., the sol-to-gel transition) is particularly useful for drug delivery applications. This property allows for the injection of the material into a body cavity as readily flowing liquid. Upon heating in the body the polymer turns to a semisolid gel, forming a depot. Although the mechanism of gelation for these polymers is not fully understood, it has been attributed to a decrease in the solubility of the copolymer in water as the temperature increases. This causes an abrupt increase in aggregation number, resulting in micellar growth, close micelle packing, and partial phase mixing of PEG and PLGA at the CGT [196, 200].

The PLGA–PEG–PLGA triblock, currently marketed as ReGel, has been used for the release of proteins such as insulin, porcine growth hormone, granulocyte colony stimulating factor and recombinant hepatitis B surface antigen [197, 201, 202]. This system remains at the site of injection for up to 1 month with controlled release of the encapsulated therapeutic occurring via a combination of diffusion and polymer degradation mechanisms, which last from 1 to 6 weeks [197]. Currently, ReGel is formulated with paclitaxel as a product known as OncoGel and is being investigated for the treatment of pancreatic [203], breast [197], and esophageal and primary brain cancers [204].

9.4.9 Drug-Eluting Coronary Artery Stents

Coronary artery disease is the leading cause of death in the United States with one in every five Americans being affected [205]. Percutaneous coronary angioplasty (PTCA) has become the mainstay in the surgical intervention of coronary artery disease and has recently overtaken bypass surgery in terms of the number of annual procedures [206]. A common complication of PTCA is the development of restenosis, which is a wound healing response that results in the renarrowing of the vessel wall due to elastic recoil, neointimal proliferation, and negative remodelling [207]. The use of coronary artery stents, which are metal cagelike structures deployed during PTCA and used to prop open the vessel, can significantly decrease the occurrence of restenosis and subsequent major adverse cardiac events (MACE), myocardial infarction, and death (Fig. 9.6) [208]. In many cases, restenosis still occurs, resulting in the overgrowth of the stent in a

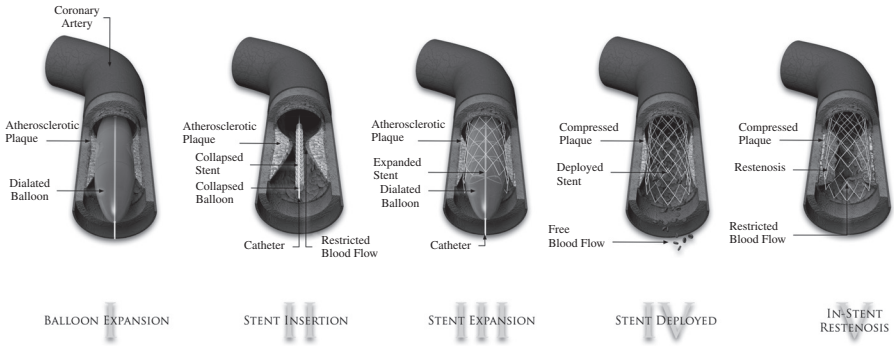


FIGURE 9.6 After the treatment of an atherosclerotic plaque with balloon angioplasty (I), a bare metal coronary artery stent is inserted using an inflated balloon (II–III). Bare metal stents maintain free blood flow by decreasing rates of restenosis due to angioplasty (IV); however, use of these devices may result in significant rates of in-stent restenosis (V).

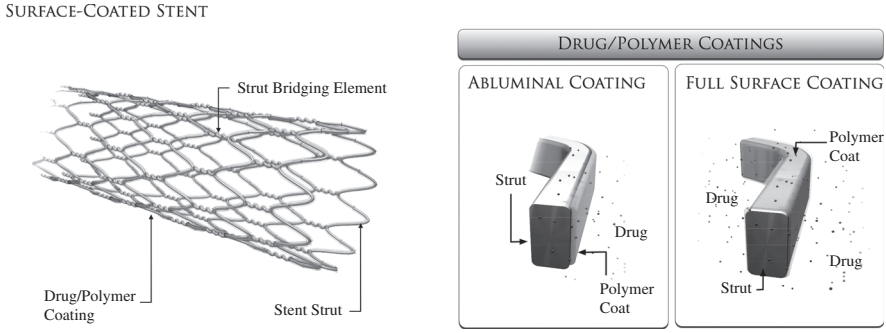


FIGURE 9.7 Polymer/drug coating on a drug-eluting stent may either fully cover the surface of the strut or only coat the abluminal side.

process termed in-stent restenosis (Fig. 9.6). This often results in the need for further angioplasty or bypass surgery. Drug-eluting stents emerged in the 1990s as an elegant method of reducing in-stent restenosis. Typically, these devices are metal stents that are either fully coated or abluminally coated with a drug-loaded polymeric coating, which allows for the local delivery of the therapeutic agent at a controlled rate (Fig. 9.7). The drugs selected for this application inhibit the proliferation of vascular smooth muscle cells. The first of these devices were the Cypher and Taxus stents using the drugs sirolimus and paclitaxel, respectively. Both of these devices employed nondegradable polymer coatings, a copolymer of ethylene and vinyl acetate for Cypher and a block copolymer of polystyrene and polyisobutylene for Taxus. Compared to bare metal stents,

these devices have demonstrated significantly decreased rates of restenosis [146, 209]. However, clinical trials have shown that there is an increased risk of death and myocardial infarction up to 3 years after treatment due to late stent thrombosis [210]. Late stent thrombosis involves rapid formation of a blood clot within the stent due to incomplete endothelialization of the device. Although the exact mechanisms causing late stent thrombosis have not been determined, several factors have been implicated, including hypersensitivities to the coating polymers [211] and delayed healing of the injury site due to the use of potent drugs such as paclitaxel and sirolimus [212]. The next generation of stents are aimed at reducing the risk of late stent thrombosis by utilizing new stent designs and drugs tailored for the treatment of restenosis as well as more biocompatible, biodegradable polymers including PLA and PLGA as coating and strut materials.

The BioMatrix stent uses a stainless steel stent coated with PLA loaded with Biolimus A9, a sirolimus analog that was specifically designed for the treatment of restenosis. The drug is loaded only on the abluminal surface of the stent at a concentration of 15.6 μg/mm of stent. The coating is reported to degrade over a period of 6–9 months providing controlled drug release. In the first human trials (STEALTH I trial) it was shown that the BioMatrix stent was more effective at preventing in-stent late lumen loss and in-stent restenosis as compared to bare metal stents [213]. Whether this design results in a lower rate of late stent thrombosis will require a longer study.

The majority of stent platforms are retrofitted to enable the delivery of drugs to the arterial wall by simply coating the strut with polymer–drug mixtures. This can lead to deformation of the polymer coating during stent deployment, possibly causing cracking and disruption of the coating. The CoStar stent developed by Conor Medsystems is unique in that the stent strut is not coated but rather contains polymer and drug-loaded wells (Fig. 9.8). This design prevents polymer deformation during stent deployment. Furthermore, since the polymer is

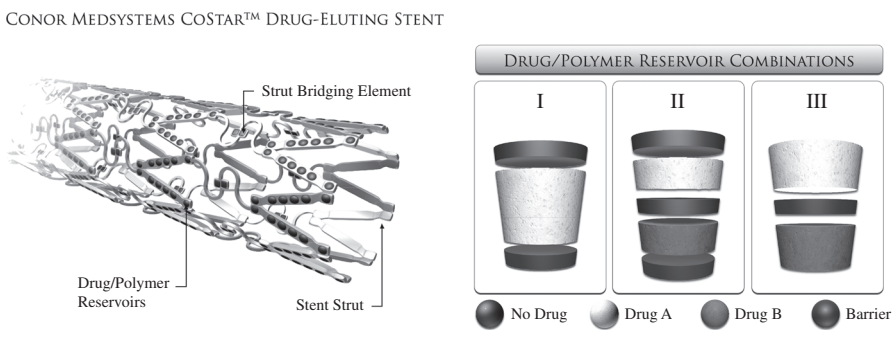


FIGURE 9.8 Medsystems CoStar stent contains polymer/drug-filled wells that can be customized to directionally release one drug only abluminally (I) or two drugs abluminally but at different times (II) or at the same time with one released abluminally and the other luminally (III) [216].

contained in a well, it limits the contact between the tissue and polymer, potentially minimizing any polymer-induced inflammation and hypersensitivity reactions [214]. These wells provide several options for customization to enable directional and temporal release of drugs as illustrated in Figure 9.8 [215]. This stent system also provides the capability to deliver two drugs simultaneously by filling alternating wells with the agents.

The Conor stent has undergone several clinical trials, the first of which was the paclitaxel in-stent controlled elution study (PISCES trial), which investigated the safety and performance of different doses and release rates from the stent [216]. This study found that target lesion revascularization and MACE were lowest with stents designed to release drug for 30 days as opposed to those that released drug for 10 days, regardless of the paclitaxel loading. A follow-up study (COSTAR trial) also found promising results with the long-release formulation showing less late loss and binary restenosis. In a recent trial, COSTAR II, it was shown that CoStar failed to show noninferiority over the Taxus stent [217] and has since been removed from the market. Since this time, Conor has been using the same stent platform to develop a drug-eluting stent that will deliver two drugs, paclitaxel and primecrolimus, as well as sirolimus as a single agent.

It is speculated that the occurrence of late stent thrombosis in the case of drug-eluting stents may be due to the delay of growth of healthy endothelium over the stent struts and the nondegradable coating [218]. The need for scaffolding of the vessel and delivery to prevent initial neointimal growth is temporary and thus, the use of fully bioresorbable stents may result in less late stent thrombosis as they allow for the complete disappearance of the biomaterial [219]. The safety of such stents was demonstrated by Tamai and co-workers who tested a PLLA stent in humans, showing a similar safety profile as bare metal stents [220]. Abbott Pharmaceuticals is currently developing a bioabsorbable drug-eluting stent composed of a PLLA structure coated with everolimus-loaded PDLLA. The stent is designed to release 80% of its 98-μg loading over 28 days with a peak tissue concentration of 15 ng/mL occurring 3 hours after implantation. In a recent clinical trial (ABSORB trial) the stent had a low rate of MACE of 3.3% and no target lesion revascularizations or late stent thromboses after one year [219]. Although it is too soon to determine whether these new stents truly are safer than their metal counterparts, these early findings are promising.

9.4.10 Pulmonary Microparticles

The delivery of drugs via the pulmonary route has been used for decades for the local delivery of drugs in treating respiratory diseases such as asthma and chronic obstructive pulmonary disease. For several reasons, this route of administration also holds promise for the delivery of therapeutics for systemic circulation. The lungs have a high blood flow and provide a large surface area for drug uptake [221]. Furthermore, the lungs have lower enzymatic activity than other areas of the body and inhaled therapeutics avoid first-pass metabolism [222]. Therefore, the lungs have been investigated for the delivery

of several biopharmaceticals that suffer from poor bioavailability as a result of degradation. In addition, drug administration via the pulmonary route is considerably less invasive than parenteral administration, which is often required for biopharmaceticals such as proteins and peptides. Inhalation therapy has the potential to be better accepted by the patient and does not carry the same risks as injections.

In order for a therapeutic agent to be systemically absorbed via the lungs, the carrier must reach the alveolar epithelium; however, several mechanisms exist to prevent foreign bodies from penetrating deep within the respiratory tract. The airway continually bifurcates through 23 stages before the alveoli are reached. This tortuous pathway makes it difficult for aerosolized formulations to reach their target. If the particles are not propelled at the correct velocity or are not of the correct size, impact with the mouth and throat will occur and clearance by the mucociliary escalator will remove the particles. If particles do reach the deep lung, they are subject to subsequent removal by macrophages. In order to maximize particle deposition in the lungs, several inhaler devices have been developed; however, the intelligent design of the aerosolized particles has also been pivotal in the successful delivery of therapeutics via the pulmonary route.

It is well known that the deposition of particles in the lungs is governed by the aerodynamic diameter, which is defined as the geometric diameter that a particle possesses on the basis of its in-flight speed, if it is assumed to be spherical and possess a density of 1 g/cm^3 [223]. This factor is dependent on the size and mass density of the particle. Experimentally, it has been demonstrated that aerosolized liquid drugs with a mean aerodynamic diameter of 1–3 μm reach the deep lungs and have the maximal therapeutic effect [224, 225]. Curiously, larger aerosolized particles with geometric diameters of tens of microns such as coal dust and pollen are capable of achieving lung deposition [223]. This is attributed to their low mass densities and thus smaller aerodynamic diameter. Since the phagocytosis and clearance of particles larger than 2–3 μm is decreased [226], it is advantageous for controlled-release formulations to be as large as possible while retaining lung deposition. In addition, larger particles are less prone to aggregation, thus requiring less energy to aerosolize. With these concepts in mind, Edwards et al. designed large porous PLGA microparticles for the delivery and controlled release of proteins to the lungs for systemic uptake [227]. These large porous particles with a diameter of > 5 μm were first reported for the encapsulation and sustained pulmonary delivery of insulin [227]. The microspheres were composed of 50 : 50 PLGA and formed by a double emulsification technique. Lung deposition and systemic bioavailability studies were conducted in rats, demonstrating that a greater number of porous particles reached and were retained in the deep lung as compared to nonporous microspheres of the same aerodynamic diameter. Porous microspheres maintained elevated insulin levels in blood for 4 h and decreased serum glucose levels for up to 96 h. This technology was used by Alkermes, Inc. as its AIR pulmonary drug delivery technology for the delivery of insulin as its product AIR insulin. Due to the low cohesive forces the

particles can be aerosolized by a small breath-activated device unlike larger bulky devices such as that of Exubera, which may have been a contributing factor in its commercial failure. In clinical studies in collaboration with Eli Lily and Company, AIR insulin was shown to be rapidly absorbed with t_{max} values similar to subcutaneously administrated insulin lispro (Humalog) but with a longer median time for plasma levels to return to baseline [228]. Patients suffering from moderate asthma or chronic obstructive pulmonary disease had lower and more variable plasma area under the curve values and decreased glucodynamic response [229, 230]. Despite the positive efficacy and safety data of AIR insulin and the promise to provide an alternative treatment for diabetics, the development of the product was terminated in phase III trials. The AIR delivery system is currently being investigated for the delivery of trospium chloride for the treatment of chronic obstructive pulmonary disease and is undergoing phase II trials. Alkermes is also looking at the feasibility of delivering parathyroid hormone using the same technology.

9.5 SUMMARY

Lactide and glycolide polymers constitute a diverse and extensive family of bio-derived polyesters that are commercially available and have been widely investigated for medical uses. By means of specific synthetic routes, including the formation of copolymers, these polymers provide the benefit that properties can potentially be tuned for specific medical requirements. Furthermore, polylactide, polyglycolide, and their copolymers are resorbable and, since the ultimate breakdown products are carbon dioxide and water, they have generally presented few indications of significant toxicity in medical trials. This has led to FDA approval for specific formulations and the widespread investigation of these polymers for uses in drug delivery, orthopedics, sutures, wound dressings, post-operative barrier films, and various other applications. In view of its significance, this chapter has focused specifically on the use of lactide and glycolide polymers and their copolymers in drug delivery, which is already advanced on many fronts either through clinical trials or clinical development. Given the history of research and development in this field to date, it seems certain that new developments in existing applications and a wide variety of new uses in drug delivery and other areas of medicine will open up in the coming years.

REFERENCES

1. Amass, W., A. Amass, and B. Tighe. A review of biodegradable polymers: Uses, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradation studies. *Polym. Int*. 1998;**47**:89–144.
2. Leenslag, J. W., A. J. Pennings, R. R. M. Bos, F. R. Rozema, and G. Boering. Resorbable materials of poly(L-lactide) VII. In vivo and in vitro degradation. *Biomaterials* 1987:**8**:311–314.

3. Cutright, D. E. and E. E. Hunsack. Tissue reaction to the biodegradable polylactic acid suture. *Oral Surg., Oral Med., Oral Path*. 1971;**31**:134–139.
4. Nakamura, T., S. Hitomi, T. Shimamoto, S.-H. Hyon, S. Ikada, S. Watanabe, and Y. Shimizu. Surgical application of biodegradable films from lactide-caprolactone copolymers. In *Biomaterials and Clinical Applications*. A. Pizzoferrato, P. G. Marchetti, and A. J .C. Lee (Eds.). Elsvier Science: Amsterdam, 1987, pp. 759–764.
5. Greisler, H. P., D. U. Kim, J. B. Price, and A. B. Voorhee. Arterial regenerative activity after prosthetic. *Arch. Surg*. 1985;**120**:315–323.
6. Hutchinson, F.G. and B. J. A. Furr. Biodegradable carriers for the sustained release of polypeptides. *Trends Biotechnol*. 1987:**5**:102–106.
7. Chaubal, M. Polylactides/glycolides—Excipients for injectable drug delivery & beyond. *Drug Deliv. Technol*. 2002:**2**:34–36.
8. http://www.puracbiomaterials.com; accessed October 21, 2008.
9. http://www.absorbables.com; accessed October 21, 2008.
10. http://www.boeringer-ingelheim.com; accessed October 21, 2008.
11. http://www.polysciences.com/Catalog/Department/81/categoryId__286/; accessed November 23, 2008.
12. http://www.bpi-sbs.com/; accessed November 24, 2008.
13. http://www2.dupont.com/Glycolic_Acid/en_US/; accessed November 26, 2008.
14. http://www.intox.org; accessed October 23, 2008.
15. Nieuwenhuis, J. Synthesis of polylactides, polyglycolides and their copolymers. *Clin. Mater*. 1992;**10**:59–67.
16. Holten, C. H., A. Müller, and D. Rehbinder. *Lactic Acid: Properties and Chemistry of Lactic Acid and Derivatives*. Chemie: Weinheim, 1971.
17. Kleine, J. and H.-H. Kleine. Über hochmolekulare, insbesondere optisch aktive polyester der milchsäure, ein betrag zur stereochemie makromolekularer verbindungen. *Macromolecules Chem*. 1959;**30**:23–38.
18. Tsuji, H. and Y. Ikada. Crystallization from the melt of poly(lactide)s with different optical purities and their blends. *Macromolecules Chem. Phys*. 1996;**197**:3483–3499.
19. Huang, J., M. S. Lisowski, J. Runt, E. S. Hall, R. T. Kean, N. Buehler, and J. S. Lin. Crystallization and microstructure of poly(L-lactide-*co*-*meso*-lactide) copolymers. *Macromolecules* 1998;**31**:2593–2599.
20. Nieuwenhuis, J. Synthesis of polylactides, polyglycolides and their copolymers. *Clin. Mater*. 1992;**10**:59–67.
21. Spassky, N. Ring-opening polymerisation. *Rapra Rev. Rep*. 1995;**8**:1–29.
22. Penczec, S., A. Duda, R. Szymanski, and T. Biela. What we have learned in general from cyclic esters polymerization. *Macromolecules Symp*. 2000;**153**:1–15.
23. Nijenhuis, A. J., D. W. Grijpma, and A. J. Pennings. Lewis acid catalyzed polymerization of L-lactide. Kinetics and mechanism of the bulk polymerization. *Macromolecules* 1992;**25**:6419–6424.
24. Dahlmann, J. and G. Rafler. Biodegradable polymers. 7th comm. On the mechanism of ring-opening polymerization of cyclic esters of aliphatic hydroxycarboxylic acids by means of different tin compounds. *Acta Polym*. 1993;**44**:103–107.
25. Dubois, P., C. Jacobs, R. Jérôme, and P. Teyssié. Macromolecular engineering of polylactones and polylactides. 4. Mechanism and kinetics of lactide homopolymerization by aluminum isopropoxide. *Macromolecules* 1991;**24**:2266–2270.

26. Chabot, F., M. Vert, S. Chapelle, and P. Granger. Configurational structures of lactic acid stereocopolymers as determined by ^{13}C-[^{1}H] n.m.r. *Polymer* 1983; **24**:53–59.
27. Stolt, M. and A. Sodergard. Use of monocarboxylic iron derivatives in the ring-opening polymerization of L-lactide. *Macromolecules* 1999;**32**:6412–6417.
28. Chamberlain, B. M., B. A. Jazdzewski, M. Pink, M. A. Hillmyer, and W. B. Tolman. Controlled polymerization of D,L-lactide and ε-caprolactone by structurally well-defined alkoxo-bridged di- and triyttrium(III) complexes. *Macromolecules* 2000;**33**:3970–3977.
29. Garlotta, D. A literature review of poly(lactic acid). *J. Polym. Environ.* 2004:**9**:63–84.
30. Kowalski, A., A. Duda, and S. Penczek. Kinetics and mechanism of cyclic esters polymerization initiated with tin(II) octoate. 3. Polymerization of L,L-Dilactide. *Macromolecules* 2000;**33**:7359–7370.
31. Joziasse, C. A. P., H. Grablowitz, and A. J. Pennings. Star-shaped poly[(trimethylene carbonate)-*co*-(ε-caprolactone)] and its copolymers with lactide/glycolide: Synthesis, characterization and properties. *Macromolecules Chem. Phys.* 2000;**201**:107–112.
32. Grijpma, D. W. and A. J. Pennings. Polymerization temperature effects on the properties of L-lactide and ε-caprolactone copolymers. *Polym. Bull.* 1991;**25**: 335–341.
33. Gilding, D. K. and A. M. Reed. Biodegradable polymers for use in surgery—polyglycolic/poly(lactic acid) homo- and copolymers: 1. *Polymer* 1979;**20**: 1459–1464.
34. Grijpma, D. W., C. A. P. Joziasse, and A. J. Pennings. Star-shaped polylactide-containing block copolymers. *Makromol. Chem. Rapid Commun.* 1993;**14**:155–161.
35. http://www.viscotek.com/pdf/maltodextrins.pdf; accessed August 21, 2008.
36. Middleton, J. C. and A. J. Tipton. Synthetic biodegradable polymers as medical devices. *Med. Plast. Biomat.* 1998;**2**:30–38.
37. Södergård, A. Modification of polylactide. *Recent. Res. Develop. Polym. Sci.* 1998;**2**:263-275.
38. Kharas, G.B., F. Sanches-Riera, and D. K. Severson. In *Plastics from Microbes*, D. P. Mobley, (Ed.) Hanser: Munich, 1994, p. 93.
39. Törmälä, P. Biodegradable self-reinforced composite materials; manufacturing, structure and mechanical properties. *Clin. Mater.* 1992;**10**:29–34.
40. Eling, B., S. Gogolewski, and A. J. Pennings. Biodegradable materials of poly-(L-lactic acid): 1 Melt-spun and solution-spun fibres. *Polymer* 1982;**23**:1587–1593.
41. Penning, J. P., H. Dijkstra, and A. J. Pennings. Preparation and properties of adsorbable fibres from L-lactide copolymers. *Polymer* 1993;**34**:942–951.
42. Postema, A. R. Studies on the preparation of strong/stiff polymeric fibres. Ph.D. Thesis. University of Groningen, The Netherlands, 1988.
43. Hu, Y., D. Bai, Z. Zhang, Y. Wang, and B. He. Degradation and processing of PLA and its application in fixation of bone fracture. *Chinese J. React. Polym.* 1999;**8**:102–109.
44. Sosnowski, S., M. Gadzinowski, S. Slomkowski, and S. Penczek. Synthesis of bioerodible poly(ε-caprolactone) latexes and poly(D,L-lactide) microspheres by ring-opening polymerization. *J. Bioact. Comp. Polym.* 1994;**9**:345–366.

45. Benoit, J.-P., H. Marchais, H. Rolland, and V. Vande Velde. Biodegradable microspheres: Advances in production technology. In *Microencapsulation: Methods and Industrial Applications*. S. Benita (Ed.) Marcel Dekker: New York, 1996, pp. 35–72.
46. Arshady, R. Preparation of biodegradable microspheres and microcapsules: 2. Polylactides and related polyesters. *J. Control. Release* 1991;**17**:1–22.
47. Holmbom, J., A. Södergård, E. Ekholm, M. Märtson, A. Kuusilehto, P. Saukko, and R. Penttinen. Long term evaluation of porous poly(ε-caprolactone-*co*-L-lactide) as bone-filling material. *J. Biomed. Mat. Res.* 2005:**75A**:308–315.
48. Whang, K., C. H. Thomas, K. E. Healy, and G. Nuber. A novel method to fabricate bioabsorbable scaffolds. *Polymer* 1995;**36**:837–842.
49. Nam, Y.S., J. J. Yoon, and T. G. Park. A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive. *J. Biomed. Mater. Res. (Appl. Biomater.)* 2000;**53**:1–7.
50. Mooney, D. J., D. F. Baldwin, N. P. Suh, J. P. Vacanti and R. Langer. Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. *Biomaterials* 1996;**17**:1417–1422.
51. Zoppi, R. A., S. Contant, E. A. R. Duek, F. R., Marques, M. L. F. Wada, and S.P. Nunes. Porous poly(L-lactide) films obtained by immersion precipitation process: Morphology, phase separation and culture of VERO cells. *Polymer* 1999;**40**:3275–3289.
52. Lee, D., F. J. Hua, and G. E. Kim. Preparation of macroporous biodegradable poly-(L-lactic acid) scaffolds via thermally induced phase separation. *Polym. Mater. Sci. Eng.* 2001;**85**:399–400.
53. Tsuji, H., R. Smith, W. Bonfield, and Y. Ikada. Porous biodegradable polyesters. I. Preparation of porous poly(L-lactide) films by extraction of poly(ethylene oxide) from their blends. *J. Appl. Polym. Sci.* 2000;**75**:629–637.
54. Engelberg, I. and J. Kohn, Physico-mechanical properties of degradable polymers used in medical applications: A comparative study, *Biomaterials* 1991;**12**:292–304.
55. Fischer, E. W., H. J. Sterzel, and G. Wegner. Investigation of the structure of solution grown crystals of lactide copolymers by means of chemical reactions. *Kolloid Z. Z. Polym.* 1973;**251**:980–990.
56. Jamshidi, K., S.-H. Hyon, and Y. Ikada. Thermal characterization of polylactides. *Polymer* 1988;**29**:2229–2234.
57. Migliaresi, C., A. De Lollis, L. Fambri, and D. Cohn. The effect of thermal history on the crystallinity of different molecular weight PLLA biodegradable polymers. *Clin. Mater.* 1991;**8**:111–118.
58. Tsuji, H. and Y. Ikada. Crystallization from the melt of poly(lactide)s with different optical purities and their blends. *Macromolecules Chem. Phys.* 1996;**197**:3483–3499.
59. Ikada, Y., K. Jamshidi, H. Tsuji, and S.-H. Hyon. Stereocomplex formation between enantiomeric poly(lactides). *Macromolecules* 1987;**20**:904–906.
60. Tsuji, H. Poly(lactide) stereocomplexes: Formation, structure, properties, degradation, and applications. *Macromolecules Biosci.* 2005;**5**:569–597.
61. Tsuji, H. and Y. Ikada. Stereocomplex formation between enantiomeric poly(lactic acid)s. X. Binary blends from poly(D-lactide-*co*-glycolide) and poly(L-lactide-*co*-glycolide). *J. Appl. Polym. Sci.* 1994;**53**:1061–1071.

62. Södergård, A. and M. Stolt, Properties of lactic acid based polymers and their correlation with compositions, *Prog. Polym. Sci.* 2002;**27**:1123–1163.
63. Montes de Oca, H., I. M. Ward, R. A. Chivers, and D. F. Farrar. Structure development during crystallization and solid-state processing of poly(glycolic acid). *J. Appl. Polym. Sci.* 2009;**111**:1013–1018.
64. Tsuji, H. and Y. Ikada. Stereocomplex formation between enantiomeric poly(lactic acid)s. XI. Mechanical properties and morphology of solution-cast films. *Polymer* 1999;**40**:6699–6708.
65. Eling, B., S. Gogolewski, and A. J. Pennings. Biodegradable materials of poly(L-lactic acid): 1 Melt-spun and solution-spun fibres. *Polymer* 1982;**23**:1587–1593.
66. Grijpma, D. W., J. P. Penning, and A. J. Pennings. Chain entanglement, mechanical properties and drawability of poly(lactide). *Colloid Polym. Sci.* 1994;**272**:1068–1081.
67. Bergsma, J. E., W. C. de Bruijn, F. R. Rozema, R. R. M. Bos, and G. Boering. Late degradation tissue response to poly(L-lactide) bone plates and screws. *Biomaterials* 1995;**16**:25–31.
68. Li, S. M., H. Garreau, and M. Vert. Structure-property relationships in the case of the degradation of massive aliphatic poly-(α-hydroxy acids) in aqeous media, Part 1. Poly(D,L-lactic acid). *J. Mater. Sci. Mater. Med.* 1990;**1**:123–130.
69. Grijpma, D. W. High impact strength poly(lactide)—tough biodegradable materials. Ph.D. Thesis. University of Groningen, The Netherlands, 1993.
70. Cooper, D. R., G. J. Sutton, and B. J. Tighe. Poly α-ester degradation studies. V. Thermal degradation of polyglycolide. *J. Polym. Sci. Part A: Polym. Chem.* 1973;**11**:2045–2056.
71. von Oepen, R. and W. Michaeli. Injection moulding of biodegradable implants. *Clin. Mater.* 1992;**10**:21–28.
72. Zhang, X., U. P. Wyss, D. Pichora and M. F. A. Goosen. An investigation of the synthesis and thermal stability of poly(DL-lactide). *Polym. Bull.* 1992; **27**:623–629.
73. McNeill, I. C. and H. A. Leiper. Degradation studies of some polyesters and polycarbonates—2. Polylactide: Degradation under isothermal conditions, thermal degradation mechanism and photolysis of the polymer. *Polym. Degrad. Stab.* 1985;**11**:309–326.
74. Kopinke, F.-D., M. Remmler, K. Mackenzie, M. Möder, and O. Wachsen. Thermal decomposition of biodegradable polyesters—II. Poly(lactic acid). *Polym. Degrad. Stab.* 1996;**53**:329–342.
75. Sadler, S. Wirtschftliche und technologische Aspekte bei der reaktiven Polymer verarbeitung. Diploma Thesis. *Technische Universität Berlin, Germany*, 1992, pp. 65–69.
76. Södergård, A. and J. H. Näsman. Melt stability study of various types of poly(L-lactide). *Ind. Eng. Chem. Res.* 1996;**35**:732–735.
77. Cam, D. and M. Marucci. Influence of residual monomers and metals on poly(L-lactide) thermal stability. *Polymer* 1997;**38**:1879–1884.
78. D'Alelio, G. F., R. Häberli, and F. G. Pezdirtz. Effect of ionizing radiation on a series of saturated polyesters. *J. Macromolecules Sci. Chem.* 1968;**A2**:501–588.
79. Collet, J. H., L. Y. Lim, and P. L. Gould. Gamma-irradiation of biodegradable polyesters in controlled physical environments. *Polym. Prep.* 1989;**30**:468–469.

80. Birkinshaw, C., Buggy, M., G. G. Henn, and E. Jones. Irradiation of poly-D,L-lactide. *Polym. Degrad. Stab*. 1992;**38**:249–253.
81. Gupta, M. C. and V. G. Deshmukh. Radiation effects on poly(lactic acid). *Polymer* 1983;**24**:827–830.
82. Pittman, Jr., C. U., M. Iqbal, C. Y. Chen, and J. N. Helbert. Radiation degradation of poly(α-hydroxyisobutyric acid) and poly(glycolic ester). *J. Polym. Sci. Part A: Polym. Chem*. 1978;**16**: 2721–2724.
83. Chu, C. C. Degradation phenomena of two linear aliphatic polyester fibres used in medicine and surgery. *Polymer* 1985;**26**:591–594.
84. Volland, C., M. Wolff, and T. Kissel. The influence of terminal gamma-sterilization on captopril containing poly(D,L-lactide-*co*-glycolide) microspheres. *J. Control. Release* 1994;**31**:293–305.
85. Vert, M., S. Li, and H. Garreau. More about the degradation of LA/GA-derived matrices in aqueous media. *J. Control. Release* 1991;**16**:15–26.
86. Fischer, E. W., H. J. Sterzel, and G. Wegner. Investigation of the structure of solution grown crystals of lactide copolymers by means of chemical reactions. *Kolloid Z. Z. Polym*. 1973;**251**:980–990.
87. Middleton, J. C. and A. I. Tipton. Synthetic biodegradable polymers as medical devices. *Med. Plastics Biomater*. 1998;**5**:30–39.
88. Joziasse, C. A. P., D. W. Grijpma, J. E. Bergsma, F. W. Cordewener, R. R. M. Bos, and A. J. Pennings. The influence of morphology on the hydrolytic degradation of as-polymerized and hot-drawn poly(L-lactide). *Colloid. Polym. Sci*. 1998;**276**: 968–975.
89. Li, S. and S. McCarthy. Further investigations on the hydrolytic degradation of poly(D,L-lactide). *Biomaterials* 1999;**20**:35–44.
90. Kenley, R. A., M. O. Lee, T. R. Mahoney II, and L. M. Sanders. Poly(lactide-*co*-glycolide) decomposition kinetics in vivo and in vitro. *Macromolecules* 1987;**20**: 2398–2403.
91. Li, S. M., H. Garreau, and M. Vert. Structure-property relationships in the case of the degradation of massive aliphatic poly-(α-hydroxy acids) in aqueous media, Part 1. Poly(D,L-lactic acid). *J. Mater. Sci. Mater. Med*. 1990;**1**:123–130.
92. Grijpma, D. W. and A. J. Pennings. (Co)polymers of L-lactide, 1: Synthesis, thermal properties and hydrolytic degradation. *Macromolecules Chem. Phys*. 1994;**195**: 1633–1647.
93. Emerich, D. F., M. A. Tracy, K. L., Ward, M. Figueiredo, R. Qian, C. Henschel and R. T. Bartus. Biocompatibility of poly (DL-lactide-co-glycolide) microspheres implanted into the brain. *Cell Transplant*. 1999; **8**:47–58.
94. Alexender, H., A. B. Weiss, J. R. Parsons, I. D. Stauchler, S. F. Corcoran, O. Gona, and C. Mayott. Canine patellar tendon replacement with a polylactic acid polymer-filamentous carbon tissue degrading scaffold. *Orthopad. Rev*. 1981; **10**:41–51.
95. Gupta, A. P. and V. Kumar. New emerging trends in synthetic biodegradable polymers—Polylactide: A critique. *Eur. Polym. J*. 2007; **43**:4053–4074.
96. van Sliedregt, A., C. A. van Blitterswijk, S. C. Hesseling, J. J. Grote, and K. de Groot. The effect of the molecular weight of polylactic acid on in vitro biocompatibility. *Adv. Biomater*. 1990; **9**:207–211.

97. van Sliedregt, A., A. M. Radder, K. de Groot, and C. A. van Blitterswijk. In vitro biocompatibility testing of polylactides. Part 1. Proliferation of different cell types. *J. Mater Sci.: Mater. Med.* 1992;**3**:365–370.

98. Taylor, M. S., A. U. Daniels, K. P. Andriano, and J. Heller. Six bioabsorbable polymers. In vitro acute toxicity of accumulated degradation products. *J. Appl. Biomater.* 1994;**5**:151–157.

99. Vert, M., G. Schwach, R. Engel, and J. Coudane. Something new in the field of PLA/GA bioresorbable polymers? *J. Control. Release* 1998;**53**:85–92.

100. Ueda, H. and Y. Tabata. Polyhydroxyalkanonate derivatives in current clinical applications and trials. *Adv. Drug Deliv. Rev.* 2003;**55**:501–518.

101. Athanasiou, K. A., G. G. Niederauer, and C. M. Agrawal. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* 1996;**17**:93–102.

102. Jain, R., N. H. Shah, A. W. Malick, and C. T. Rhodes. Controlled drug delivery by biodegradable poly(ester) devices: Different preparative approaches. *Drug. Dev. Ind. Pharm.* 1998;**24**:703–727.

103. Moghini, S. M., A. C., Hunter, and J. C. Murray. Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacol. Rev.* 2001;**53**:383–318.

104. Jostel, A., A. Mukherjee, J. Alenfall, L. Smethurst, and S. M. Shalet. A new sustained-release preparation of human growth hormone and its pharmokinetic, pharmacodynamic and safety profile. *Clin. Endocrinol.* 2005;**62**:623–627.

105. Cleland, J. L.Protein delivery from biodegradable microspheres. *Pharm. Biotechnol.* 1997;**10**:1–43.

106. Jiang, W., R. K. Gupta, M. C. Deshpande, and S. P. Schwendeman. Biodegradable poly(lactic acid-*co*-glycolic acid) microparticles for injectable delivery of vaccine antigens. *Adv. Drug Deliv. Rev.* 2005;**57**:391–410.

107. Hedley, M. L. Formulations containing poly(lactide-*co*-glycolide) and plasmid DNA expression vectors. *Expert. Opin. Biol. Ther.* 2003;**3**:903–910.

108. Olivier, J.-C. Drug transport to brain with targeted nanoparticles. *NeuroRx* 2005;**2**:108–109.

109. Menei, P., V. Daniel, C. Montero-Menei, M. Brouillard, A. Pouplard-Barthelaix, and J. P. Benoit, Biodegradation and brain tissue reaction to poly(D,L-lactide-*co*-glycolide) microspheres. *Biomaterials* 1993; **14**:470–478.

110. Bazile, D. V., C. Ropert, P. Huve, T. Verrecchia, M. Marlard, A. Frydman, M. Veillard, and G. Spenlehauer. Body distribution of fully biodegradable [14 C]-poly(lactic acid) nanoparticles coated with albumin after parenteral administration to rats. *Biomaterials* 1992;**13**:1093–1102.

111. Plard, J. P. and D. Bazile, Comparison of the safety profiles of PLA50 and Me. PEG-PLA50 nanoparticles after single dose intravenous administration to rat. *Colloids Surfaces B: Biointerf.* 1999;**16**:173–183.

112. Uurto, I., J. Mikkonen, J. Parkkinen, L. Keski-Nisula, T. Nevalainen, M. Kellomaki, P. Tormala, and J. P. Salenius. Drug-eluting biodegradable poly-D/L-lactic acid vascular stents. An experimental pilot study. *J. Endovasc. Ther.* 2005;**12**:371–379.

113. Gunja, N. J. and K. A. Athanasiou. Biodegradable materials in arthroscopy. *Sports Med. Arthro. Rev.* 2006;**14**:112–119.

114. Bostman, O., E. Hirvensalo, E. Partio, P. Tormala, and P. Rokkanen. Resorbable rods and screws of polyglycolide in stabilizing malleolar fractures. A clinical study of 600 patients. *Der Unfallchirurg* 1992;**95**:109–112.

115. Bucholz, R. W., S. Henry, and M. B. Henley. Fixation with bioabsorbable screws for the treatment of fractures of the ankle. *J. Bone Joint Surg. Am.* 1994; **76**:319–324.

116. Claes, L. and A. Ignatius. Development of new, biodegradable implants. *Chirurg* 2002;**73**:990–996.

117. Bostman, O. M. Absorbable implants for the fixation of fractures. *J. Bone Joint Surg. Am.* 1991;**73**:148–153.

118. Gautier, S. E., M. Oudega, M. Fragaso, P. Chapon, G. W. Plant, M. B. Bunge, and J.-M. Parel. Poly(alpha-hydroxy acids) for application in the spinal cord: Resorbability and biocompatibility with adult rat Schwann cells and spinal cord. *J. Biomed. Mater. Res.* 1998;**42**:642–654.

119. de Medinaceli, L., R. al Khoury, and M. Merle. Large amounts of polylactic acid in contact with divided nerve sheaths have no adverse effects on regeneration. *J. Reconstr. Microsurg.* 1995;**11**:43–49.

120. Deguchi, M., B. C. Cheng, K. Sato, Y. Matsuyama, and T. A. Zdeblick. Biomechanical evaluation of translaminar facet joint fixation. A comparative study of poly-L-lactide pins, screws and pedicle fixation. *Spine* 1998;**23**:1307–1312.

121. Iera, D., A. J. Haddad, G. K. Sandor, and N. Ashammakhi. Bioabsorbable fixation devices. *Ann. Chir. Plast. Esthet.* 2005;**50**:723–732.

122. Suuronen, R., R. Kontio, N. Ashammakhi, C. Lindqvist, and P. Laine. Bioabsorbable self-reinforced plates and screws in craniomaxillofacial surgery. *Biomed. Mater Eng.* 2004;**14**:517–524.

123. Holmes, R. E., S. Cohen, G. B. Cornwall, K. A. Thomas, K. K Kleinhenz, and M. Z. Beckett. MacroPore resorbable devices in craniofacial surgery. *Clin. Plast. Surg.* 2004;**31**:393–406.

124. Suuronen, R., I. Kallela, and C. Lindqvist. Bioabsorbable plates and screws: Current state of the art in facial fracture repair. *J. Craniomaxillofac. Trauma* 2000;**6**:19–27.

125. Bostman, O. M. and H. K. Pihlajmaki. Adverse tissue reactions to bioabsorbable fixation devices. *Clin. Orthop. Relat. Res.* 2000;**371**:216–227.

126. Ambrose, C.G. and T. O. Clanton. Bioabsorbable implants: Review of clinical experience in orthopedic surgery. *Ann. Biomed. Eng.* 2004;**32**:171–177.

127. Corey, J. M., C. C. Gertz, B. S. Wang, L. K. Birrell, S. L. Johnson, D. C. Martin, and E. L. Feldmann. The design of electrospun PLLA nanofiber scaffolds compatible with serum-free growth of primary motor and sensory neurons. *Acta Biomater.* 2008;**4**:863–875.

128. Murukami, Y., M. Yokohama, T. Okano, H. Nishida, Y. Tomizawa, M. Endo, and H. Kurusawa. A novel synthetic tissue-adhesive hydrogel using a cross-linkable polymeric micelle. *J. Biomed. Mat. Res. Part A* 2007;**80A**: 421–427.

129. Ashammakhi, N., A. Ndreu, Y. Yang, H. Ylikauppila, and L. Nikkola. Nanofiber-based scaffolds for tissue engineering. *Eur. J. Plast. Surg.* 2008. Available on-line: DOI 10.1007/s00238-008-0217-3

130. McLaren, A. C. Alternative materials to acrylic bone cement for delivery of depot antibiotics in orthopaedic infections. *Clin. Orthoped. Rel. Res.*2004; **427**:101–116.

131. El-Beyrouty, C., V. Huang, C. J. Darnold, and P. G. Clay. Poly-L-lactic acid for facial lipoatrophy in HIV. *Ann. Pharmacother.* 2006; **40**:1602–1606.

132. Holland, S. J., B. J. Tighe, and P. L. Gould. Polymers for biodegradable medical devices. 1. The potential of polyesters as controlled macromolecular release systems. *J. Control. Release* 1986;**4**:155–180.

133. Alexis, F. Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly[(lactic acid)-*co*-(glycolic acid)]. *Polym. Int.* 2005;**54**: 36–46.

134. Lewis, D. H. Controlled release of bioactive agents from lactide/glycolide polymers. *Drugs Pharma. Sci.* 1990;**45**:1–41.

135. Kissel, T., S. Maretschek, C. Packhaeuser, J. Schnieders, and N. Seidel. Microencapsulation techniques for parenteral depot systems and their application in the pharmaceutical industry. *Drugs Pharma. Sci.* 2006;**158**:99–122.

136. Lu, W. and T. G. Park. Protein release from poly(lactic-co-glycolic acid) microspheres: Protein stability problems. *PDA J. Pharma. Sci. Tech.* 1995;**49**: 13–19.

137. Chen, L., R. N. Apte, and S. Cohen. Characterization of PLGA microspheres for the controlled delivery of IL-1alpha for tumor immunotherapy. *J. Control. Release.* 1997;**43**:261–272.

138. Pean, J.-M., F. Boury, M.-C. Venier-Julienne, P. Menei, J.-E. Proust, and J.-P. Benoit. Why does PEG 400 co-encapsulation improve NGF stability and release from PLGA biodegradable microspheres? *Pharma. Res.* 1999;**16**:1294–1299.

139. Uchida, T., K. Shiosaki, Y. Nakada, K. Fukada, Y. Eda, S. Tokiyoshi, N. Nagareya, and K. Matsuyama. Microencapsulation of hepatitis B core antigen for vaccine preparation. *Pharma. Res.* 1998;**15**:1708–1713.

140. Cleland, J. L., O. L. Johnson, S. Putney, and A. J. S. Jones. Recombinant human growth hormone poly(lactic-*co*-glycolic acid) microsphere formulation development. *Adv. Drug Deliv. Rev.* 1997;**28**:71–84.

141. Cleland, J. L. and A. J. S. Jones. Stable formulations of recombinant human growth hormone and interferon-gamma for microencapsulation in biodegradable microspheres. *Pharma. Res.* 1996;**13**:1464–1475.

142. Johnson, O. L., W. Jaworowicz, J. L. Cleland, L. Bailey, M. Charnis, E. Duenas, C. Wu, D. Shepard, S. Magil, T. Last, A. J. S. Jones, and S. D. Putney. The stabilization and encapsulation of human growth hormone into biodegradable microspheres. *Pharma. Res.* 1997;**14**:730–735.

143. Zhu, G., S. R. Mallery, and S. P. Schwendeman. Stabilization of proteins encapsulated in injectable poly(lactide-*co*-glycolide). *Nature Biotech.* 2000; **18**:52–57.

144. Zhang, L., F. X. Gu, J. M. Chan, A. Z. Wang, R. S. Langer, and O. C. Farokhzad. Nanoparticles in medicine: Therapeutic applications and developments. *Clin. Pharma. Therapeu.* 2008;**83**:761–769.

145. Diwan, M. and T. G. Park. Pegylation enhances protein stability during encapsulation in PLGA microspheres. *J. Control. Release.* 2001;**73**:233–244.

146. Diwan, M. and T. G. Park. Stabilization of recombinant interferon-alpha by pegylation for encapsulation in PLGA microspheres. *Int. J. Pharma.* 2003; **252**:111–122.

147. Hinds, K. D., K. M. Campbell, K. M. Holland, D. H. Lewis, C. A. Piche, and P. G. Schmidt. PEGylated insulin in PLGA microparticles. In vivo and in vitro analysis. *J. Control. Release* 2005;**104**:447–460.

148. Kim, D., L. MacConell, D. Zhuang, A. Kothare Prajakti, M. Trautmann, M. Fineman, and K. Taylor. Effects of once-weekly dosing of a long-acting release formulation of exenatide on glucose control and body weight in subjects with type 2 diabetes. *Diabetes Care* 2007;**30**:1487–1493.

149. Drucker, D. J., J. B. Buse, K. Taylor, D. M. Kendall, M. Trautmann, D. Zhuang, and L. Porter. Exenatide once weekly versus twice daily for the treatment of type 2 diabetes: A randomised, open-label, non-inferiority study. *Lancet* 2008;**372**:1240–1250.

150. Gefvert, O., B. Eriksson, P. Persson, L. Helldin, A. Bjoerner, E. Mannaert, B. Remmerie, M. Eerdekens, and S. Nyberg. Pharmacokinetics and D2 receptor occupancy of long-acting injectable risperidone (Risperdal Consta) in patients with schizophrenia. *Int. J. Neuropsychopharma.* 2005;**8**:27–36.

151. Weiss, R. D. Adherence to pharmacotherapy in patients with alcohol and opioid dependence. *Addiction* 2004;**99**:1382–1392.

152. Dean, R. L. The preclinical development of Medisorb naltrexone, a once a month long-acting injection, for the treatment of alcohol dependence. *Front. Biosci.* 2005;**10**:643–655.

153. Garbutt, J. C., H. R. Kranzler, S. S. O'Malley, D. R. Gastfriend, H. M. Pettinati, B. L. Silverman, J. W. Loewy, and E. W. Ehrich. Efficacy and tolerability of long-acting injectable naltrexone for alcohol dependence. A randomized controlled trial. *J. Am. Med. Assoc.* 2005;**293**:1617–1625.

154. Dunn, R. L. The Atrigel drug delivery system. *Drugs Pharma. Sci.* 2003;**126**: 647–655.

155. Ravivarapu, H. B., K. L. Moyer, and R. L. Dunn. Parameters affecting the efficacy of a sustained release polymeric implant of leuprolide. *Int. J. Pharma.* 2000; **194**:181–191.

156. Ravivarapu, H. B., K. L. Moyer, and R. L. Dunn. Sustained suppression of pituitary-gonadal axis with an injectable, in situ forming implant of leuprolide acetate. *J. Pharma. Sci.* 2000;**89**:732–741.

157. Perez-Marreno, R., F. M. Chu, D. Gleason, E. Loizides, B. Wachs, and R. C. Tyler. A six-month, open-label study assessing a new formulation of leuprolide 7.5 mg for suppression of testosterone in patients with prostate cancer. *Clin. Therapeu.* 2002;**24**:1902–1914.

158. Chu, F. M., M. Jayson, M. K. Dineen, R. Perez, R. Harkaway, and R. C. Tyler. A clinical study of 22..5 mg. LA-2550: A new subcutaneous depot delivery system for leuprolide acetate for the treatment of prostate cancer. *J. Urol.* 2002; **168**:1199–1203.

159. Crawford, E. D., O. Sartor, F. Chu, R. Perez, G. Karlin, and J. S. Garrett. A 12-month clinical study of LA-2585 (45.0 MG): A new 6-month subcutaneous delivery system for leuprolide acetate for the treatment of prostate cancer. *J. Urol.* 2006;**175**:533–536.

160. Alexandridis, P. and A. T. Hatton. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling. *Coll. Surf. A.* 1995;**96**:1–46.

161. Alexandridis, P., J. F. Holzwarth, and A. T. Hatton. Micellization of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers in aqueous solutions: Thermodynamics of copolymer association. *Macromolecules* 1994; **27**:2414–2425.

162. Tuzar, Z. and P. Kratochvil. In *Micelles of Block and Graft Copolymers in Solution*. E. Matijevic (Ed.). Plenum Press: New York, 1993, pp. 1–192.

163. Martin, A. N. *Colloids*. In *Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences*. A. N. Martin (Ed.). Williams and Wilkins: Baltimore, 1993, pp. 393–422.

164. Kwon, G. S. Diblock copolymer nanoparticles for drug delivery. *Crit. Rev. Therapeu. Drug Carrier Syst.* 1998;**15**:481–512.

165. Zhang, X., J. K. Jackson, and H. M. Burt. Development of amphiphilic diblock copolymers as micellar carriers of taxol. *Int. J. Pharma.* 1996;**132**:195–206.

166. Aliabadi, H. M., A. Mahmud, A. D. Sharifabadi, and A. Lavasanifar. Micelles of methoxy poly(ethylene oxide)-*b*-poly(caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A. *J. Control. Release* 2005;**104**:301–311.

167. Bazile, D., C. Prud'Homme, M. T. Bassoullet, M. Marland, G. Spenlehauer, and M. Veillard. Stealth MePEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. *J. Pharma. Sci.* 1995;**84**:493–498.

168. Gref, R., M. Luck, P. Quellec, M. Marchand, E. Dellacherie, S. Harnisch, T. Blunk, and R. H. Muller. "Stealth" corona-core nanoparticles surface modified by polyethylene glycol (PEG): Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Coll. Surf. B: Biointerfaces* 2000;**18**:301–313.

169. Kwon, G., S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka. Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly(ethylene oxide-aspartate) block copolymer-Adriamycin conjugates. *J. Control. Release* 1994;**29**:17–23.

170. Park, J. W., D. B. Kirpotin, K. Hong, R. Shalaby, Y. Shao, U. B. Nielsen, J. D. Marks, D. Papahadjopoulos, and C. C. Benz. Tumor targeting using anti-HER2 immunoliposomes. *J. Control. Release* 2001;**74**:95–113.

171. Xiong, X-B., A. Mahmud, H. Uludag, and A. Lavasanifar. Conjugation of arginine-glycine-aspartic acid peptides to poly(ethylene oxide)-*b*-poly(ε-caprolactone) micelles for enhanced intracellular drug delivery to metastatic tumor cells. *Biomacromolecules* 2007;**8**:874–884.

172. Farokhzad, O. C., S. Jon, A. Khademhosseini, T.-NT. Tran, D. A. LaVan, and R. Langer. Nanoparticle-aptamer bioconjugates: A new approach for targeting prostate cancer cells. *Cancer Res.* 2004;**64**:7668–7672.

173. Sparreboom, A., J. Verweij, M. E. L. Van der Burg, W. J. Loos, E. Brouwer, L. Vigano, A. Locatelli, A. I. De Vos, K. Nooter, G. Stoter, and L. Gianni. Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype in vivo. *Clin. Cancer Res.* 1998;**4**:1937–1942.

174. Kim, S. C., D. W. Kim, Y. H. Shim, J. S. Bang, H. S. Oh, S. W. Kim, and S. H. Seo. In vivo evaluation of polymeric micellar paclitaxel formulation: Toxicity and efficacy. *J. Control. Release* 2001;**72**:191–202.

175. Kim, T-Y., D-W. Kim, J-Y. Chung, S. G. Shin, S. -C. Kim, D. S. Heo, N. K. Kim, and Y. J. Bang. Phase I and pharmacokinetic study of genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. *Clin. Cancer Res.* 2004;**10**:3708–3716.

176. Lee, K. S., H. C. Chung, S. A. Im, Y. H. Park, C. S. Kim, S. -B. Kim, S. Y. Rha, M. Y. Lee, and J. Ro. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. *Breast Cancer Res. Trea.* 2008;**108**:241–250.

177. Newman, M. G, K. S. Kornman, and F. M. Doherty. A 6-month multi-center evaluation of adjunctive tetracycline fiber therapy used in conjunction with scaling and root planing in maintenance patients: Clinical results. *J. Periodontol.* 1994;**65**:685–691.

178. Garrett, S., L. Johnson, C. H. Drisko, D. F. Adams, C. Bandt, B. Beiswanger, G. Bogle, K. Donly, W. W. Hallmon, E. B. Hancock, P. Hanes, C. E. Hawley, R. Kiger, W. Killoy, J. T. Mellonig, A. Polson, F. J. Raab, M. Ryder, N. H. Stoller, H. L. Wang, L. E. Wolinsky, G. H. Evans, C. Q. Harrold, R. M. Arnold, and G. L. Southard. Two multi-center studies evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene, and scaling and root planing in the treatment of periodontitis. *J. Periodontol.* 1999;**70**:490–503.

179. Williams, R. C., D. W. Paquette, S. Offenbacher, D. F. Adams, G. C. Armitage, K. Bray, J. Caton, D. L. Cochran, C. H. Drisko, J. P. Fiorellini, W. V. Giannobile, S. Grossi, D. M. Guerrero, G. K. Johnson, I. B. Lamster, I. Magnusson, R. J. Oringer, G. R. Persson, T. E. Van Dyke, L. F. Wolff, E. A. Santucci, B. E. Rodda, and J. Lessem. Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. *J. Periodontol.* 2001;**72**:1535–1544.

180. del Amo, E. M. and A. Urtti. Current and future ophthalmic drug delivery systems. *Drug Discov. Today* 2008;**13**:135–143.

181. Velez, G. and S. M. Whitcup. New developments in sustained release drug delivery for the treatment of intraocular disease. *Br. J. Ophthalmol.* 1999;**83**:1225–1229.

182. Kuppermann, B. D., M. S. Blumenkranz, J. A. Haller, G. A. Williams, D. V. Weinberg, C. Chou, and S. M. Whitcup. Randomized controlled study of an intravitreous dexamethasone drug delivery system in patients with persistent macular edema. *Arch. Ophthalmol.* 2007;**125**:309–317.

183. Herrero-Vanrell, R. and I. T. Molina-Martinez. PLA and PLGA microparticles for intravitreal drug delivery: An overview. *J. Drug Deliv. Sci. Technol.* 2007;**17**:11–17.

184. Giordano, G. G., P. Chevez-Barrios, M. F. Refojo, and C. A. Garcia. Biodegradation and tissue reaction to intravitreous biodegradable poly(D,L-lactic-*co*-glycolic)acid microspheres. *Curr. Eye Res.* 1995;**14**:761–768.

185. Moritera, T., Y. Ogura, Y. Honda, R. Wada, S. H. Hyon, and Y. Ikada. Microspheres of biodegradable polymers as a drug-delivery system in the vitreous. *Invest. Ophthalmol. Visual Sci.* 1991;**32**:1785–1790.

186. Yasukawa, T., Y. Ogura, Y. Tabata, H. Kimura, P. Wiedemann, and Y. Honda. Drug delivery systems for vitreoretinal diseases. *Progr. Retinal Eye Res.* 2004; **23**:253–281.

187. Moritera, T., Y. Ogura, N. Yoshimura, Y. Honda, R. Wada, S. H. Hyon, and Y. Ikada. Biodegradable microspheres containing adriamycin in the treatment of proliferative vitreoretinopathy. *Invest. Ophthalmol. Visual Sci.* 1992;**33**: 3125–3130.

188. Giordano, G. G., M. F. Refojo, and M. H. Arroyo. Sustained delivery of retinoic acid from microspheres of biodegradable polymer in PVR. *Invest. Ophthalmol. Visual Sci.* 1993;**34**:2743–2751.

189. Veloso, A. A., Jr., Q. Zhu, R. Herrero-Vanrell, and M. F. Refojo. Ganciclovir-loaded polymer microspheres in rabbit eyes inoculated with human cytomegalovirus. *Invest. Ophthalmol. Visual Sci.* 1997;**38**:665–675.

190. Bourges, J-L., E. Gautier Sandrine, F. Delie, A. Bejjani Riad, J. -C. Jeanny, R. Gurny, D. BenEzra, and F. F. Behar-Cohen. Ocular drug delivery targeting the retina and retinal pigment epithelium using polylactide nanoparticles. *Invest. Ophthalmol. Visual Sci.* 2003;**44**:3562–3569.

191. Bejjani, R. A., D. BenEzra, H. Cohen, J. Rieger, C. Andrieu, J. -C. Jeanny, G. Gollomb, and F. F. Behar-Cohen. Nanoparticles for gene delivery to retinal pigment epithelial cells. *Mol. Vision* 2005;**11**:124–132.

192. Bhardwaj, R. and J. Blanchard. Controlled-release delivery system for the alpha-MSH analog melanotan-I using poloxamer 407. *J. Pharma. Sci.* 1996;**85**:915–919.

193. Miyazaki, S., Y. Ohkawa, M. Takada, and D. Attwood. Antitumor effect of pluronic F-127 gel containing mitomycin C on sarcoma-180 ascites tumor in mice. *Chem. Pharma. Bull.* 1992;**40**:2224–2226.

194. Davidorf, F. H., R. B. Chambers, O. W. Kwon, W. Doyle, P. Gresak, and S. G. Frank. Ocular toxicity of vitreal pluronic polyol F-127. *Retina* 1990;**10**:297–300.

195. Lin, H. R. and K. C. Sung. Carbopol/Pluronic phase change solutions for ophthalmic drug delivery. *J. Control. Release* 2000;**69**:379–388.

196. Jeong, B., Y. H. Bae, and S. W. Kim. Thermoreversible gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions. *Macromolecules* 1999;**32**:7064–7069.

197. Zentner, G. M., R. Rathi, C. Shih, J. C. McRea, M. H. Seo, H. Oh, B. G. Rhee, J. Mestecky, Z. Moldoveanu, M. Morgan, and S. Weitman. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Control. Release* 2001;**72**:203–215.

198. Jeong, B., D. S. Lee, J-I. Shon, Y. H. Bae, and S. W. Kim. Thermoreversible gelation of poly(ethylene oxide) biodegradable polyester block copolymers. *J. Polym. Sci. Part A: Polym. Chem.* 1999;**37**:751–760.

199. Jeong, B., Y. H. Bae, and S. W. Kim. Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. *J. Control. Release* 2000;**63**:155–163.

200. Jeong, B., M. R. Kibbey, J. C. Birnbaum, Y-Y. Won, and A. Gutowska. Thermogelling biodegradable polymers with hydrophilic backbones: PEG-*g*-PLGA. *Macromolecules* 2000;**33**:8317–8322.

201. Choi, S. and S. W. Kim. Controlled release of insulin from injectable biodegradable triblock copolymer depot in ZDF rats. *Pharma. Res.* 2003;**20**:2008–2010.

202. Kim, Y. J., S. Choi, J. J. Koh, M. Lee, K. S. Ko and S. W. Kim. Controlled release of insulin from injectable biodegradable triblock copolymer. *Pharma. Res.* 2001;**18**:548–550.

203. Matthes, K., M. Mino-Kenudson, D. V. Sahani, N. Holalkere, K. D. Fowers, R. Rathi, and W. R. Brugge. EUS-guided injection of paclitaxel (OncoGel) provides therapeutic drug concentrations in the porcine pancreas (with video). *Gastrointest Endosc*. 2007;**65**:448–453.

204. http://www.protherics.com/Products/cancer.aspx; accessed October 8th, 2008.

205. Pendyala, L., R. Jabara, T. Shinke, N. Chronos, K. Robinson, J. Li, and D. Hou. Drug-eluting stents: present and future. *Cardiovas. Hematolog. Agents Medi. Chem*. 2008;**6**:105–115.

206. Hunter, W. L. Drug-eluting stents: Beyond the hyperbole. *Adv. Drug Deliv. Rev*. 2006;**58**:347–349.

207. Mintz G. S., J. J. Popma, A. D. Pichard, K. M. Kent, L. F. Satler, C. Wong, M. K. Hong, J. A. Kovach, and M. B. Leon. Arterial remodeling after coronary angioplasty: A serial intravascular ultrasound study. *Circulation* 1996; **94**:35–43.

208. Kimmel, S. E., A. R. Localio, R. J. Krone, and W. K. Laskey. The effects of contemporary use of coronary stents on in-hospital mortality. Registry Committee of the Society for Cardiac Angiography and Interventions. *J. Am. Coll. Cardiol*. 2001;**37**:499–504.

209. Regar, E., P. W. Serruys, C. Bode, C. Holubarsch, J. L. Guermonprez, W. Wijns, A. Bartorelli, C. Constantini, M. Degertekin, K. Tanabe, C. Disco, E. Wuelfert, and M. C. Morice. Angiographic findings of the multicenter randomized study with the sirolimus-eluting Bx velocity balloon-expandable stent (RAVEL): sirolimus-eluting stents inhibit restenosis irrespective of the vessel size. *Circulation* 2002;**106**:1949–1956.

210. Camenzind, E., P. G. Steg, and W. Wijns. Stent thrombosis late after implantation of first-generation drug-eluting stents: A cause for concern. *Circulation* 2007;**115**:1440–1455; discussion 1455.

211. Nebeker, J. R., R. Virmani, C. L. Bennett, J. M. Hoffman, M. H. Samore, J. Alvarez, C. J. Davidson, J. M. McKoy, D. W. Raisch, B. K. Whisenant, P. R. Yarnold, S. M. Belknap, D. P. West, J. E. Gage, R. E. Morse, G. Gligcoric, L. Davidson, and M. D. Feldman. Hypersensitivity cases associated with drug-eluting coronary stents: A review of available cases from the Research on Adverse Drug Events and Reports (RADAR) project. *J. Am. Coll. Cardiol*. 2006;**47**:175–181.

212. Joner, M., V. Finn Aloke, A. Farb, K. Mont Erik, D. Kolodgie Frank, E. Ladich, R. Kutys, K. Skorija, K. Gold Herman, and R. Virmani. Pathology of drug-eluting stents in humans: Delayed healing and late thrombotic risk. *J. Am. Coll. Cardiol*. 2006;**48**:193–202.

213. Costa, R. A., A. J. Lansky, A. Abizaid, R. Mueeller, Y. Tsuchiya, K. Mori, E. Cristea, M. B. Leon, J. E. Sousa, T. Schmidt, K. E. Hauptmann, and E. Grube. Angiographic results of the first human experience with the biolimus A9 drug-eluting stent for de novo coronary lesions. *Am. J. Cardiol*. 2006; **98**:443–446.

214. Ramcharitar, S., S. Vaina, and P. W. Serruys. The next generation of drug-eluting stents: What's on the horizon? *Am. J. Cardiovas. Drugs* 2007;**7**:81–93.

215. Finkelstein, A., D. McClean, S. Kar, K. Takizawa, K. Varghese, N. Baek, K. Park, C. Fishbein Michael, R. Makkar, F. Litvack, and N. L. Eigler. Local drug delivery

via a coronary stent with programmable release pharmacokinetics. *Circulation* 2003;**107**:777–784.

216. Serruys, P. W., G. Sianos, A. Abizaid, P. den Heijer, H. Bonnier, P. Smits, D. McClean, S. Verheye, J. Belardi, J. Condado, M. Pieper, L. Gambone, M. Bressers, J. Symons, E. Sousa, and F. Litvack. The effect of variable dose and release kinetics on neointimal hyperplasia using a novel paclitaxel-eluting stent platform: The paclitaxel in-stent controlled elution study (PISCES). *J. Am. Coll. Cardiol.* 2005;**46**:253–260.
217. Krucoff, M. W., D. J. Kereiakes J. L. Petersen, R. Mehran, V. Hasselblad, A. J. Lansky, P. J. Fitzgerald, J. Garg, M. A. Turco, C. A. Simonton, S. Verheye, C. L. Dubois, R. Gammon, W. B. Batchelor, C. D. O'Shaughnessy, J. B. Hermiller, J. Schofer, M. Buchbinder, and W. Wijns. A novel bioresorbable polymer paclitaxel-eluting stent for the treatment of single and multivessel coronary disease. *J. Am. Coll. Cardiol.* 2008;**51**:1543–1552.
218. Finn, A. V., M. Joner, G. Nakazawa, F. Kolodgie, J. Newell, M. C. John, H. K. Gold, and R. Virmani. Pathological correlates of late drug-eluting stent thrombosis: Strut coverage as a marker of endothelialization. *Circulation* 2007;**115**:2435–2441.
219. Ormiston, J. A., P. W. Serruys, E. Regar, D. Dudek, L. Thuesen, M. W. Webster, Y. Onuma, H. M. Garcia-Garcia, R. McGreevy, and S. Veldhof. A bioabsorbable everolimus-eluting coronary stent system for patients with single de-novo coronary artery lesions (ABSORB): A prospective open-label trial. *Lancet* 2008;**371**:899–907.
220. Tamai, H., K. Igaki, E. Kyo, K. Kosuga, A. Kawashima, S. Matsui, H. Komori, T. Tsuji, S. Motohara, and H. Uehata. Initial and 6-month results of biodegradable poly(L-lactic acid) coronary stents in humans. *Circulation* 2000;**102**:399–404.
221. Shoyele, S. A. and S. Cawthorne. Particle engineering techniques for inhaled biopharmaceuticals. *Adv. Drug Deliv. Rev.* 2006;**58**:1009–1029.
222. Patton, J. S. Mechanisms of macromolecule absorption by the lungs. *Proc. Int. Symp. Control. Release Bioactive Mater.* 1997;**24**:65–66.
223. Edwards, D. A., A. Ben-Jebria, and R. Langer. Recent advances in pulmonary drug delivery using large, porous inhaled particles. *J. Appl. Physiol.* 1998; **85**:379–385.
224. Johnson, M. A., S. P. Newman, R. Bloom, N. Talaee, and S. W. Clarke. Delivery of albuterol and ipratropium bromide from two nebulizer systems in chronic stable asthma. Efficacy and pulmonary deposition. *Chest* 1989;**96**:6–10.
225. Ruffin, R. E., M. B. Dolovich, F. A. Oldenburg, Jr., and M. T. Newhouse. The preferential deposition of inhaled isoproterenol and propranolol in asthmatic patients. *Chest* 1981;**80**:904–907.
226. Tabata, Y. and Y. Ikada. Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. *Biomaterials* 1988;**9**:356–362.
227. Edwards, D. A., J. Hanes, G. Caponetti, J. Hrkach, A. Ben-Jebria, M. L. Eskew, J. Mintzes, D. Deaver, N. Lotan, and R. Langer. Large porous particles for pulmonary drug delivery. *Science* 1997;**276**:1868–1871.
228. Rave, K. M., L. Nosek, A. de la Pena, M. Seger, C. S. Ernest, II, L. Heinemann, R. P. Batycky, and D. B. Muchmore. Dose response of inhaled dry-powder insulin

and dose equivalence to subcutaneous insulin lispro. *Diabetes Care* 2005; **28**:2400–2405.

229. Rave, K., A. de la Pena, S. Tibaldi Fabian, L. Zhang, B. Silverman, M. Hausmann, L. Heinemann, and D. B. Muchmore. AIR inhaled insulin in subjects with chronic obstructive pulmonary disease: Pharmacokinetics, glucodynamics, safety, and tolerability. *Diabetes Care* 2007;**30**:1777–1782.
230. Wolzt, M., A. de la Pena, P-Y. Berclaz, S. Tibaldi Fabian, R. Gates Jeffrey, and D. B. Muchmore. AIR inhaled insulin versus subcutaneous insulin: Pharmacokinetics, glucodynamics, and pulmonary function in asthma. *Diabetes Care* 2008;**31**:735–740.

CHAPTER 10

POLYANHYDRIDES-POLY(CPP-SA), FATTY-ACID-BASED POLYANHYDRIDES

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10.1 INTRODUCTION

Drugs given routinely by oral administration and by intramuscular (IM) or intravenous (IV) injection are distributed nonselectively to body parts, including target and nontarget sites, whereas the requirement is only at a particular site in the body. Several old and new drugs and new molecular entities are administered to improve safety and efficacy in new therapies [1]. Disease such as cancer (solid tumors) [2], thrombosis, restenosis [3], osteomyelitis [4], local infection [5], glaucoma, and retinal disorders [6] are complicated to treat by systemic therapy. For example, retinal diseases are complicated to treat with systemically administered drugs because of the blood–retinal barrier and potential systemic toxicity. Hemorrhagic complications occur when antithrombotic agents are administered systemically in cancer treatment for a localized tumor, resulting in serious side effects [7]. Many strategies have been explored to deliver the drug to a specific site using a polymer; they act as the best and simple approach. Polymers for localized application can play structural, functional, or both roles. Functionally active polymers enhance biocompatibility and medical device operation or the delivery of pharmacologically active agents [8].

The advantages of using polymers should be considered even given the following aspects: (1) the toxicity of polymers and their degradation products in the body, that is, biocompatibility; (2) problems associated with release, that is, dose dumping or release failure; (3) the discomfort caused by the system itself because of insertion of the delivery system; and (4) the overall cost of polymeric drug delivery systems [9]. The polymers used in drug delivery systems generally are divided into two types: nonbiodegradable and biodegradable [10]. Biodegradable polymers undergo self-elimination and avoid the removal of the polymer system from the site of implantation after its use. Biodegradation can be enzymatic, microbial, chemical, or simply by hydrolysis [11].

Generally, the polymeric matrix used for drug delivery is hydrophobic, stable, solid, flexible, and soluble in an organic solvent and degrades in an aqueous environment [12]. These biodegradable polymers need to be removed, once the drug has been released [13]. Biodegradable polymers are usually classified on the basis of type of chemical linkage in the backbone. It includes polyesters, polyanhydrides [13, 14] polyorthoesters, polyphosphoesters, poly(cyanoacrylates), polyphophazenes [15], and natural polysaccharides and polyamides. The homo- and heteropolymers of these classes has given a new outlook to polymer therapeutics, so that the modified polymer with all desirable properties can be prepared and used.

The most essential property of any biodegradable polymer is its degradation pattern in the biological system, physical state, hydrophobicity, flexibility, and

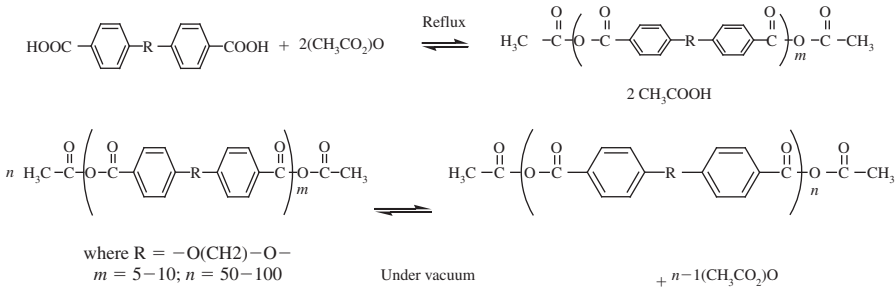
ability to retain the encapsulated or entrapped compound. These attributes preside over the end use of the polymer. Fatty acids are obtained from natural sources and incorporated in biodegradable polymer chains to obtain the desired property of flexibility, hydrophobicity, and injectability [16–18]. Any polymer used for biomedical application is developed on the basis of certain reasonable medical application. Thus, the polymer should be hydrophobic enough so that the drug is released in a predictable and controlled manner; be biocompatible when implanted in the target organ; completely eliminated from the implantation site in predictable time; have suitable physical properties for device fabrication (low melting point, usually, solubility); in case of injectable pasty polymers, the polymer should be fluid enough after drug incorporation to allow easy injection. More flexible before and during degradation so that it integrates or fragments during use; and easy to manufacture at a reasonable cost.

Fatty-acid-based polymers possess many properties to claim their utility as drug delivery carriers. Fatty acids are prepared from oils and fats consisting of saturated and unsaturated oils such as castor oil constitute a glycerol triester of 12-hydroxyoleic acid (ricinoleic acid). Oils and fats hydrolyze into fatty acids and glycerol or fatty alcohols. This process is done by hydrolysis of triesters and then derivatized into other forms [19].The overall share of the fatty acid and its derivatives is important in biodegradable polymers for drug delivery. Fatty acids are a good choice to be included as part of the polymer chain, apart from the points discussed above, and there is an abundance of availability.

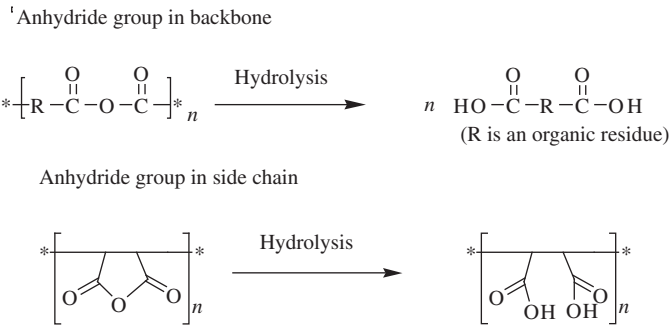
Polyanhydrides have been used for short-term release of drugs [11, 20, 21]. Recently, intensive research has been carried out describing new polymer structures, studies on chemical and physical characterization, degradation and stability properties, toxicity studies, and applications of polymers for controlled delivery of bioactive agents. It also yielded a tool (Gliadel) in clinical use for treating brain cancer [22]. Fast degradation and limited mechanical properties enable the use of these polymers in short-term controlled delivery of bioactive agents. First, synthesis of polyanhydrides was reported by Bucher and Slade [23]. Years later, Hill and Carothers [24, 25] synthesized aliphatic polyanhydrides and studied the actions of diacids toward anhydride formation. They prepared superanhydrides from homologous aliphatic dicarboxylic acid and used them to spin fibers of excellent mechanical strength. Polyanhydrides prepared from aromatic acids are found to be hydrolytically stable and have outstanding film and fiber-forming properties [26]. Conix gives a general method for the preparation of aromatic polyanhydrides as shown next.

Conix [26] studied a number of aromatic polyanhydrides that had glass transition temperatures in the range of 50–100°C and were formed into dense porcelainlike solids that resist hydrolysis even on exposure to alkaline solutions. Yoda [27, 28] introduced a new class of heterocyclic crystalline compounds in the polyanhydrides family. He synthesized a variety of five-membered heterocyclic dibasic acids and polymerized these compounds with acetic anhydride at 200–300°C under vacuum and nitrogen atmosphere. These heterocyclic polymers

have melting points in the range of 70–190°C and good fiber and fiber-forming properties. Aliphatic polyanhydrides were considered as nonimportant materials due to their unstable nature against hydrolysis. In 1980, Langer was the first to use the hydrolytically unstable polyanhydrides for controlled drug delivery applications [29] and used them as biodegradable carriers in various medical devices.



This class of polyanhydrides belongs to those polyanhydrides that have the anhydride bond in the polymer backbone and degrade to shorter chains after breakdown of the anhydride bonds. In other polyanhydrides the anhydride is a side group and is not a part of the polymer backbone. For example, poly(malic anhydride) is a polyethylene chain having anhydride groups as side groups to the polymeric backbone. After the breakdown of the anhydride bond in malic anhydride, no change in the initial molecular weight is expected. The difference in degradation performance of both types of polyanhydrides is shown in the following:



Polyanhydrides form a new category of biodegradable polymers in the biomaterials family having a hydro backbone with hydrolytically labile anhydride linkages. The hydrolytic degradation can be controlled by manipulation of the polymer composition. These polymers merit attention because they show no evidence of inflammatory reaction. They degrade in vitro as well as in vivo to their acid counterparts as nonmutagenic and noncytotoxic products [30, 31]. Polyanhydrides are biocompatible with outstanding controlled-release characteristics [32, 33]. Pharmaceutical research has been focused on polyanhydrides derived from sebacic acid (SA), 1,3-bis(*p*-carfilm boxyphenoxy) propane (CPP)

and the fatty acid dimer (FAD). Recently, the Food and Drug Administration (FDA) has approved the use of the polyanhydride poly(sebacic acid-*co*-1,3-bis (*p*-carboxyphenoxy) propane) [P(CPP-SA)] to deliver the chemotherapeutic release of BCNU (1,3-bis[2-chloroethyl]-1-nitroso-urea) for the treatment of brain cancer [34]. Introduction of the *imide group* into polyanhydrides enhances the mechanical properties of the polymers [35–38], while the presence of poly(ethylene glycol) (PEG) groups in polyanhydrides increases hydrophilicity and induces fast drug release [39]. The major drawback of polyanhydrides is their storage stability, which requires refrigeration. A number of research articles published on polyanhydrides have established different aspects with special emphasis on controlled drug delivery applications [40–43]. Degradation, biocompatibility, drug release behavior, and various applications of polyanhydrides are described herein.

Polyanhydrides are suitable polymers for controlled drug delivery as they degrade uniformly into nontoxic metabolites that are nonmutagenic, noncytotoxic, and noninflammatory [12]. In 1980, Langer was first to explaining the hydrolytically unstable nature of polyanhydrides for sustained release of drug in controlled drug delivery applications [30]. Since then, few polyanhydride products have reached the market or are in different clinical stages. Gliadel, a device to deliver carmustine (BCNU) to the malignant glioma tumor, is the most successful application of polyanhydrides [44]. Polyanhydrides are the class of biodegradable polymers that release the drug by simple hydrolysis; hence, general enzymatic disparity does not play an effective role on the polymer erosion and drug release or its pharmacological effects [45]. These have been investigated as an important biomaterial, used for short-term release of drugs and have been systematically investigated for their chemical and physical properties, degradation and stability, toxicity, and applications in the delivery of bioactive agents [13]. The degradation profile of these polymers can be modulated from days to months by varying the type and ratio of the monomers [12, 18].

Due to all the aspects of localized delivery and disease situation, this chapter focuses on the use of polyanhydrides in localized delivery with special consideration to their degradability behavior in vitro and in vivo, toxicological profile, their uses in different disease condition, and recent advances in the medical field.

10.2 IMPORTANCE OF POLYANHYDRIDES

There is a need to develop more rational approaches for creating superior biomaterials for drug delivery, particularly biodegradable polymers. To maximize the control over release, it is desirable for a system to degrade only from its surface. These surface-eroding polymers are predictable to release the drug at a constant release rate; thus, the rate is directly proportional to the polymer erosion. For a surface-eroding device, the polymer must be hydrophobic with water labile linkages. Polyanhydrides are believed to predominantly undergo

surface erosion due to (i) the high water labiality of the anhydride bonds on the surface and (ii) hydrophobicity, which restricts water penetration into the bulk. A decrease in the device thickness throughout the erosion process, maintenance of the structural integrity, and the nearly zero-order degradation kinetics suggest the dominancy of heterogeneous surface erosion [11]. High hydrolytic reactivity of the anhydride linkage provides an intrinsic advantage in versatility and control of degradation rates. By varying the types of monomer and their ratios, surface-eroding polymers with degradation of 1 week to several years can be designed and synthesized. The hydrolytic degradation rates can be obtained varying several thousand folds by simple changes in the polymer backbone and by altering the hydrophobic and hydrophilic balance of the polymer [46, 47]. Aliphatic polyanhydrides degrade in a few days while aromatic polyanhydrides degrade over a few years. Degradation rates of copolymers of aliphatic and aromatic polyanhydrides vary between these extremes, and this feature of polyanhydrides gives an opportunity for making a drug delivery system that can provide the release of drugs in a desired time length of treatment.

Polyanhydrides are important polymer material in various aspects of drug delivery. The merit and demerit of these polymers are due to the hydrolytic instability of the anhydride bond that degrades rapidly to form nontoxic diacid monomers. The main advantages of this class of polymers are as follows:

- They are prepared from easily available low-cost materials and considered as safe dicarboxylic acid building blocks; many are body constituents or metabolites.
- They, are prepared in a one-step synthesis without purification.
- They have a definite polymer structure with controlled molecular weight and degrade hydrolytically at a predictable rate.
- These polymers can be consequently manipulated to release bioactive agents at a predictable rate for periods of weeks.
- They are easily processable at low-temperature injection molding or extrusion molding for mass production and have versatile properties, which can be varied by monomer selection, composition, surface area, and additives.
- They degrade to their respective diacids and are completely eliminated from the body within a period of weeks to months..
- They can be sterilized by terminal γ-irradiation with minimal effect on polymer properties.

Besides the benefits of polyanhydrides, they have some limitations. They require storage under moisture-free frozen conditions, low mechanical strength, and film or fiber-forming properties. These polymers undergo spontaneous depolymerization to low-molecular-weight polymers in organic solutions or upon storage at room temperatures and above.

10.3 TYPES OF POLYANHYDRIDES

In the development of erodible materials, the use of copolymers is important. These copolymers are obtained by using various proportions of monomer ratios for their different erosion rates, which enables achieving different targets with the same monomers [11]. This flexibility of the polyanhydrides is due to the hydrophobicity of the polymer backbone, which provides a wide range of backbones and ensures biodegradability of the polymer. Due to hydrolytic sensitivity of the anhydride bond, a minor change in chemical structure of the backbone would significantly affect the degradation rate of the polymer. The extent of hydrophobicity of the monomer makes the polymer more stable due to less penetration of water molecules [13]. The most extensively studied and used copolymer of polyanhydride is P(CPP-SA) [48, 49]. FDA approved the use of polyanhydride [P(CPP-SA)] to deliver drugs for the treatment of brain cancer. This is an example wherein an implantable synthetic degradable polymer has been accepted for human use [50]. Furthermore, alkane hydroxides studied in this series are 1,6-bis(p-carboxyphenoxy)hexane (CPH); (carboxyphenoxy) methane (CPM); and 5-@-carboxyphenoxy)- valeric acid (CPV) and their copolymers with SA (Table 10.1). Among other aliphatic-aromatic polyanhydride copolymers, some are based on the common diacids isophthalic acid (IPA), terephthalic acid (TA), and fumaric acid (FA). These copolymers are extremely soluble in chloroform or dichloromethane, melt at temperatures above 250°C, and are stable upon storage at 25°C and exposure to 2.5 Mrad of μ-radiation [51]. Copolymers of fumaric acid with aliphatic diacids such as sebacic acid are less crystalline and soluble in chlorinated hydrocarbons. The complete degradation of 14×1.5 mm disks of PFA and PSA occurred in 2 and 15 days, respectively, while their copolymers also degraded within this range [52].

Another important group of polyanhydrides used for controlled drug release applications is the fatty-acid-based polyanhydrides [16, 53, 54]. This new class of aliphatic copolyanhydrides was synthesized from nonlinear hydrophobic dimers (FAD) of erucic acid and sebacic acid, which have desired physicochemical and mechanical properties for their use as a carrier for drugs [16]. Oleic acid and linoleic acids are different fatty acid dimers used with sebacic acid as drug delivery systems [54, 55]. Polyanhydrides synthesized from nonlinear hydrophobic fatty acid esters, based on ricinoleic acid and sebacic acid, have the desired physicochemical properties such as a low melting point, hydrophobicity, and flexibility of the polymer for their localized injectable use [13]. The properties of polyanhydrides have been modified by the inclusion of long-chain fatty acid terminals such as stearic acid into the polymer, which alters its hydrophobicity and decreases the degradation rate. Polyanhydrides are modified by the introdution of amino acids such as glycine and alanine into the polymer backbone to increase the mechanical properties. The amino acids are incorporated by imide bonds at the amino terminus, leaving the terminal carboxylic acids available for activation. This poly(anhydride-imides) appears to undergo mainly surface erosion [11]. Crosslinked amino-acid-containing

polyanhydrides based on trimethylimido alanine (TMA-ala) or trimethylimido glycene (TMA-gly) and sebacic acid were synthesized by copolycondensation using BTC prepolymer as a crosslinking agent [56].

The improvement of polyanhydride including poly(anhydride-esters), includes two different types of hydrolytically cleavable bonds in the polymer backbone: ester and anhydride. In a study, low-molecular-weight carboxylic acid terminated prepolymers of poly(ε-caprolactone) were coupled via anhydride linkages; and in another example poly(lactic acid) (PLA) was coupled with PSA to form a triblock copolymer of PLA (polylactic acid)–PSA (polysebacic acid)–PLA [57] and used as stereocomplex-based drug delivery carriers.

10.4 SYNTHESIS OF POLYANHYDRIDES

Polyanhydrides have been synthesized by a variety of techniques, namely melt condensation, ring-opening polymerization, interfacial condensation, dehydroion chlorination, and dehydrative coupling agents [58]. Solution polymerization in general yields low-molecular-weight polymers. A range of catalysts have been used in the synthesis of a variety of polyanhydrides by melt condensation. Mainly, coordination catalysts facilitate anhydride interchange in the polymerization and improve the nucleophilicity of the carbonyl carbon. Significantly high molecular weights were achieved in shorter reaction time by utilizing cadmium acetate, earth metal oxides, and $ZnEt_2H_2O$. With the exception of calcium carbonate, which is a safe natural material, but the use of these catalysts for the production of medical-grade polymers is limited due to its high toxicity [46].

10.5 BIOCOMPATIBILITY AND TOXICITY OF POLYANHYDRIDES

Incorporation of the fatty acid in the biodegradable polymer backbone is advantageous and restricted for monofunctionality of naturally occurring fatty acid. Polyanhydrydes based on sebacic acid terminated with oleic, stearic, linoleic, or lithocholic acid was synthesized [58]. These polymers were developed to use in situ gelling injectable carriers. Positive results were obtained with the polymer having more than 70% of ricinoleic acid (RA) content [59, 60] These were synthesized by transeterification of the PSA chain with RA. It was found that upon addition of the liquid polymer to water it solidifies to form a stable semisolid. Polymers obtained by this method were loaded with cisplatin (5%) and paclitaxel (5–20%). Drug release was found faster with the pasty polymers as compared to the solid polymer and the reason is the same as for low-molecular-weight polymers. Low SA content decreases the crystallinity of the polymer and allows water to penetrate into the matrix, causing a faster release [61].

Complete release of cisplatin was observed in 400 h for pasty formulation (Fig. 10.2) [59] Paclitaxel was released for over 100 days while the polymer carrier was being degraded [60, 62]. The release rate was affected by the paclitaxel content; at a higher content, the release rate was lowered. High affinity of the

drug to the hydrophobic matrix and low solubility of the drug in aqueous medium lead to slow and controlled release of the drug from an in situ formed implant. As the content of paclitaxel in the polymer is higher, the formulation becomes more hydrophobic and does not allow water to penetrate and dissolve the drug and degrade the polymer. The polymer formulations containing anticancer agents (paclitaxel and cisplatin) were evaluated in vivo in heterotrophic (mouse bladder tumor) and orphotrophic (rat prostate cancer) models. The single administration of the polymer–pactlitaxel formulation intratumorally in a mouse bladder tumor model increased the survival rate of the animals as compared to untreated animals and to animals treated with paclitaxel dispersion (conventional administration method).

Some new studies have included ricinoleic acid in the polymer backbone and most of these reports are of ricinoleic-acid-based-copolyester. One of these use ricinoleic lactone for the synthesis of copolyester by ring-opening polymerization (ROP) [63, 64]. Ricinoleic acid lactones were synthesized using dicyclohexylcarbodimide and (dimethylamino)pyridine as catalyst. Mono- to Hexalactones were obtained and polymerized with catalysts commonly used for ring-opening polymerization of lactones, under specific reaction conditions, resulted in oligomers. ROP of ricinoleic acid lactones is difficult even when using a highly reactive ring-opening catalyst such as tin octoate, yttrium isopropoxide, trimethylsilanolates, or (2,4-di-*tert*-butyl-6-{[(2′-dimethylaminoethyl)methylamino]-methyl} phenol)ethylzinc. Polymerization of ricinoleic acid lactones one repetition maximum-six repetition maximum (1RM-6RM) with more reactive catalysts, yttrium isopropoxide Y(OiPr), resulted in oligomers. Polymerization of chromatography-purified 1RM ($M_n = 280$) with Me3SiONa resulted in short oligomers of 5 units ($M_n \sim 1400$). The number of end groups (OH or COOH) that were noticed by proton nuclear magnetic resonance (^{1}H–NMR) is lower than the case of low-molecular-weight oligomers (according to ^{1}H-NMR, degree of polymerization (DP) = 16.5), due to reversible cyclization to lactones. Polymerization of chromatography-purified dilactone 2RM with Sn(Oct) resulted in the formation of longer oligomers ($M_n = 4400$, $M_w = 5700$, 15- to 20-unit oligomer). However, copolymerization with lactide resulted in copolymers of low molecular weight. Polymers of molecular weights in the range of 5000–16,000 were obtained with melting temperatures of 100–130°C for copolymers containing 10–50% w/w ricinoleic acid residues. The polymers were off-white in color, which became yellow with an increase of the RA content. The molecular weights of the polymers decreased with an increase in the content of the ricinoleic acid lactone. It was hypothesized that more reactive lactide, activated first by catalyst, polymerizes and only in the end does some ricinoleic acid lactones react.

The reaction is terminated because of the ricinoleic acid lactones' low reactivity. This low reactivity can be attributed to the low ring strain and to the steric hindrance of the ester bond by the fatty acid side chain. In vitro degradation of RA(*cis*-12-hydroxyoctadeca-9-eonoic acid)-LA (lactic acid) copolymers showed that copolymerization with RA had some effect on the degradation rate and polymer physical properties, which is related to the low

incorporation of RA in the polymer. Addition of RA to PLA is expected to improve the hydrophobicity of the polymer and thus the drug release profile.

Polyesters synthesized were compared for release of hydrophilic and hydrophobic drug, namely 5′-Fluoro uracil and triamcinolone, respectively. 5-FU release was faster in all cases; the total release occurred in 17 days from polymers prepared by transesterification and melt-condensation. Slower 5-FU release was found in polymers prepared by ROP (40% in 17 days). The same pattern was observed for triamcinolone, only 5% in 17 days from ROP polymer in contrast to the 30% from polymer synthesized by transesterfication. The difference was attributed to the diblock nature of the ROP polymer. Its high crystallinity and melting point inhibits the water penetration and polymer degradation, which finally shows up in release profiles [64–66].

Whenever synthetic polymer material is utilized in vivo, the possible tissue implant interactions were also taken in to consideration. In case of biodegradable matrices, the toxicity of the polymer and its degradation products have to be evaluated. Moreover, biodegradation to nontoxic products is considered as the foundation for the biocompatibility of degradable polymer systems. Thus, poly(L-lactic acid) (PLLA) is defined as a safe biomaterial for in vivo use. This is because its degradation product L-lactic acid is a natural metabolite of the body. Even though poly(lactide-co-glycolide) (PLGA) is extensively used and represents the gold standard of degradable polymers, increased local acidity due to its degradation leads to irritation at the site of the polymer implant. The aliphatic–aromatic polyanhydride P(CPP-SA) with a molar ratio of 20 : 80 has been extensively investigated in vivo as a drug delivery matrix. This polymer is used clinically for the delivery of the antineoplastic drug carmustine (BCNU) for the treatment of malignant gliomas in rat brain. The polymer degraded completely, and no polymer remnants were found after 6 weeks [67].

Poly(esteranhydride)s synthesized from ricinoleic and sebacic acids were used as a potential controlled delivery carrier for paclitaxel. Toxicity of these polymers and their formulations with paclitaxel was examined by subcutaneous injection of liquid polymer samples or by implantation of solid polymer specimens into mice for different time periods. Histopathological examination of the tissue surrounding the implant showed minor inflammation 1 week after the injection and no inflammation 3 weeks after the implantation. Injection of the polymer without paclitaxel showed no adverse effects [68].

Polyanhydride toxicological aspects deal with the host response in terms of cytotoxicity, allergic responses, irritation, inflammation, and systemic and chronic toxicity. Cytotoxicity study of any polymer has been done first in a sequential program for assessing biocompatibility of a polymer. This is done by using a tissue culture method [69, 70]. In one of the studies, bovine aortic endothelial cells and bovine smooth muscle cells were used to evaluate in vitro biocompatibility of three polyanhydrides P(CPP-SA) 45 : 55, P(TA-SA) 50 : 50, and P(TA). These cultured mammalian cells were sensitive to the changes in growth medium and substrate [31]. Study showed the absence of acute toxicity of these polymers or their degradation products to sensitive mammalian

cells. Chemical carcinogenesis usually proceeds by a mutagenic route; therefore mutagenicity testing has been used as a rapid screening test for neoplastic transformation. Mutagenicity in vitro results and the cytotoxicity results of the polyanhydride degradation products P(CPP-SA) showed that they are noncytotoxic, nonmutagenic, and have a very low teratogenic potential [31].

In intramuscular or dermal applications, polyanhydrides are tested for local tissue irritation and inflammation by muscle and skin tests. Leong et al. studied the local tissue response of polyanhydrides [P(CPP) and P(TA-SA) 50 : 50] by the implantation of polymer samples into the cornea of rabbits [31]. No discernible inflammatory characteristics were reported in a 6-week implantation period of polymers in rabbit corneas. Cornea clarity was maintained throughout and the proliferation of new blood vessels was absent. Histological examinations confirmed the absence of inflammatory cells throughout the corneas. Laurencin et al. administered high doses of P(CPP-SA) 20 : 80 subcutaneously in rats to study the acute systemic toxicity of the polyanhydrides [71]. Disk polymer implants were administered subcutaneously for a period of 8 weeks at two different doses in two groups. One group was implanted with one matrix each, and the other group implanted with three matrices each, whereas the control group did not have a polymer matrices. The toxicological effects on each individual organ were evaluated on the basis of blood clinical chemistry, hematological parameters, and histological evaluation of the organ sites and implant sites. Prenecropsy examination of all the rats in the study showed no changes in physical appearance or activity due to implantation of polyanhydride matrices. At the time of necropsy, gross examination of the body cavities and tissues did not show any evidence of changes due to the polymer implantation (Fig. 10.1).

Similarly, a histological examination of all organ tissues revealed no histomorphological evidence of induced systemic toxicity on polyanhydride copolymer implantation. No reports are available regarding the long-term carcinogenicity studies on polyanhydrides or their degradation products. However, Leong et al. showed from histological examination that subcutaneous implantation of P(CPP) in rats over a 6-month period showed no evidence of tumor formation [31]. Brain biocompatibility of P(CPP-SA) 20 : 80 was established in rat brain by Tamorgo et al. [33]. It was experimentally proved that none of the animals showed any behavioral changes or neurological deficits suggestive of either systemic or localized toxicity from biodegradable polyanhydrides P(CPP-SA) 20 : 80 after implantation in rat brain. Brem et al. also evaluated the brain biocompatibility of polyanhydride P(CPP-SA) 50 : 50 by implantation in rabbit brain [33]. The animals were evaluated daily after the surgery for behavioral changes such as decreased alertness, passivity, impaired grooming, restlessness, irritability, fearfulness, and focal motor neurological deficits. None of the animals showed any behavioral changes or neurological deficits and all the animals survived until they were sacrificed.

It was concluded that P(CPP-SA) 50 : 50, a polyanhydride matrix, can be used for the interstitial delivery of drugs and is biocompatible in the rat brain. Thus, various types of in vitro and in vivo toxicity studies on polyanhydrides

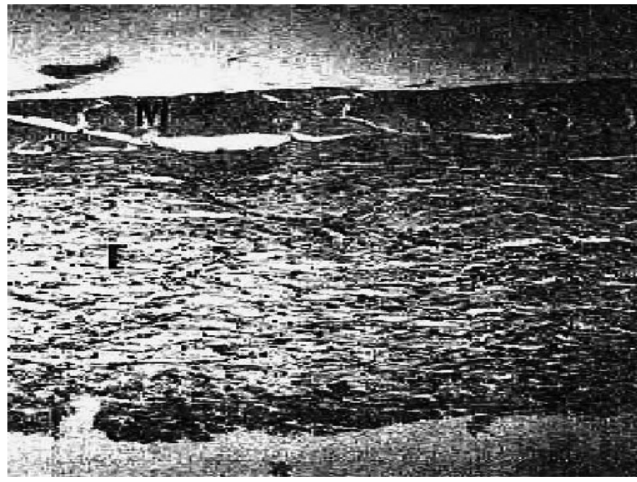


FIGURE 10.1 Light micrograph of local tissue surrounding the implantations of group of rats implanted with three polyanhydride copolymer matrices at 8 weeks postimplantation.

showed these polymers are well tolerated by the body and can be considered biocompatible [14].

10.6 POLYANHYDRIDES AND DISEASE CONDITIONS

Various polyanhydrides and copolymers play an important role as drug carriers in localized drug delivery applications in various disease conditions, as shown in Table 10.1

10.6.1 Cancer

Cancer, or neoplasm, is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated as compared to the normal tissues. It persists in the same excessive manner after the cessation of the stimuli that evoked the changes. Malignant tumors grow very fast, sometimes at an unpredictable speed. It is accompanied by progressive infiltration, invasion and destruction of surrounding tissue, and metastasis, which unequivocally marks a tumor malignant [72]. Various options such as surgical excision, irradiation, and chemotherapy have been tried on cancer [73] and emphasized that the treatment of most cancer patients requires a skillful interdigitation of these multiple modalities. Each form of the treatment carries its own risks and benefits. Chemotherapy is an important component of the armory, but systemically giving chemotherapy can be curative for some tumors and not particularly effective in treating many malignant

TABLE 10.1 Different Polanhydrides Studied for Various Applications as Drug Carriers

Disease	Polymer	Delivery system	Drugs
Cancer	Ricinoleic-acid-based polyanhydride	Matrix	Methotrexate
	P(RA-SA)	Matrix	Cisplatinum
	P(CPP-SA)	Implant	Bromodeoxyuridine *N*-(phosphonacetyl)-aspartic acid
	P(CPP-SA)	Implant	5-Fluorouracil
	P(FAD-SA)	Implant	Taxol
	P(CPP-SA)	Implant	Camptothecin
	P(RA-SA)	Injectable paste	Paclitaxel
Head and neck cancer	P(FAD-SA)	Matrix	Cisplatin, 5-FU, methotrexate, paclitaxel
Osteomyelitis	P(OA/LAD-SA)	Matrix	Gentamicin
Local anesthesia	P(FAD-SA)	Matrix	Bupivacaine HCl
	P(CPP-SA)	Implant	Dibucaine, bupivacaine
Local infection	P(DDDA-TA), P(BA-PA)	Matrix	Ciprofloxacin hydrochloride
	P(FAD-SA)	Implant	Cefazolin sodium
Restenosis	P(EAD-SA)	Implants	Heparin
Gene delivery	Photocrosslinked polyanhydride	Matrix	DNA
Glaucoma	P(CPP-SA)	Implants	Etoposide
Inflammatory bowel disease	Poly(anhydride-esters)	Microspheres	Aminosalicylates
Inflammation	PLA-PSA-PLA	Microspheres	Triamcinolone
Hormone therapy	P(FAD-SA)	Microspheres	GnRHa
Alzheimer's disease	SA copolymer	Microspheres	Bethanechol

Source: From [135–142].

tumors, such as brain and other localized tumors [74]. At the same time, few common medicines in use have a narrow therapeutic index and a greater potential for causing harmful side effects, for example, paclitaxel [75]. Of the 60% of cancer patients with localized disease 32% have been estimated to face recurrence following initial treatment. Around 66% relapse due to local recurrence and 34% relapse due to distant metastasis [76]. Most of the anticancer drugs in clinical use do not have specific effects on invasiveness or the tendency to metastasize; but they are only antiproliferative [73]. Therefore, these drugs rapidly affect dividing cells, including normal tissues and show dose-limiting toxic effects. Furthermore, in systemic anticancer drug administrations,

enormous toxicity, patient-related factors such as renal and hepatic function, and bone marrow reserve must be considered, as well as the status of general performance assessing medical problems. To improve safety and efficacy, drug targeting at different levels should be also done. Increased delivery of a drug to the body compartment is the first priority; while the second target is to increase drug delivery to tumor cells; and intracellular delivery is the third order of target [73]. First- and second-order targeting is easy to achieve by local delivery of the drugs through systems such as implants, and the like surgical paste, microchips, and the like [77]. The addition of polymers to cancer therapeutics is one of the simple approaches for targeting.

Brain Tumor. Glioblastoma multiforme (GBM) is responsible for 80% of primary brain tumors, usually found in the cerebral hemispheres [78]. Around 17,000 people are diagnosed with primary brain tumors every year in the United States. About half of the primary brain tumors are malignant and about 30% are GBM [79]. The conventional therapy used for tumor includes diagnosis by a surgical biopsy and a surgical debulking of accessible tumors followed by radiation therapy and chemotherapy [80]. Chances of survival are low after surgical resection alone, the additional radiation therapy extends the survival for a further 9 months. The systemic chemotherapy has also been found less effective [81], although neurosurgery and neuroradiation therapy have been improved. The limitations of removal of functional brain tissue and an increased radiation dose or size of the irradiated field cause acute and chronic adverse effects [77]. Hence some other novel approaches to treat the condition are needed. To attain a high intratumoral drug concentration in the brain, without systemic side effects, five potential approaches are suggested. These enhance drug permeability through the blood–brain barrier (BBB), temporary disruption of the BBB, the interstitial delivery of drugs via catheters, enhanced delivery of drugs to the central nervous system (CNS), and the use of polymers or microchips directly to achieve the therapy. Direct localized delivery using polymers is one of the simplest approaches [82]. Some anticancer drugs are not capable of crossing the BBB because of their large size; intolerably high systemic drug levels are required to achieve the therapeutic doses within the CNS [83]. Localized delivery resolves the problem associated with permeability of chemotherapeutic agents through the BBB [84]. Using biodegradable polymers, the loaded drug will release when polymers degrade and preclude the need for removal of the delivery system after exhaustion, which is an important advantage in case of drug delivery to brain. Various polyanhydrides and drugs in combination have been used to obtain the optimum release profile and treat the brain tumor or glioma.

P(CPP-SA)-based delivery systems have been characterized for a variety of drugs and used clinically. Controlled-release systems based on an implantable wafer have been studied with the drugs mitoxantrone, carmustine (BCNU), 4-hydroperoxycyclophosphamide (4-HC), paclitaxel, carboplatin, and adriamycin [85, 86]. The characteristics of drug release from wafers depend

on the polymer and drug used. A typical controlled release curve for BCNU is shown in Figure 10.2 [87].

The linear region in Figure 10.2 indicates that the drug release is diffusion controlled [88]. In drug–polymer systems loaded with a high degree of surface-associated drug, a burst release phase is observed. P(CPP-SA) wafers of BCNU and 4-HC have demonstrated release for 50 days. The release of paclitaxel proceeded at a much lower rate, less than 0.01 mg/day, and for a much longer period of time (160 days). The slower release rate can be attributed to the hydrophobicity of the drug, which causes a strong affinity for the polymer. The controlled release of drugs from these wafers has demonstrated performance in many in vitro and in vivo glioma models. Studies have demonstrated improved performance of these wafers when compared to free drug administration in a rat model challenged with an intracranial 9L tumor [89]. Small cylindrical wafers have been also studied in the same way [85].

Gliadel has been studied for clinical use in initial treatment, treatment of recurrences, and in conjunction with radiotherapy for malignant gliomas [90]. In all cases, therapy with Gliadel was well tolerated with no significant increase in toxicity, infection, or inflammation. The P(CPP-SA) wafer loaded with BCNU has undergone further characterization and is available clinically as Gliadel [91]. The Gliadel wafer is 14 mm in diameter and 1 mm thick. It is

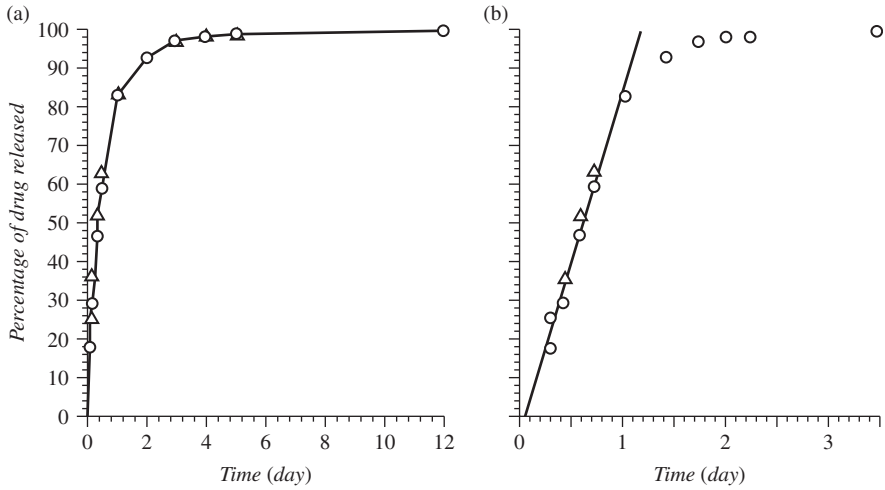


FIGURE 10.2 The cumulative release of BCNU into a well-stirred reservoir as a function of time (a) and the square root of time (b). The linear portion indicated in b is indicative of diffusion controlled release from a planar geometry. Reprinted with permission from Springer Science and Business Media: Fung, L. K. et al. Chemotherapeutic drugs released from polymers: distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat brain. Figure 2. Pharmaceutical Research. 1996;13:671-82 (From [85].)

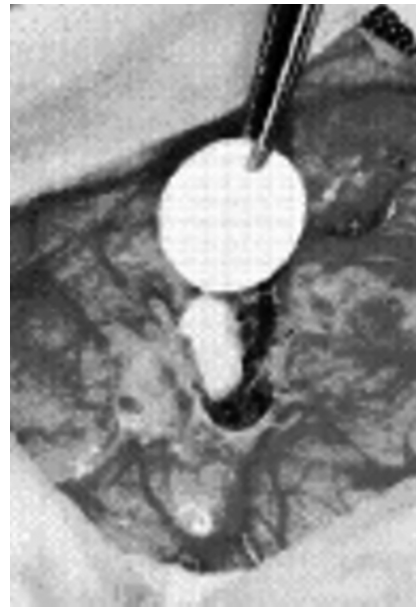


FIGURE 10.3 Intracranial placement of Gliadel® wafer. The wafer is implanted in the cavity left after surgical resection of the tumor. Wafers are dime-sized and impregnated with carmustine. (Reprinted with permission from [88].)

loaded with 7.7 mg of the drug carmustine and implanted intracranially after surgical debulking of the tumor (Fig.10.3). The majority of studies indicated a modest improvement in survival for patients with malignant gliomas that received Gliadel [92]. Further research is directed to improve the Gliadel wafers by examining dosing and combination therapy [93].

Fatty acid dimer copolymers used the same polyanhydride linkages, offering a distinct advantage over carboxyphenoxy propane polymer systems. FAD-SA is typically formed into a disk shape and then implanted intracranially. The primary use of FAD-SA is the controlled delivery of the drug 4-hydroperoxycyclophosphamide (4-HC) [94]. FAD-SA is used for delivery of this drug because 4-HC is hydrolytically unstable in P(CPP-SA) wafers [95]. The ability to tailor the chemical and physical properties of the drug vehicle is one of the advantages of polymeric delivery.

Gliadel. Gliadel is a polyanhydride implantable polymer drug delivery system, containing 3.85% carmustine (BCNU). The polymer is composed of polycarboxyphenoxy propane : sebacic acid in a 20 : 80 copolymer [poly(CPP:SA)20:80]. The final product is formulated as a 200-mg, round disk wafer, 14 mm in diameter by 1 mm thick, which is approximately the same size as a dime. It is a new drug product for the treatment of brain cancer. It is a biodegradable polymer implant,

used in the treatment of brain cancer in over 20 years. Gliadel polymer implants are small, white wafers that are left in the surgical cavities created when a brain tumor is removed. As the wafer slowly erodes in the brain, it releases the cancer chemotherapeutic drug carmustine (BCNU) directly to the tumor site in high concentrations over an extended period of time.

The wafers are placed directly into the brain at the time of surgical removal of a brain tumor, permitting BCNU to be delivered directly at the site of tumor for an extended period of time. Up to 8 wafers can be placed at one time. This results in much higher local concentrations of BCNU than the systemic administration. As a result, antineoplastic activity is significantly increased, and side effects decreased as compared to systemic therapy. Approximately 62 mg of BCNU delivered as Gliadel achieves substantially higher local brain tissue concentrations than a 3000-mg intravenous dose. Gliadel provides brain concentrations of BCNU 100–1000 times higher than the systemic administration. In the current Gliadel formulation, the duration of drug delivery is 2–3 weeks (the ratio of PCPP : SA can be varied to shorten or lengthen the delivery).

Prior to its release, the treatment protocol was tested extensively in several studies worldwide. The first phase III study was in 222 patients (at 27 centers) with malignant glioma undergoing reoperation for recurrent disease; 145 of these patients had glioblastoma multiforme. The primary endpoint of the reoperation trial was survival over 6 months, using a Cox multiple regression statistical analytical model to adjust for prognostic factors. At the 6-month time point, 60% of Gliadel patients were alive as compared with 47% of placebo. These results are statistically significant at $p = 0.01$ adjusted for prognostic factors. Median survival time was increased from 24 weeks with placebo to 32 weeks with Gliadel, statistically significant at $p = 0.05$. Independent statistical analysis, using 45-month survival as the primary endpoint, showed a statistically significant benefit for Gliadel at $p = 0.005$ in a multiple regression analysis. Long-term follow-up through 71 months showed Gliadel to continue to have a statistically significant benefit, $p = 0.05$ (Cox regression model). No serious adverse effects caused by Gliadel were identified. No clinically important adverse events attributable to Gliadel were identified. The results of this clinical trial were published in *The Lancet,* April 22, 1995.

The second placebo-controlled phase III study was conducted in patients undergoing surgery upon initial diagnosis of malignant glioma. This study was conducted in 32 patients in Scandinavian centers. The primary endpoints used to assess efficacy in this study were of one-year survival and overall survival. In this study, median survival was 58 weeks for Gliadel and 40 weeks for placebo; 18-week improvement in the survival was statistically significant to $p = 0.01$. One-year survival was 63% for Gliadel (10/16) and only 19% for placebo (3/16). This was also statistically significant to $p = 0.029$. After accounting for the effects of age, Karnofsky score (a measure of patient performance), and tumor type in a Cox regression model, the effects of Gliadel treatment remained statistically significant ($p = 0.006$). The side effects profile for Gliadel did not statistically differ from placebo.

The targeted treatment of cancer with polymer-based products are important for other cancers beyond brain cancer to treat tumor recurrence after surgical resection. In this endeavor Guilford is also investigating a number of other next-generation polyanhydride polymer oncology products for the use in other cancers. Among other technologies, Guilford is developing a series of polyanhydride polymers in collaboration with The Hebrew University of Jerusalem, invented by Dr. Avi Domb, the original inventor of the P(CPP-SA) polymer used for Gliadel. These polymers include polymers derived from hydrolyzable diacid fats, such as PRAM : SA (polyricinoleic acid maleate : sebacic acid). Guilford plans to develop these polymers with a variety of chemotherapeutic agents for the targeted treatment of other cancers including prostate, breast, head and neck, lung, esophageal, hepatic, pancreatic, colon, and others.

Guilford is also developing other physical formulations of polymers for stereotactic implantation. Such polymer products may potentially be used for the treatment of inoperable tumors, or for reimplantation of a polymer therapeutic after the initial surgery and implant. Such products in the form of a gel or microspheres are implanted by stereotactic injection, without conventional surgery. These stereotactic products will be explored both for brain and other forms of cancer [96, 97]. The complications of using the Gliadel wafer seem to be comparable with those of craniotomy alone. However, increased difficulty with wound healing, and rare and potential fatal incidents of malignant brain edema, as well as of chemical meningitis followed by obstructive hydrocephalus, have been reported. Therefore, the survival benefits of Gliadel placement should be balanced by the clinician with the concern for possible toxicity [98].

The limitations of the BCNU wafers are directly related to the limited penetrance of BCNU, as most of the tumors recur locally, at a short distance from the wafers. Therefore, it is critical to evaluate valid systemic treatments that can both address the local and the distant brain involvement and safely be used in this setting. A number of promising therapies are currently progressing through clinical trials, based on the good safety profile demonstrated in the combinations of different systemic chemotherapeutic agents such as temozolomide, carboplatin, PCV (lomustine/procarbazine/vincristine), and so forth [96].

In conclusion, the unique properties of Gliadel, its lack of systemic side effects, and its documented safety and efficacy justify its use in malignant gliomas and its potential applications in other intracranial tumors. Large-scale trials addressing ways to overcome local resistance to BCNU, as well as multiagent chemotherapy combinations, are currently in different stages. Further work needs to focus on developing better local delivery systems, with improved penetrance in the brain tissue by multifactorial mechanisms of BCNU resistance, and attempting the interstitial delivery of improved chemotherapeutic agents [99].

Frazier et al. studied the efficacy of minocycline local delivery, an antiangiogenic agent, and systemic BCNU on intracranial glioma [100]. Minocycline was incorporated in P(CPP : SA) in a ratio of 50 : 50 by weight and found the

combination of intracranial minocycline and systemic BCNU extended median survival by 82% as compared to BCNU (bis-chloronitrosourea) alone (Pb0.0001) and 200% as compared to no treatment (Pb0.004). Tamargo et al. used a polyanhydride matrix to deliver heparin and cortisone acetate as antineoplastic agents. They have reported the inhibition of 9 l glioma growth and found that in the presence of heparin and cortisone, and of cortisone alone, there was a 4.5- and 2.3-fold reduction, respectively, in the growth of 9 l glioma [101]. Paclitaxel (taxol) is a potent antiangiogenic having molecular weight of 853.9 Da and poor BBB permeability. Hence, the use of the implant for intracerebral delivery was suggested for delivery of taxol [73, 75]. Walter et al. have formulated a polymeric disk of poly(CPP : SA, 20 : 80) with 20–40% of taxol loading and found the taxol was released up to 1000 h, in vitro [102]. In the study the concentration of polyanhydride was maintained to 75–125 ng taxol/mg brain tissue, within a 1–3 mm radius of the disk and concluded that taxol shows promising results in malignant glioma when delivered via the polyanhydride system [102]. Another polyanhydride system for delivery of taxol has been formulated using poly(FAD : SA, 50 : 50). But due to the hydrophobic nature of the FAD, the release rate was very slow and therapeutic concentration could not be achieved [5]. 4-HC, a hydrophilic derivative of cyclophosphamide, with or without *T*-buthionine sulfoxine was incorporated in poly(FAD : SA) and found to be effective in rat intracranial 9 L gliosarcoma and F98 glioma model [103]. Fluorodeoxyuridine, an antimetabolite, has been optimally released from poly(FAD-SA) in vitro and in vivo [104]. Aldriamycin incorporated in poly (CPP: SA) has shown improved (Fig. 10.4) median survival in rat intracranial 91 glioma model [96]. Williams et al. [105] have incorporated 5-iodo-2V deoxyuridine (IUdR) for radiosensitization of experimental human malignant gliomas. After the implantation of 50% IUdR-P (CPP : SA, 20 : 80) either contralateral to the tumor or inside the tumors, followed by radiation, tumor regression, growth delays to 14 + 3.6 or 24.2 + 0.2 (Pb0.01) days, respectively. The results evidenced that the biodegradable polyanhydride holds promise for the controlled release and local delivery for gliomas radiosensitization. Methotrexate (MTX), a folate antagonist used against several types of malignancies, shows lack of permeability across the BBB and systemic side effects, including myelosupression and gastrointestinal necrosis [106]. MTX–dextran conjugate on incorporation in poly(FAD : SA) offered significant improvement over controls in rat intracranial 9 L glioma [96].

A platinum analog hydrophilic carboplatin was optimally released by the polymer and found to be effective in rat intracranial F98 glioma model [107]. Na-camptothecin, a topoisomerase inhibitor, has been incorporated into poly (CPP : SA) and tested in rat intracranial 9 l glioma model. The median survival time with 50% (w/w) camptothecin-loaded polymers was 69 days as compared to 17 days in the control animals (Pb0.0001), and in vitro release of intact camptothecin was 1000 h [108]. An immunotherapeutic local delivery to combat malignant gliomas mainly involves the use of cytokines. Several experiments have recently examined the interaction between local paracrine IL-2 transduced cells using mouse intracranial F16-B10 melanoma model. It

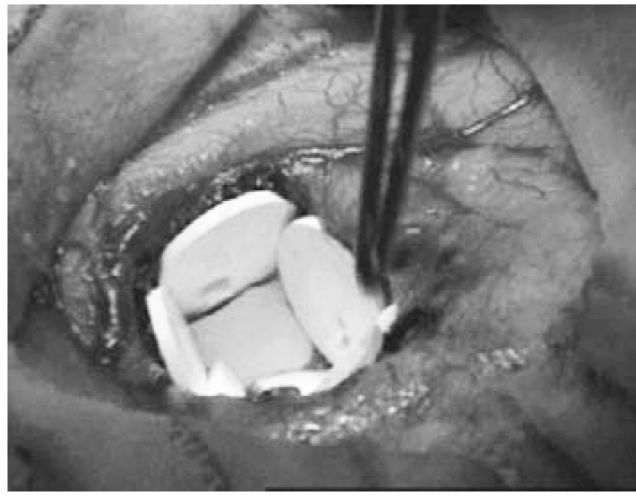


FIGURE 10.4 Gliadel wafer implantation at tumor resection site. Around 7–8 wafers are placed for localized delivery of BCNU via P(CPP:SA) polymer. (From [107].)

shows 70% of animals receiving therapy survived N72 days as compared to none in controls (Pb0.01).

Head and Neck Cancer. Head and neck cancer, also called squamous cell carcinoma (SCC) of head and neck (SCCHN) is one of the frequently occurring cancers in the world [109]. Chemotherapeutic treatment for SCCHN is limited because of its systemic toxicity. Shikani et al. have used poly(FAD : SA) incorporated with 5 and 7% w/w cisplatin. In vivo testing was performed on a nude mouse carrying human floor-of-mouth squamous cell carcinoma xenografts, as an animal model. After 70 days of implantation, the treated tumor size was found to be 41.4% of control in the 5% group and 38.1% in the 7% group, indicating a significant delay of tumor growth as compared to control or intraperitoneally injected cisplatin [109]. Recently, Li et al. have incorporated cisplatin in poly (ester-anhydride) prepared by insertion of ricinoleic acid in poly(sebacic acid) and reported the total drug release after 400 h [110]. This high sensitivity of all three head and neck SCCs is of interest since these types of tumors clinically require very large doses for chemotherapy to be effective, which is associated with substantial systemic toxicity. Polymers do offer the advantage of delivering high concentrations of anticancer agents directly to the tumor target in a sustained and continuous fashion, minimizing toxicity. The potential applications of polymer chemotherapy for the head and neck area are multiple. After resection of SCC, the surgeon frequently finds a situation where part of the tumor is judged to be unresectable (e.g., because of involvement of the carotid artery, skull base, prevertebral fascia, or the like). In these cases, implanting polymers in proximity of the tumor may

provide palliation to these patients, without the toxicity of systemic chemotherapy [111].

Patients who present with locoregional cancer recurrence despite aggressive therapy, including surgery and maximal doses of external irradiation, may also benefit from polymer implantation. In these patients, microspheric polymers may be injected transcutaneously into and around the tumor stroma. Moreover, multiple anticancer drugs may be delivered through polymers (either simultaneously or sequentially), simulating clinical combination chemotherapy protocols. These injections may be performed repeatedly. An additional application of polymer chemotherapy includes prophylaxis against tumor recurrence following resection. Viable SCC cells have been recovered from the surgical wound following neck dissection and were shown to be capable of growing as colonies in vitro; theoretically, these may implant and cause cancer recurrence [111]. MTX, an effective treatment for head and neck cancer, has been incorporated in RA-based polyanhydride as a model drug [53]. The drug release followed first-order kinetics and the initial molecular weight of the polymer did not affect the release rate also. It indicates that these carriers are used effectively for cancer treatment.

10.6.2 Osteomyelitis

Osteomyelitis is a bone infection usually caused by bacteria and rarely by fungi. This bone infection can occur after hip or knee replacement surgery [102]. Bone infection can also be caused by an adjacent soft tissue infection that has been infected from other parts of the body through the blood. The treatment of bone infection mainly involves operative debridement, removal of foreign bodies, and antibiotic therapy [112]. However, osteomyelitis is a complicated infection to treat because a sufficient concentration of antibiotic at the site of infection by systemic administration is not reached. A high parenteral dose of antibiotic is required for the effective therapeutic drug levels in the bone. In addition, the prolonged course of treatment can lead to a systemic toxicity of the antibiotic. Besides, the short half-life of antibiotics and the poor blood circulation to the infected area makes treatment difficult by systemic therapy. Management of chronic osteomyelitis using local antibiotic delivery is a novel therapeutic modality, which achieves elevated antibiotic concentrations at the site of the infection without systemic toxicity. The methods of local delivery generally involve the use of nondegradable drug carriers. For example, PMMA-gentamicin, CHAS antibiotics, and calcium hydroxyapatite ceramic (CHAC) and apatite-wollastonite containing glass ceramics (AWGC) antibiotics. But the problem of these delivery systems is that they are not biodegradable and have to be removed finally [54].

Polyanhydride has been evaluated for local delivery of antibiotics in the treatment of osteomyelitis [4, 113]. Septacin is a polyanhydride implant of a copolymer of Erucic acid dimer (EAD) and sebacic acid in a 1 : 1 weight ratio developed for osteomyelitis. It is a controlled-release implant containing gentamicin sulfate dispersed into a polyanhydride polymer matrix. Sebacic acid is the

hydrophilic monomer while EAD forms the hydrophobic component. The release of gentamicin was observed for a period of about 3 weeks. No residual drug is detected in the polymer remnants 8 weeks postimplantation. In vivo drug release from Septacin in rats showed that gentamicin plasma levels were extremely lowered, indicating the low systemic exposure to gentamicin Figure 10.5. Septacin samples have also demonstrated efficacy in the rat skin abscess and horse-joint infection models. Results from the human in vivo study also showed high local drug concentrations at implant sites while systemic exposure to the drug was minimal [112]. The drug in the polymer matrix was stable for at least 12 months when stored at 25.8°C, but the molecular weight of the copolymer declined rapidly at this temperature [113].

Recently, another drug carrier using polyanhydride, poly (oleic/linoleic acid dimer: sebacic acid) poly(OAD/LOAD:SA) was synthesized and mixed with 20% gentamicin to get poly(OAD/LOAD:SA) gentamicin beads. These beads were shown to release gentamicin over 7 weeks and indicated its usefulness to treat chronic osteomyelitis [114].

Laboratory-scale injection-molding equipment was utilized to fabricate an implant consisting of poly(FAD : SA, 1 : 1) and 20% (w/w) gentamicin sulfate. Characterizations were performed to determine the molecular weight and glass transition temperature of poly (FAD : SA, 1 : 1). A study was carried out to investigate the relationships between the in vitro performance, morphology, and microstructures of the molded implants. It was found that implants produced with different structures exhibited different physical integrities in water, that is, cracking or noncracking. For the noncracking implants, a skin-core structure formed by an oriented skin layer was observed under a polarized light microscope. The same morphology was not seen in the cracking implants. The crystal orientation in the skin layer of the noncracking implants was further identified using a wide-angle X-ray diffraction method (WAXD). No crystal orientation could be found in the cracking implants by WAXD. Furthermore, studies were carried out to evaluate the in vitro drug release for implants showing different degrees of integrity in water. The in vitro drug

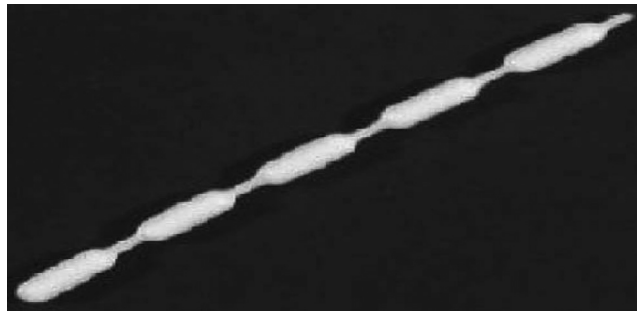


FIGURE 10.5 Septacink beads. (From [112].)

release of the cracking implants was markedly faster than the noncracking implants due to the pronounced initial drug-burst effect as a result of crack formation in the implants.

10.6.3 Local Anesthesia

Local anesthesia has wide applications in various clinical areas involving acute or chronic pain. It is useful to deal with the debilitating postsurgical pain requiring analgesic medications for 3–5 days after surgery. The desired period of nerve blockade varied from as little as one day to as long as one week. A long-acting local anesthetic solution provides local analgesia for only 4–9 h. Therefore, to provide regional blockade for more than 1 day, a drug delivery system is required that releases slowly a fixed amount of local anesthesia at the surgical site for a prolonged period of time. Therefore, controlled-release devices based on poly(FAD-SA) were prepared. The study was conducted with rectangular devices consisting of bupivacaine HCl dispersed homogenously in the polymer at 10% w/w of drug loading. The drug released from the copolyanhydrides of the fatty acid dimer and sebacic acid primarily by surface erosion and followed the first-order release profiles. The preknown erosion rate of the fatty-acid-dimer-based polyanhydride achieved the desired drug release kinetics by altering the geometry of the device [45].

Another polyanhydride matrix has been successfully used for sustained release of local anesthetics such as dibucaine free base, dibucaine HCl, and bupivacaine HCl. The drugs were incorporated into polyanhydride copolymer poly(CPP-SA) matrices (1 : 4). Local anesthetics were released in a sustained manner yielding 90% drug release over a period of 3–14 days. Local anesthetic containing a polyanhydride matrix device on implantation near the sciatic nerve in rats, a reversible neural blockade was observed for 4 days. The study suggested this technology could lead to prolonged blockade of peripheral nerves [115].

10.6.4 Gene Therapy

In gene therapy, a sustained DNA (deoxyribonucleic acid) delivery is very useful with control of the wide range of DNA release profiles. Photocrosslinked polyanhydrides allowed repeated transfection, with an appropriate amount of DNA for controlled release. In a study, multifunctional anhydride monomers (methacrylated end groups) were photocrosslinked and its suitability for DNA encapsulation and delivery was demonstrated. The polymer showed surface erosion, suggesting the polymers could deliver molecules of a wide range of sizes at a sustained rate, useful for the high-molecular-weight DNA delivery. Previous reports on DNA delivery showed that photopolymerization was compatible with DNA encapsulation and the protective agent used reduced the damaging effects of photoinitiated radicals. To improve the DNA recovery and reduce the damaging effects of polymer degradation,

DNA was preencapsulated in alginate microparticles to serve as a temporary coating that quickly dissolved upon microparticle release from the polyanhydride matrix. The results suggested that the reliability of plasmid DNA is maintained on exposure to ultraviolet (UV) light under the photopolymerizing condition. The photocrosslinked polyanhydrides have highly predictable drug release profiles, depending on the polymer erosion rates and implant geometry [116].

10.6.5 Restenosis

Angioplasty, athrectomy, or stenting procedures cause an adhesion of inflammatory cells at the injury site, and these cells have migrated into the arterial wall [4, 117]. Insertion of a foreign body, such as a stent, aggravates this inflammatory response. Furthermore, elastic recoil, smooth muscle cell migration, and proliferation enhance the extracellular matrix synthesis, vessel wall remodeling, and thrombus formation [4, 118]. Ultimately, all these conditions result in the reobstruction of the artery [119]. To block the initial events in pathogenesis of restenosis, conventional therapeutic approaches involve the inhibition of platelet deposition and thrombus formation. It is achieved by using anticoagulants, anti-inflammatory agents, and antiplatelet agents, whereas the proliferation of smooth muscle cells and matrix formation are to be prevented by using antiproliferative agents [120].

Prevention of restenosis by systemic delivery of pharmacologically active compounds is not effective because of many reasons. It includes drug inactivation during absorption, patient inability to tolerate the high systemic concentrations of active agents required to achieve therapeutic levels at the desired site, and unwanted side effects on organs and tissue that are not directly involved in the pathology of restenosis [121]. During the clinical trial of localized high dose, unfractioned heparin showed that the hourly dose of heparin needs to be reduced by 10-fold [122] than what is demonstrated in the rat carotid model of angioplasty.

Local delivery of the active agent is found to be beneficial due to the localized nature of restenosis and other cardiovascular diseases. Different strategies have been tried by various workers to deliver the drug locally [123]. One of the simple and commonly used approaches is to implant the drug-loaded polymer around the area of the interventional procedure so that the drug can be released at a predetermined rate. In this process, a biocompatible and biodegradable polymer is needed that is eliminated from the body within 6 months of implantation. All these criteria are fulfilled by several polymers such as collagen, copolymers of lactide and glycolide, and polyanhydrides [19]. Poly(FAD : SA) has been applied perivascularly to release heparin to microvascular anastomoses [124]. The vessel patency rates were found significantly greater in polyanhydride-heparin-treated vessels as compared to controls at 24 h and 7 days after surgery [125]. PLA has been used to coat the poly(FAD : SA) sheets to improve the release profile and strength of the films. Animal studies of the heparin-releasing devices showed a

significant reduction of the internal artery diameter in the control group as compare to the treated group [120].

10.6.6 Glaucoma and Other Eye Disorders

A successful surgery of glaucoma filtration can be prolonged by administering agents with antifibrotic activity. Lee et al. investigated that fluorouracil 20% by weight was incorporated in a poly(CPP : SA) disk and tested on 18 rabbits in a prospective, randomized, double-masked, and placebo-controlled fashion. The results showed an intraocular pressure (IOP) was lowered in the experimental eyes during the 5th–17th postoperative day. But, finally, both experimental and control eyes returned to preoperative levels, and filtration surgery failed in both the experimental and control rabbit eyes [125]. Jampel et al. incorporated 5-fluorouridine in a polyanhydride disk and reported the success of filtration surgery in glaucomatus monkeys was significantly longer in the eye that received polyanhydride with 5-fluorouridine (mean $\pm$ 26.0 $\pm$ 9.2 days) than in control (8.5 $\pm$ 4.0 days) [125]. Jampel et al. have also tried taxol and etoposide in a polyanhydride disk but only taxol had shown remarkable benefits on intraocular pressure and belb appearance. Taxol–polymer disks had low IOP than control eyes from 20 days after experimental filtration surgery until death in cynomologus monkey eyes [6]. Uppal et al. have studied pharmacokinetics of etoposide contained in a poly(CPP : SA) disk, after glaucoma filtration surgery. The release of etoposide from the implant was nearly over time, at 30 Ag/day completed except for a burst between days 6 and 7. After the 12th postoperative day 92% of etoposide was released. Drug released in aqueous humor, other ocular tissue, and in the contralateral eye were negligible [126]. Polyanhydride microspheres have been used to deliver the drug in a controlled manner in vitreoretinal disorders, to achieve intraocular drug levels within the therapeutic range [127].

10.6.7 Miscellaneous Applications

Polyanhydrides have been also used for many other applications. Delivery of proteins and peptides macromolecules via polymer is an important issue. Polyanhydride matrices are used for a controlled delivery of proteins or polymer–drug conjugates [128]. Fluorescent-labeled, macrosized dextran release can be controlled from a polyanhydride matrix by adjusting the particles size dispersed in the matrix [42]. According to Hanes et al., poly(anhydride-*co*-imides) have an erosion mechanism that leads to predictable drug release. They used bovine serum albumin as a model compound and suggested that the polymer may be appropriate for delivery of many therapeutic proteins, including vaccine antigens [129]. Moreover, stability and activity of proteins and peptides can also be maintained using polyanhydride carrier [41]. Lucas et al. reported water-soluble protein possessing chondrogenic stimulating activity incorporated in polyanhydride polymeric vehicles. The delivery system was capable of inducing cartilage and bone up to 50% of the time. It was concluded that polyanhydride could be

used as a controlled-release delivery vehicle for soluble bioactive factors that interacts with local cell populations [130]. PSA-*b*-PEG and poly(TMA-glycine-*co*-SA)-*b*-PEG were used as isolating layers for their good processing properties at room temperature. These polymers were used in protein delivery due to their pH sensitivity and appropriate erosion duration [131]. Kubek et al. incorporated neuropeptide TRH (protirelin) in polyanhydride matrix for anticonvulsant effects. Rat kindeling model was assessed for a number of stimulations required to reach each behavioral stage and become fully kindled [132]. The report provides evidence in support of in situ pharmacotherapy for potential delivery in intractable epilepsy and possibly other neurological disorders. Recently, Allard et al. reported a process for an efficient immobilization of proteins onto copolymers with optimal biological activity and for the controlled production of active bioconjugates [133]. Cai et al. have synthesized a novel polyanhydride, P[(CBF)-ASA] poly(5-carboxybutylformamide)-2-acetyl salicylic anhydride, with 5-ASA incorporated in the backbone. They found the potential of colon-specific 5-ASA delivery and degradation characteristics [134]. Using a sebacic acid copolymer, localized intracerebral neurotransmitter delivery has also been tried and found that intracerebral polymeric drug delivery successfully reversed lesion-induced memory deficit and can potentially be used in the treatment for Alzheimer's disease and other neurological disorders [135].

The use of polymeric materials for the administration of pharmaceuticals and in biomedical devices has been increased significantly. Important applications of biodegradable polymers are in controlled drug delivery systems [135, 136] and in the form of implants and devices for fracture repairs [137]. Ligament reconstruction [138], surgical dressings [139], dental repairs, artificial heart valves, contact lenses, cardiac pacemakers, vascular grafts [140], tracheal replacements [141], and organ regeneration are some of the devices used [142]. Thus, widening the scope of these polymers from practical and clinical usability points of view is necessary. Overall these polymers have remarkable possibilities for use in drug delivery and other implantation purposes.

REFERENCES

1. Langer, R. New methods of drug delivery. *Science*, 1990;**249**(4976):1527–1533.
2. Brem, H. Polymers to treat brain tumours. *Biomaterials* 1990;**11**(9):699–701.
3. Kavanagh, C. A., Y. A. Rochev, W. M. Gallagher, K. A. Dawson, and A. K. Keenan. Local drug delivery in restenosis injury: Thermoresponsive co-polymers as potential drug delivery systems. *Pharmacol. Ther. (Oxf.)*, 2004;**102**(1):1–15.
4. Stephens, D., L. Li, D. Robinson, S. Chen, H. Chang, R. M. Liu, Y. Tian, E. J. Ginsburg, X. Gao, and T. Stultz. Investigation of the in vitro release of gentamicin from a polyanhydride matrix *J. Control. Release* 2000:**63**(3):305–317.
5. Park, E. S., M. Maniar, and J. C. Shah. Biodegradable polyanhydride devices of cefazolin sodium, bupivacaine, and taxol for local drug delivery: Preparation, and kinetics and mechanism of in vitro release. *J. Control. Release* 1998;**52**: (1–2):179–189.

6. Jampel, H. D., D. Thibault, K. W. Leong, P. Uppal, and H. A. Quigley. Glaucoma filtration surgery in nonhuman primates using taxol and etoposide in polyanhydride carriers. *Invest. Ophthalmol. Visual Sci.* 1993;**34**(11): 3076–3083.
7. Dhanikula, A. B. and R. Panchagnula. Localized paclitaxel delivery. *Int. J. Pharm.* 1999;**183**(2):85–100.
8. Surmodics. Enabling local drug delivery/implant device combination therapies. www.surmodics.com; accessed on July 15, 2004.
9. Langer, R. Drug delivery and targeting. *Nature* 1998;**392**(6679 Suppl.):5–10.
10. Angelova, N., and D. Hunkeler. Rationalizing the design of polymeric biomaterials. *Tech. Innov. Berufl. Bild.* 1999;**17**:411–421.
11. Uhrich, K. E., S. M. Cannizzaro, R. S. Langer, and K. M. Shakesheff. Polymeric systems for controlled drug release. *Chem. Rev.* 1999;**99**:3181–3198.
12. Domb, A. J., M. Maniar, and A. S. T. Haffer. Biodegradable polymer blends for drug delivery. U.S. Patent 5, 919, 835, 1999.
13. Kumar, N. R. S Langer, and A. J. Domb. Polyanhydrides: An overview. Adv. Drug Deliv. Rev. 2002;**54**(7):889–910.
14. Jain, J. P., S. Modi, A. J. Domb, and N. Kumar. Role of polyanhydrides as localized drug carriers *J. Control. Release* 2005;**103**:541–563.
15. Qiu, L. In vivo degradation and tissue compatibility of polyphosphazene blend films. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 2002;**19**:191–205.
16. Domb, A. J. and M. Maniar. Absorbable biopolymers derived from dimer fatty acids. *J. Polym. Sci: Part A: Polym. Chem.* 1993;**31**:1275–1285.
17. Domb, A. J. R. Nudelman. Biodegradable polymers derived from natural fatty acids. *J. Polym. Sci., Part A: Polym. Chem.* 1995;**33**:717–725.
18. Kumar, N., M. Krishnan, T. Azzam, A. Magora, M. N. V. Ravikumar, and D. R. Flanagan.et al. Analysis of fatty acid anhydrides and polyanhydrides. *Anal. Chim. Acta.* 2002;**465**:257–272.
19. Domb, A. J. Polymers for site-specific drug delivery. In *Polymeric Site-Specific Pharmacotherapy*. A. J. Domb. (Eds.). Wiley: Chichester, 1994, pp. 1–26.
20. Chasin, M., D. Lewis, and R. Langer. Polyanhydrides for con trolled drug delivery, *Biopharm. Manuf.* 1988;**1**:33–35, see also pp. 38–40, 46.
21. Mader, K., G. Bacic, A. Domb, and H. M. Swartz. Characterization of microstructures in drug delivery systems by EPR polyspectroscopy. *Proc. Int. Symp. Controll Release Bioact. Mater.* 1995;**22**:780–781.
22. Brem, H., R. J. Tamargo, and A. Olivi. Delivery of drugs to the brain by use of a unstained-release polyanhydride polymer system. *New Technol. Concepts Reducing Drug Toxic.* 1993; 33–39.
23. Bucher, J. E. and W. C. Slade. The anhydrides of isophthalic and terephthalic acids. *J. Am. Chem. Soc.* 1909;**31**:1319–1321.
24. Hill, J. W. Studies on polymerization and ring formation. VI. Adipic anhydride. *J. Am. Chem. Soc.* 1930;**52**:4110–4114.
25. Hill, J. W. and H. W. Carothers. Studies on polymerization and ring formation. XIV. A linear superpolyanhydride and a cyclic macrodimeric anhydride from sebacic acid. *J. Am. Chem. Soc.*, 1932;**54**:5169.

26. Conix, A. Aromatic poly(anhydrides): A new class of high fiber-forming polymers, *J. Polym. Sci. Part A* 1958;**29**:343–353.
27. Yoda, N. Synthesis of poly(anhydrides). Poly (anhydrides) of five-membered heterocyclic dibasic acids. *Makromol. Chem.* 1962;**55**:174–190.
28. Yoda, N. Synthesis of poly(anhydrides). Crystalline and high poly(amide) poly (anhydrides) of methylene bis(*p*-carboxyphenyl) amide. *J. Polym. Sci. Part A* 1963;**1**:1323–1338.
29. Rosen, H. B., J. Chang, G. E. Wnek, R. J. Linhardt, and R. Langer. Bioerodible polyanhydrides for controlled drug delivery. *Biomaterials*, 1983;**4**:131–133.
30. Leong, K. W., J. Kost, E. Mathiowitz, and R. Langer. Polyanhydrides for controlled release. *Biomaterials* 1986;**7**:364–371.
31. Leong, K. W., P. D'Amore, M. Marletta, and R. Langer. Bioerodible polyanhydrides as drug-carrier matrices. II. Biocompatibility and chemical reactivity, *J. Biomed. Mater. Res.* 1986;**20**:51–64.
32. Chasin, M., A. Domb, E. Ron, E. Mathiowitz, R. Langer, K. Leong, C. Laurencin, H. Brem, and S. Grossman. Polyanhydrides as drug delivery systems, *Drugs Pharm. Sci.* 1990;**45**:43–70.
33. Tamargo, R. J., J. I. Epstein, C. S. Reinhard, M. Chasin, and H. Brem. Brain biocompatibility of a biodegradable controlled-release polymer in rats. *J. Biomed. Mater. Res.* 1989;**23**:253–266
34. Dang, W. B., T. Daviau, and H. Brem. Morphological characterization of polyanhydride biodegradable implant Gliadel® during in vitro and in vivo erosion using scanning electron microscopy. *Pharm. Res.* 1996;**13**:683–691.
35. Uhrich, K.E., A. Gupta, T. T. Thomas, C. T. Laurencin, and R. Langer. Synthesis and characterization of degradable poly-(anhydride-*co*-imides). *Macromolecules*, 1995;**28**, 2184–2193.
36. Uhrich, K. E., T. T. Thomas, C. T. Laurencin, and R. Langer. In vitro degradation characteristics of poly(anhydride-*co*-imides) containing trimellityimidoglycine. *J. Appl. Polym. Sci.* 1997;**63**:1401–1411.
37. Young, J. S., K. D. Gonzales, and K. S. Anseth. Photopolymers in orthopedics: Characterization of novel crosslinked anhydrides. *Biomaterials*. 2000;**110**: 1181–1188.
38. Muggli, D. S., A. K. Burkoth, and K. S. Anseth. Crosslinked poly anhydrides for use in orthopedic applications: Degradation, behavior and mechanics. *J. Biomed. Mater. Res.* 1999;**46**:271–278.
39. Jiang, H. L.K. J. Zhu. Preparation, characterization and degradation characteristics of polyanhydrides containing poly(ethylene glycol). *Polym. Int.* 1999;**48**: 47–52.
40. Linhardt, R. J., H. B. Rosen, and R. Langer. Biodegradable polyanhydrides for controlled drug delivery. *Polym. Prepr.* 1983;**24**:47–48.
41. Tabata, Y., S. Gutta, and R. Langer. Controlled delivery systems for proteins using polyanhydride microspheres. *Pharm. Res.* 1993;**10**:487–496.
42. Dang, W. B., and W. M. Saltzman. Controlled-release of molecules from a degradable polyanhydride matrix. *J. Biomater. Sci. Polym. Ed.* 1994**6**:297–311.
43. Mahoney, M. J. and W. M. Saltzman. Controlled release of proteins to tissue transplants for the treatment of neurodegenerative disorders. *J. Pharm. Sci.*,1996;**85**:1276–1281.

44. Guilford Pharmaceuticals, About Gliadel® wafer. www.gliadel.com; accessed on July 10, 2004.
45. Gopferich, A. and J. Tessmar. Polyanhydride degradation and erosion. *Adv. Drug Deliv. Rev.* 2002;**54**(7):911–931.
46. Domb, A. J., R. Langer, and I. Polyanhydrides. Preparation of high molecular weight polyanhydrides. *J. Polym. Sci A, Polym. Chem.* 1987;**25**:3373–3386.
47. Domb, A.J. and R. Langer Poly(anhydrides). 3. Poly(anhydrides) based on aliphatic-aromatic diacids. *Macromolecules.* 1989;**22**:3200–3204.
48. D'Emanuele, A., J. Hill, J. A. Tamada, A. J. Domb, and R. Langer. Molecular weight changes in polymer erosion. *Pharm. Res.*, 1992;**9**(10):1279–1283.
49. Mathiowitz, E., W. M. Saltzman, A. Domb, P. Dor, and R. Langer. Polyanhydride microspheres as drug carriers. II. Microencapsulation by solvent removal. *J. Appl. Polym Sci.* 1988;**35**:755–774.
50. Wang, P. P., J. Frazier, H. Brem. Local drug delivery to the brain. *Adv. Drug Deliv. Rev.* 2002;**54**(7):987–1013.
51. Deng, J. S., L. Li, D. Stephens, Y. Tian, F. W. Harris, and S. Z. Cheng. Effect of gamma-radiation on a polyanhydride implant containing gentamicin sulfate. *Int. J. Pharm.* 2002;**232**(1–2): 1–10.
52. Domb, A. J., E. Mathiowitz, E. Ron, S. Giannos, and L. Langer. Polyanhydrides. IV. Unsaturated and crosslinked polyanhydrides. *J. Polym. Sci. A, Polym. Chem.* 1991;**29**:571–579.
53. Teomim, D., A. Nyska, and A. J. Domb. Ricinoleic acid-based biopolymers. *J. Biomed. Mater. Res.* 1999;**45**(3):258–267.
54. Teomim, D. and A. J. Domb. Nonlinear fatty acid terminated polyanhydrides. *Biomacromolecules.* 2001;**2**(1):37–44.
55. Krasko, M. Y., A. Shikanov, N. Kumar, and A. J. Domb. Polyanhydrides with hydrophobic terminals. *Polym. Adv. Technol.* 2002;**13**:960–968.
56. Kipper, M. J., S. Seifert, P. Thiyagarajan, and B. Narasimhan. Understanding polyanhydride blend phase behavior using scattering, microscopy, and molecular simulations. *Polymer.* 2004;**45**(10):3329–3340.
57. Silviniak, R. and A. J. Domb. Stereocomplexes of enantiomeric lactic acid and sebacic acid ester-anhydride triblock copolymers. *Biomacromolecules.* 2002;**3**:2002, 754–760.
58. Leong, K. W., A. Domb, and R. Langer. Polyanhydrides. In *Encyclopedia of Polymer Science and Technology*. H. F. Mark, and J. I. Kroschwitz, (eds.), Wiley: New York, 1989, p. 648.
59. Krasko, M. Y., A. Shikanov, A. Ezra, and A. J. Domb. Poly(ester anhydride)s prepared by the insertion of ricinoleic acid into poly(sebacic acid). *J. Polym. Sci. Polym. Chem.* 2003;**41**(8):1059–1069.
60. Shikanov A., S. Shikanov, B. Vaisman, J. Golenser, and A. J. Domb. Cisplatin Tumor Biodistribution and Efficacy after Intratumoral Injection of a Biodegradable Extended Release Implant, *Chemotherapy Research and Practice*, 2011;**2011**:9 Pages, doi:10.1155/2011/175054.
61. Domb, A. J. and M. Maniar. Fatty acid terminatedpolyanhydride., U.S. Patent 5,317,079, 1994.
62. Shikanov, A., A. Ezra, and A. J. Domb. Poly(sebacic acid-coricinoleic acid) biodegradable carrier for paclitaxel—effect of additives. *J. Control. Release* 2005;**105**:52–67.

63. Shikanov, A. and A. J. Domb. Poly(sebacic acid-co-ricinoleic acid) biodegradable injectable in situ gelling polymer. *Biomacromolecules* 2006;**7**:288–296.
64. Slivniak, R. and A. J. Domb. Macrolactones and polyestersfrom ricinoleic acid. *Biomacromolecules* 2005;**6**:1679–1688.
65. Slivniak, R., A. Ezra, and A. J. Domb. Hydrolytic degradation and drug release of ricinoleic acid-lactic acid copolyesters. *Pharm. Res.* 2006;**23**:1306–12.
66. Sokolsky-Papkov, M. A. Shikanov, N. Kumar, B. Vaisman, and A. J. Domb. Fatty acid based biodegradable polymers—synthesis and applications. *Bull. Israel Chemi. Soc.* 2008; Issue 2008;**23**:12–17.
67. Domb, A. J., M. Rock, C. Perkin, G. Yipchuck, B. Broxup, and J. G. Villemure. *Biomaterials* 1995;**16**:1069–1072.
68. Shikanov, A., B. Vaisman, M. Y. Krasko, A. Nyska, and A. J. Domb. *J. Biomed. Mater. Res.* 2004;**69A**:47–54.
69. Hakkarainen, M. *Degradable Aliphatic Polyesters* 2002;**157**:113–138.
70. Katti, D. S., S. Lakshmi, R. Langer, and C. T. Laurencin. Toxicity, biodegradation and elimination of polyanhydrides. *Adv. Drug Deliv. Rev.* 2002;**54**(7):933–961.
71. Giralami, U. D., D. C. Anthony, and M. P. Frasch. The central nervous system. In Robins, Pathologic Basis of Disease. R.S. Cotran, V. Kumar, and T. Collins (Eds). Saunders. Philadelphia, 2000, pp. 1293–1357.
72. Dhanikula, A. B. and R. Panchagnula. Localized paclitaxel delivery. *Int. J. Pharm.* 1999; **183**(2):85–100.
73. Calabresi, P. and B. A. Chabner. Chemotherapy of neoplastic diseases. In Goodman and Gilman's the Pharmacological Basis of Therapeutics. J. G. Hardman, and L. E. Limbrid (Eds.). McGraw-Hill: New York, 2001, pp. 1381–1388.
74. Singla, A. K., A. Garg, and D. Aggarwal. Paclitaxel and its formulations. *Int. J. Pharm.* 2002;**235**:(1–2):179–192.
75. Moses, M.A., H. Brem, and R. Langer. Advancing the field of drug delivery: Taking aim at cancer. *Cancer Cell Int.* 2003;**4** (5):337–341.
76. Senior, K. Glioblastoma: Encouraging the body to fight back, *PSTT.* 2000;**3** (6):189–190.
77. Wang, P. P., J. Frazier, and H. Brem. Local drug delivery to the brain. *Adv. Drug Deliv. Rev.* 2002;**54**(7):987–1013.
78. Rostomily, R. C., G. E. Keles, and M. S. Berger. Radical surgery in the management of low-grade and high-grade gliomas. *Bailliere's Clin. Neurol.* 1996;**5**(2):345–369.
79. Chang, C. H., J. Horton, D. Schoenfeld, O. Salazer, R. Perez-Tamayo, S. Kramer, A. Weinstein, J. S. Nelson, and Y. Tsukada. Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. A joint radiation therapy oncology group and eastern cooperative oncology group study, *Cancer.* 1983;**52** (6):997–1007.
80. Barker II, F. G., S. M. Chang, P. H. Gutin, M. K. Malec, M. W. McDermott, M. D. Prados, and C. B. Wilson. Survival and functional status after resection of recurrent glioblastoma multiforme. *Neurosurgery* 1998;**42**(4):709–720.
81. Mohan, D. S., J. H. Suh, J. L. Phan, P. A. Kupelian, B. H. Cohen, and G. H. Barnett. Outcome in elderly patients undergoing definitive surgery and

radiation therapy for supratentorial glioblastoma multiforme at a tertiary care institution. *Int. J. Radiat. Oncol. Biol. Phys.* 1998;**42**(5):981–987.

82. Saltzman W. M. In *Drug Delivery: Engineering Principles for Drug Therapy*. K. E. Gubbins(Ed). Oxford University Press: New York: 2001.
83. DiMeco, F., K. W. Li, B. M. Tyler, A. S. Wolf, H. Brem, and A. Olivi. Local delivery of mitoxantrone for the treatment of malignant brain tumors in rats. *J. Neurosurg.* 2002;**97**(5):1173–1178.
84. Fung, L. K., M. G. Ewend, A. Sills, E. P. Sipos, R. Thompson, M. L. Watts, O. M. Colvin, H. Bre, and W. M. Saltzman. Pharmacokinetics of interstitial delivery of carmustine, 4 hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer implant in the monkey brain. *Cancer Res.* 1998;**58**(4): 672–684.
85. Fung, L. K., M. Shin, B. Tyler, H. Brem, and W. M. Saltzman. Chemotherapeutic drugs released from polymers: Distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat brain. *Pharm Res.* 1996;**13**(5):671–682.
86. Buahin, K. G. and H. Brem. Interstitial chemotherapy of experimental brain tumors: Comparison of intratumoral injection versus polymeric controlled release. *J. Neurooncol.* 1995;**26**(2):103–110.
87. Fleming, A. B. and W. M. Saltzman. Pharmacokinetics of the carmustine implant. *Clin. Pharmacokinet.* 2002;**41**(6):403–419.
88. Westphal, M., D. C. Hilt, E. Bortey, P. Delavault, R. Olivares, P. C. Warnke, I. R. Whittle, J. Jaaskelainen, and Z. Ram. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-Oncol.* 2003;**5**(2):79–88.
89. Engelhard, H. H. The role of interstitial BCNU chemotherapy in the treatment of malignant glioma. *Surg. Neurol.* 2000;**53**(5):458–464.
90. Kleinberg, L. R., J. Weingart, P. Burger, K. Carson, S. A. Grossman, K. Li, A. Olivi, III, M. D. Wharam, H. Brem. Clinical course and pathologic findings after Gliadel and radiotherapy for newly diagnosed malignant glioma: Implications for patient management. *Cancer Invest.* 2004;**22**(1):1–9.
91. Brem, H., S. Piantadosi, P. C. Burger, M. Walker, R. Selker, N. A. Vick, K. Black, M. Sisti, S. Brem, G. Mohr, P. Muller, R. Morawetz, and S. C. Schold. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-Brain Tumor Treatment Group. *Lancet* 1995;**345**(8956):1008–1012.
92. Brem, H., M. S. Mahaley, Jr., N. A. Vick, K. L. Black, S. C. Schold, Jr., P. C. Burger, A. H. Friedman, I. S. Ciric, T. W. Eller, J. W. Cozzens, and J. N. Kenealy. Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas. *J. Neurosurg.* 1991;**74**(3):441–446.
93. Kwame G. Buahin[1], Kevin D. Judy[1], Carol Hartke[2], Abraham J. Domb[4], Manoj Maniar[4], O. Michael Colvin[2], Henry Brem[1,3]. Controlled release of 4 hydroxyper-oxycyclo phosphamide from the fatty acid dimer-sebacic acid coploymer. *Polym. Adv. Tech.* 1992;**3**:311–316.
94. Judy, K. D., A. Olivi, K. G. Buahin, A. J. Domb, J. I. Epstein, O. M. Colvin, and H. Brem. Effectiveness of controlled release of a cyclophosph amide derivative with polymers against rat gliomas. *J. Neurosurg.* 1995;**82**(3):481–486.

95. Nicholson, C. Diffusion and related transport mechanisms in brain tissue. *Re. Prog. Phys.* 2001;**64**:815–884.
96. Zamecnik, J. The extracellular space and matrix of gliomas. *Acta Neuropathol. (Berl).* 2005;**110**(5):435–442.
97. Westphal, M., Ram, Z., Riddle, V., Hilt, D., and E. Bortey. Gliadel wafer in initial surgery for malignant glioma: Long-term follow-up of a multicenter controlled trial. *Acta Neurochir.* 2006;**148**:269–275.
98. Frazier, J. L., P. P. Wang, D. Case, B. M. Tyler, G. Pradilla, J. D. Weingart, and H. Brem. Local delivery of minocycline and systemic BCNU have synergistic activity in the treatment of intracranial glioma, *J. Neurol.* 2003;**64**(3):203–209.
99. Tamargo, R. J., K. W. Leong, and H. Brem. Growth inhibition of the 9l glioma using polymers to release heparin and cortisone acetate. *J. Neurol.* 1990;**9** (2):131–138.
100. Walter, K. A., M. A. Cahan, A. Gur, B. Tyler, J. Hilton, O. M. Colvin, P. C. Burger, A. Domb, and H. Brem. Interstitial taxol delivered from a biodegradable polymer implant against experimental malignant glioma. *Cancer Res.* 1994;**54**(8):2207–2212.
101. Judy, K. D., A. Olivi, K. G. Buahin, A. Domb, J. I. Epstein, O. M. Colvin, H. Brem Effectiveness of controlled release of a cyclophosphamide derivative with polymers against rat gliomas. *J. Neurosurg.* 1995;**82**(3):481–486.
102. Choti, M. A., J. Saenz, X. Yang, H. Brem. Intrahepatic FUdR delivered from biodegradable polymer in experimental liver metastases from colorectal carcinoma,*Proc. Am. Assoc. Cancer Res.*, 1995;**36**:309.
103. Williams, J. A., L. E. Dillehay, K. Tabassi, E. Sipos, C. Fahlman, and H. Brem. Implantable biodegradable polymers for IUdR radiosensitization of experimental human malignant glioma*J. Neurol.*, 1997;**32**(3):181–192.
104. Chabner, B. A., D. P. Ryan, L. P. Ares, R. G. Carbonero, P. Calabresi. *Antineoplastic agents*, In *Goodman and Gilman's the Pharmacological Basis of Therapeutics*. J. G. Hardman, and L. E. Limbrid.(Eds.); McGraw-Hill: New York, 2001, pp. 1389–1459.
105. Olivi, A., M. G. Ewend, T. Utsuki, B. Tyler, A. J. Domb, D. J. Brat, and H. Brem. Interstitial delivery of carboplatin via biodegradable polymers is effective against experimental glioma in the rat. *Cancer Chemother. Pharmacol.* 1996;**39**(1–2):90–96.
106. Storm, P.B., J. L. Moriarity, B. Tyler, P. C. Burger, H. Brem, and J. Weingart. Polymer delivery of camptothecin against 9L gliosarcoma: release, distribution, and efficacy, *J. Neurol.* 2002;**56**(3):209–217.
107. Shikani, A. H., D. W. Eisele, and A. J. Domb. Polymer delivery of chemotherapy for squamous cell carcinoma of the head and neck. *Arch. Otolaryngol. Head Neck Surg.*, 1994;**120**(11):1242–1247.
108. Krasko, M. Y., A. Shikanov, A. Ezra, and A. J. Domb. Poly(ester anhydride)s prepared by the insertion of ricinoleic acid into poly(sebacic acid). *J. Polym. Sci. A, Polym. Chem.* 2003;**41**:1059–1069.
109. Shikani, A. H. and A. J. Domb. Polymer chemotherapy for head and neck cancer. *Laryngoscope* 2000;**110**(6):907–917.
110. Li, L. C., J. Deng, and D. Stephens. Polyanhydride implant for antibiotic delivery—from the bench to the clinic. *Adv. Drug Deliv. Rev.* 2002;**54**(7):963–986.

111. Tian, Y., L. Li, X. Gao, J. Deng, D. Stephens, D. Robinson, and H. Chang. The effect of storage temperatures on the in vitro properties of a polyanhydride implant containing gentamicin. *Drug Dev. Ind. Pharm.* 2002;**28**(8):897–903.

112. Yang, X. F., F. D. Zeng, Z. B. Zhou, K. X. Huang, and H. B. Xu. In vitro release and antibacterial activity of poly(oleic/linoleic acid dimer: sebacic acid)-gentamicin. *Acta Pharmacol. Sin.* 2003;**24**:306–310.

113. Nobuyoshi, M., T. Kimura, H. Nosaka, S. Mioka, K. Ueno, H. Yokoi, N. Hamasaki, H. Horiuchi, and H. Ohishi. Restenosis after successful percutaneous transluminal coronary angioplasty: Serial angiographic follow-up of 229 patients, *J. Am. Coll. Cardiol.*, 1988;**12**(3):616–623.

114. Haung, Y., L. Wang, I. Verweire, B. Qiang, X. Liu, F. Verbeken, E. Schacht, and I. De Scheeder. Optimization of local methylprednisolone delivery to inhibit inflammatory reaction and neointimal hyperplasia of coated coronary stents. *J. Invasive Cardiol.* 2002;**14**: 503–513.

115. Toutouzas, K., A. Colombo, and C. Stefanadis. Inflammation and restenosis after percutaneous coronary interventions. *Eur. Heart J.*, 2004;**25**(19):1679–1687.

116. Haudenschild, C. C. Pathobiology of restenosis after angioplasty. *Am. J. Med.* 1993;**94**(4A):40S–44S.

117. Koster, R., U. Windstetter, P. Uberfuhr, G. Baumann, S. Nikol, and B. Hofling. Enhanced migratory activity of vascular smooth muscle cells with high expression of platelet-derived growth factor A and B. *Angiology* 1995;**46**(2):99–106.

118. Orloff, L. A., A. J. Domb, D. Teomim, I. Fishbein, and G. Golomb. Biodegradable implant strategies for inhibition of restenosis. *Adv. Drug Deliv. Rev.* 1997;**24**(1):3–9.

119. Herrman, J. P., W. R. Hermans, J. Vos, and P. W. Serruys. Pharmacological approaches to the prevention of restenosis following angioplasty. The search for the Holy Grail? (Part II). *Drugs* 1993;**46**(2):249–262.

120. Mitchel, J. F., M. A. Azrin, D. B. Fram, M. K. Hong, S. C. Wong, J. J. Barry, L. M. Bow, T. M. Curley, F. J. Kiernan, and D. D. Waters. Inhibition of platelet deposition and lysis of intracoronary thrombus during balloon angioplasty using urokinase-coated hydrogel balloons. *Circulation* 1994;**90**(4):1979–1988.

121. Labhasetwar, V., C. Song, J. R. Levy. Nanoparticle drug delivery system for restenosis. *Adv. Drug Deliv. Rev.* 1997;**24**:1997; 63–85.

122. Teomim, D., I. Fishbien, G. Golomb, L. Orloff, M. Mayberg, and A. J. Domb. Perivascular delivery of heparin for the reduction of smooth muscle cell proliferation after endothelial injury. *J. Control. Release* 1999;**60**(1):129–142.

123. Lee, D. A., K. W. Leong, W. C. Panek, C. T. Eng, B. J. Glasgow. The use of bioerodible polymers and 5-fluorouracil in glaucoma filtration surgery. *Invest. Ophthalmol. Vis. Sci.*, 1988;**29**(11):1692–1697.

124. Jampel, H. D., K. W. Leong, G. R. Dunkelburger, H. A. Quigley. Glaucoma filtration surgery in monkeys using 5-fluorouridine in polyanhydride disks. *Arch. Ophthalmol.* 1990;**108**(3):430–435.

125. Uppal, P., H. D. Jampel, H. A. Quigley, and K. W. Leong. Pharmacokinetics of etoposide delivery by a bioerodible drug carrier implanted at glaucoma surgery. *J. Ocul. Pharmacol.* 1994;**10**(2):471–479.

126. Herrero-Vanrell, R., and M. F. Refojo. Biodegradable microspheres for vitreoretinal drug delivery. *Adv. Drug Deliv. Rev.* 2001;**52**(1):5–16.

127. Hanes, J., M. Chiba, and R. Langer. Degradation of porous poly(anhydride-co-imide) microspheres and implications for controlled macromolecule delivery. *Biomaterials* 1998;**19**(1–3):163–172.

128. Lucas, P. A., C. Laurencin, G. T. Syftestad, A. Domb, V. M. Goldberg, A. I. Caplan, and R. Langer. Ectopic induction of cartilage and bone by water-soluble proteins from bovine bone using a polyanhydride delivery vehicle. *J. Biomed. Mater. Res.* 1990;**24**(7):901–911.

129. Jiang, H. L., and K. J. Zhu. Pulsatile protein release from a laminated device comprising polyanhydrides and pH-sensitive complexes. *Int. J. Pharm.* 2000;**194**(1):51–60.

130. Kubek, M. J., D. Liang, K. E. Byrd, and A. J. Domb. Prolonged seizure suppression by a single implantable polymeric-TRH microdisk preparation. *Brain Res.* 1998;**809**(2):189–197.

131. Allard, L., V. Cheynet, G. Oriol, G. Gervasi, E. Imbert-Laurenceau, B. Mandrand, T. Delair, and F. Mallet. Antigenicity of recombinant proteins after regioselective immobilization onto polyanhydride-based copolymers. *Bioconjug. Chem.* 2004;**15**(3):458–466.

132. Cai, Q. X., K. J. Zhu, D. Chen, and L. P. Gao. Synthesis, characterization and in vitro release of 5-aminosalicylic acid and 5-acetyl aminosalicylic acid of polyanhydride-P(CBFAS). *Eur. J. Pharm. Biopharm.* 2003;**55**(2):203–208.

133. HowardIII, M. A., A. Gross, M. S. Grady, R. S. Langer, E. Mathiowitz, H. R. Winn, and M. R. Mayberg. Intracerebral drug delivery in rats with lesion-induced memory deficits. *J. Neurosurg.* 1989;**71**(1):105–112.

134. Amsden, B., A. Hatefi, D. Knight, and E. Bravo-Grimaldo. *Biomacromolecules* 2004;**5**:637–642.

135. Durucan, C., and P. W. Brown. *Adv. Eng. Mater.* 2001;**3**:227–231.

136. Murphy, W. L. and D. J. Mooney. *J. Period. Res.* 1999;**34**:413–419.

137. Wake, M. C., P. K. Gupta, and A. G. Mikos. *Cell Transplant.* 1996;**5**:465–473.

138. Mendak, S. H., R. J. Jensik, M. F. Haklin, and D. L. Roseman. *Ann. Thora. Surg.* 1984;**38**:488–493.

139. Vacanti, J. P. and R. Langer. *Lancet*,1999;**354**:SI32–SI34.

140. Shikanov, A., N. Kumar, A. J. Domb. *Biodegrad. Polym.* 2005;**45**:393–399.

CHAPTER 11

POLY(ε-CAPROLACTONE-*CO*-GLYCOLIDE): BIOMEDICAL APPLICATIONS OF A UNIQUE ELASTOMER

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CONTENTS

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11.1 INTRODUCTION

Poly(ε-caprolactone-*co*-glycolide) copolymers represent a unique class of absorbable copolyesters that can be tailored for required elastomeric properties using a proper composition and architecture. Poly(ε-caprolactone-*co*-glycolide) copolymers are prepared by a ring-opening polymerization of ε-caprolactone and glycolide using a catalyst (typically stannous octoate), an initiator (typically an alcohol), and temperatures that yield high-molecular-weight polymers. Careful attention is paid to the use of molar ratios of catalyst to initiator to monomers to provide high-molecular-weight polymers. An inert atmosphere is also essential as the monomers and the formed polymers are susceptible to degradation and side reactions in the presence of oxygen and water. Various process steps (as described below) can be taken to synthesize poly(ε-caprolactone-*co*-glycolide copolymers with random, segmented, or blocky architectures and unique physicochemical properties. Several synthesis procedures are given below that describe the synthesis of poly(ε-caprolactone-*co*-glycolide) copolymer with different shapes, which have been utilized for sutures, suture coatings, other implantable devices, and tissue engineering applications. This chapter provides a review of the synthesis, physicochemical characteristics, and biomedical applications of poly (ε-caprolactone-*co*-glycolide) copolymers.

11.2 MONOCRYL SUTURE

11.2.1 Introduction

Synthetic absorbable sutures are available as braided or monofilament constructs. Braided absorbable sutures are made, for example, from 90 : 10 poly (glycolide-*co*-L(−) lactide), sold by Ethicon, Inc. under the trade name Vicryl or from polyglycolide (Dexon) [1−4]. Issues with braided sutures include tissue drag and the trauma this may cause, as well as the possible infection in the interstices of the braid [5, 6]. Absorbable monofilament sutures such as PDS II [poly(*p*-dioxanone); Ethicon, Inc.] or Maxon [poly(trimethylene carbonate-*co*-glycolide)] limit these issues [7, 8]. However, these monofilament sutures do not handle as well as braids. Monocryl monofilament sutures (a segmented block copolymers of ε-caprolactone and glycolide) have been shown to display excellent handling properties, minimal resistance during passage through tissue, and excellent tensile properties [4, 9, 10]. Their preparation and physical and biological properties are reviewed.

11.2.2 Monocryl Suture Synthesis

Monocryl polymer is prepared by a two-step synthesis [11]. A prepolymer of ε-caprolactone and glycolide is first prepared to give an amorphous or low crystalline soft segment. The prepolymer is then polymerized in the presence of additional glycolide to create crystalline segments attached to the already-formed soft segments. The resulting polymer forms a segmented block copolyester. The overall composition of the copolymer is 25 mol% ε-caprolactone and 75 mol% glycolide. The chemical formula of the segmented block copolyester is shown in Figure 11.1.

In more detail, the segmented copolymer of ε-caprolactone and glycolide with a 45 : 55 mole : mole middle block and a 25 : 75 overall composition is prepared using a typical scheme to form copolyesters from lactone monomers. A flame-dried, 250 mL, three-neck, round-bottomed flask is charged with 28.54 g (0.250 mol) ε-caprolactone, 35.52 g (0.306 mol) glycolide, 0.114 mL distilled diethylene glycol (1.2 mmol per mole of total monomer), and 0.0505 mL of stannous octoate (0.33 M in toluene, 60,000 : 1 molar ratio of monomer:catalyst). The flask is fitted with a flame-dried mechanical stirrer and an adaptor. The reaction vessel is purged with nitrogen followed by vacuum and repeated three times. The reaction mixture, under a blanket of nitrogen, is heated to 190°C and maintained at this temperature for about 17 h under stirring.

In the second stage of the polymerization, 51.54 g (0.444 mol) of molten glycolide is added to the prepolymer in the reaction flask. The temperature of the reaction mixture is raised to 230°C to dissolve the prepolymer into the molten glycolide. After about 10 mm at 230°C, the copolymer is uniformly mixed with glycolide. The temperature of the reaction mixture is dropped to 200°C and maintained there for about 2 h. The copolymer is isolated, ground, and dried for 16 h at 110°C under vacuum to remove any unreacted monomers. The conversion of monomer to polymer is typically greater than 99% with inherent viscosity of more than 1.6 dL/g. Large-scale polymerizations are conducted using the same general scheme.

11.2.3 Monocryl Suture Polymer Extrusion: Filament Preparation

After the poly(ε-caprolactone-*co*-glycolide) Monocryl copolymer is synthesized, the polymer is formed into filaments for use as sutures. Monocryl is

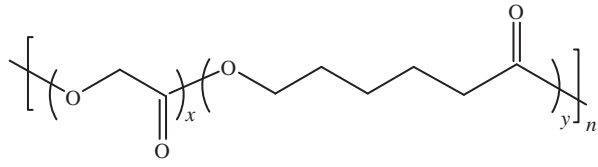

FIGURE 11.1 Monocryl: Segmented block copolyester of ε-caprolactone and glycolide.

melt spun using standard extrusion equipment. Extrusion of these copolymers occurs at temperatures of 10–50°C above their melting point. Typcially, Monocryl is extruded at a temperature of 230°C. The extrudate filaments that form from the molten polymer are cooled, rolled up on a spool, and allowed to crystallize at room temperature for up to 2 weeks. The filaments are then drawn five to seven times to improve tensile properties. The strands are cut to length and needles are attached. The needle and suture are then put into folders and placed in their packages, sterilized and sealed [9–11].

11.2.4 Results and Discussion

Mechanical Properties. The physical properties of Monocryl suture (Table 11.1) are listed along with those of Vicryl, PDS II, and Chromic Gut. Monocryl is the strongest of all the absorbable monofilament sutures (normalized for cross-sectional area). Monocryl is also more pliable than many other monofilaments. This results in excellent handling during surgical use.

Surgeons prefer a suture that can pass smoothly through tissue to minimize trauma. A rough strand presents considerable resistance during tissue passage, making it difficult to use as a continuous running suture. Smooth surface monofilaments have less tissue drag compared to braids. Monocryl has a stiffness (Table 11.2) that is considerably less than other monofilaments. This yields a suture that passes through tissue more easily.

TABLE 11.1 Physical Properties: Monocryl Suture Compared to Vicryl, PDS II, and Chromic Gut

Material/Diameter (mils)	Straight-Pull Strength (Ib) (psi)	Knot-Pull Strength (Ib) (psi)	Elongation *(%)*
Monocryl/15.03	16.14 (91,100)	8.11 (45,700)	39
PDS II/13.93	10.77 (70,700)	7.37 (48,400)	51
Chromic gut/15.70	9.07 (46,700)	4.51 (23,200)	22
Vicryl/13.74	15.28 (103,000)	7.97 (54,000)	24

Source: From [9–11].

TABLE 11.2 Stiffness Properties: Monocryl Suture Compared to Maxon, PDS II, and Chromic Gut

Suture	Diameter (mils)	Young's modulus (psi)	Stiffness (lb in2 × 10k)
Monocryl	15.03	113,000	2.8
Maxon	15.81	380,000	11.6
Chromic gut	15.70	358,000	10.7
PDS Il	13.93	211,000	3

Source: From [9–11].

TABLE 11.3 The in vivo Breaking Strength Retention of Monocryl Suture

Suture Size	Mean Breaking Strength, 0 days (Ib)	Mean Breaking Strength, 14 days (Ib)	BSR (%)
5-0	4.55	1.19	26.3
4-0	6.55	1.45	22.2
3-0	10.92	2.81	25.8
2-0	15.59	3.86	24.7
0	19.43	4.85	24.9
1	25.60	6.20	24.2

Source: From [9–11].

The in vivo breaking strength retention of Monocryl suture 14 days postoperatively is 20–30% of its initial tensile strength. This makes it ideal for healing the soft tissue since it allows the tissue some support while not shielding the tissue from stress (Table 11.3).

Biological Properties. In animal studies, Monocryl suture has been found to pass through tissues with little resistance. Knot security is good based on the tactile feedback from the suture. Animals recover uneventfully from surgery without evidence of pain or discomfort while body weights remain stable with no treatment-related changes. Upon necropsy, all surgical incision lines heal normally with no evidence of dehiscence.

Pharmocokinetic studies have also been performed on Monocryl using a ^{14}C radioactive labeled caproyl moiety. The absorption profile of the ^{14}C-labeled Monocryl suture decreased linearly (zero-order kinetics) up to week 10 with the radioactivity in the implantation site at week 10 corresponding to 5.6–9.4% of the implanted dose in males and females, respectively. By week 14, the absorption was nearly complete with the radioactivity corresponding to 0.15 and 0.24% of the implanted dose in males and females, respectively [9, 10].

The antibacterial properties of Monocryl have also been studied using Triclosan, a known antimicrobial agent [12, 13], as a coating on the suture. The study evaluated the in vitro efficacy of Monocryl suture with Triclosan against both gram-positive and gram-negative bacteria, including *Staphylococcus aureus, and Staphylococcus epidermidis*. The study used a zone of inhibition assay. The suture also was tested against *Escherichia coli* in a colonization assay in a dynamic model simulating in vivo conditions. Results showed that Monocryl with Triclosan demonstrated significant in vitro efficacy against a range of bacteria. The suture sustained in vitro efficacy for 11 days [13].

Conclusions. Monocryl suture possesses the highest straight tensile strength and best handling properties of all the available monofilament absorbable sutures. The in vivo performance of the suture has been evaluated for breaking strength retention, absorption, tissue reaction, and pharmacokinetics. Tissue reaction and absorption were assessed by intramuscular implantation of sizes

2-0 and 6-0 Monocryl suture into rats. The tissue reactions of the sutures were minimal. Absorption of the sutures was complete between 91 and 119d [3, 9–11]. Multiple surgical procedures were performed in Beagle dogs to judge the surgical performance of the sutures. Monocryl with Triclosan has been shown to be effective against a host of bacteria [13].

11.3 SUTURE COATINGS

11.3.1 Introduction

Multifilament surgical sutures such as Vicryl poly(lactide-*co*-glycolide) multifilament suture typically require a surface coating to improve the pliability and knotting characteristics of the suture. Coatings based on ε-caprolactone dissolved in an appropriate organic solvent have been used. The coating solution is typically applied to the surface of the suture using conventional techniques, and then the solvent is removed. Polycaprolactone is a biocompatable polymer with a relatively low melting point, a property that is essential for good coating characteristics. Additionally, sutures coated with polycaprolactone exhibit enhanced pliability and handling characteristics. Unfortunately, polycaprolactone homopolymer is essentially nonabsorbable because it retains some of its mass and mechanical integrity in vivo for periods up to one year, which is too long for numerous surgical applications. It was determined that compositions of caprolactone, glycolide, and glycolic acid have properties useful as suture coatings and are described below.

11.3.2 ε-Caprolactone, Glycolide, and Glycolic Acid Suture Coatings

In an effort to improve the bioabsorbability and other properties of polycaprolactone coatings, the polymer composition can be modified by incorporating copolymerizable monomers or lubricating agents. In the past, suture coatings based upon copolymers of at least 90% ε-caprolactone and a biodegradable monomer and optionally a lubricating agent have been prepared [14, 15]. Examples of monomers for biodegradable polymers include glycolic acid and glycolide, as well as other well-known monomers typically used to prepare polymer fibers or coatings for multifilament sutures. Sutures coated with such copolymers are reported to be less stiff than sutures coated with other materials, and the physical properties of the coated suture are also reported to be acceptable.

However, adequate bioabsorbability of homopolymers and copolymers of ε-caprolactone for suture coating applications was still a challenge. One solution that has been developed is a copolymer of ε-caprolactone, glycolide, and glycolic acid [14, 15]. The copolymer is characterized by a concentration of glycolic acid such that the intrinsic viscosity of the copolymer is low (0.15 to about 0.60 dL/g).

Surprisingly, the use of glycolic acid as a comonomer into the copolymers increases the rate of absorption of the copolymers. This increase in the rate of absorption is achieved while maintaining tissue drag, knot tie down, and tensile

properties. These copolymers can be used for coating bioabsorbable, multifilament surgical sutures such as Vicryl.

By adjusting the intrinsic viscosity of the copolymer by varying the concentration of glycolic acid, a copolymer coating is achieved that forms a film on the outer surface of the suture and penetrates the interstices of the multifilament fibers. The penetration of the coating polymer onto individual fibers of the multifilament increases the pliability of the suture and enhances its knotting characteristics, especially the ease with which a knot can slide down the length of a suture during surgery.

The coating is prepared by dissolving the copolymer into an organic solvent (chloroform, ethyl acetate). The concentration of the copolymer in solution is 15 wt%. Generally, concentrations greater than 20 wt% polymer provide coating solutions that are too viscous to achieve adequate penetration of the coating solution into the interstices of the fibers, and concentrations below 10 wt% are inadequate to properly coat a sufficient amount of copolymer onto the suture. Once a solution of the copolymer is prepared, a suture is coated using a dipping process. After the coating is applied, the solvent is removed by drying at an elevated temperature under vacuum.

11.4 DERMAL TISSUE REPAIR

11.4.1 Introduction

Another area that poly(ε-caprolactone-*co*-glycolide) copolymers may provide benefit is in tissue engineering. Several tissue engineering scaffold concepts with poly(ε-caprolactone-*co*-glycolide) copolymers have been developed in our laboratories. Poly(ε-caprolactone-*co*-glycolide) as an acellular tissue engineering scaffold for a wound-healing matrix in chronic wounds for dermal tissue repair has been developed. A 0.5-mm-thick porous scaffold sheet made from an elastomeric copolymer [35/65 poly (ε-caprolactone-*co*-glycolide)] using a novel phase separation process has been developed. Extensive bench-top testing and in vitro and in vivo studies resulted in the selection of an optimal material composition with excellent physical parameters of porosity, morphology, and thickness.

The approach was similar to that developed by Yannas and Burke [16]; however, the primary distinction was to focus on a completely synthetic biocompatible and bioabsorbable material instead of an animal-derived material. We also wanted to focus on chronic wounds—including venous stasis ulcers, diabetic foot ulcers and pressure sores as the target indications as opposed to burns. Most approaches so far using biodegradable synthetic scaffolds [17–19] or animal-derived materials [20] have involved cell expansion and seeding onto the scaffolds, resulting in an in vitro cultured substitute for skin. These products have had mixed clinical success and are far from optimum [21]. All of these approaches have one or more drawbacks from a viable product standpoint: limited shelf life, difficult handling and storage, and costs due to the difficult step of cell culturing process.

Earlier efforts with completely synthetic bioabsorbable scaffolds such as PGA (Dexon) and 90/10 PGA/PLA copolymers (Vicryl) meshes for skin tissue engineering met with disappointing results in terms of a poor take in a wound bed [22]. The challenge we faced was to design and develop a matrix that would have an acceptable take in a wound bed and provide a three-dimensional (3D) matrix for cellular proliferation and growth leading to dermal tissue repair and wound closure. Given this, three important aspects of the scaffold design were studied: material composition selection, scaffold porosity and morphology, and thickness and flexural rigidity of the implant.

To develop materials with these aspects, the elastomeric copolymers synthesized are more than an order of magnitude lower in stiffness and peak stress and at least an order of magnitude greater in percent elongation compared to polyglycolide (PGA), polylactide (PLA) and polycaprolactone (PCL) polymers. Our hypothesis in using these unique highly elastomeric copolymers for soft tissue applications is that the scaffolds made from these polymers will provide a compliant microenvironment in the wound bed. Such a 3D environment will provide a "provisional matrix" for cell attachment, migration, proliferation, and deposition of extracellular matrix leading to tissue repair and wound closure.

Another aspect of the material selection process was the degradation profile or time for complete absorption in vivo. Three choices would provide a range of absorption profile—from a few months to a couple of years. Table 11.4 outlines the approximate time it takes for these materials to completely resorb in vivo based on the outcome of two long-term studies (one in rat and the other in swine).

11.4.2 Scaffold Porosity and Morphology

After demonstrating the ability to make porous scaffolds from the three material compositions, we conducted a pilot in vivo study using a pig full-thickness excisional wound model. It was rationalized that we would need a 2-mm-thick scaffold for full-thickness wounds. The foams were prepared from the previously

TABLE 11.4 Physical and Biological Properties of Elastomeric Polymers

Polymer	Tensile Modulus (MPa)	Elongation at Break (%)	Degradation Profile (months)
PCL–PGA 35–65 (mol-mol %)	60	1800	3–4
PCL–PLA 40–60 (mol-mol %)	23	1100	18
PCL–PGA 35–65 (mol-mol %) & PCL–PLA 40–60 (mol-mol)			
Blend (50–50 wt%)	36	1500	18
PGA	7000	17	3–6
PLA	2700	7	24
PCL	400	80	>24

Source: From [23, 24].

listed copolymers, using a lyophilization process developed in our laboratories [25]. These scaffolds had poor tissue in-growth and a majority of the scaffolds sloughed out of the wound due to a combination of low porosity and high flexural rigidity. Based on this pilot 8-day study, the 50/50 blend was chosen as the primary composition because it provided slightly greater cellular infiltration and granulation tissue in-growth into the scaffold than the other compositions. There was no statistically significant difference between the 50/50 blend and the 35/65 PCL/PGA case. The 40/60 PCL/PLA did not provide any exceptional result and was deemed not optimal because of its extremely long-lasting absorption profile (18 months or greater).

For the next in vivo study, the scaffolds were modified in terms of porosity, morphology, and thickness. The starting polymer solution concentration was lowered from 10% (w/w) to 5% (w/w) and lyophilization cycle was modified to a quench cycle instead of a slow controlled cooling cycle. Matrices that were 0.5 and 1.0 mm thick were implanted. Strong evidence was found that scaffold physical characteristics (porosity, morphology, and thickness) play an important role in scaffold incorporation in the wound bed.

A 90-day long-term in vivo study was then conducted where scaffolds made from 35/65 PCL/PGA and the 50/50 blends were compared. The 35/65 PCL/PGA degraded much faster, and by day 28 the scaffold fragments resulted in granulation tissue overgrowth. By day 90, there was complete absorption of the 35/65 PCL/PGA scaffolds while the 50/50 blend still had approximately 50% of the material remaining in the wound and a granulomatous response was still apparent. The 35/65 demonstrated minimal response of residual inflammation following total absorption. It was concluded that shorter absorption time of 35/65 PCL/PGA provided greater advantage in a wound-healing situation than any slight benefits that the 50/50 blend might have in the initial stages of cellular infiltration and proliferation.

The safety and biocompatibility of these scaffolds made from the three compositions were evaluated in a long-term (18-month) rat study [26]. In this tissue reaction and absorption study, the scaffolds were surgically implanted at two sites—intramuscular and subcutaneous. All three compositions were deemed safe and biocompatible with acceptable tissue reaction. Both the EtO and γ-sterilized scaffolds made from 35/65 PCL/PGA copolymer were completely absorbed 90 days after implantation.

11.4.3 Results and Discussion

Scaffold Porosity and Morphology. The scaffold's porosity, pore size, and morphology form the basis of the 3D architecture that cells encounter in the wound bed. Within the wound environment the 3D structure of the scaffold would provide support for rapid cellular invasion and extracellular matrix deposition by the invading cells. The primary mechanism of action of the scaffold is through its ability to provide 3D interconnecting pores that facilitates rapid cell migration and tissue regeneration [27]. The importance of scaffold morphology

has been previously described regarding cell migration and vascularization of tissues [28, 29]. The scaffold facilitates the process of granulation tissue formation and re-epithelialization by enabling the cells to migrate and synthesize new dermal matrix in the wound defect. Despite recent advances in tissue engineering, the current state of understanding on the effect of scaffold porosity and morphology in wound healing can be described at best as empirical. Our approach was to study porosity and morphology through in vivo studies.

Additionally, several techniques such as mercury porosimetry, helium picnometry, capillary flow permeability, liquid porosimetry, confocal microscopy, and image analysis to characterize the foams in terms of porosity and pore size distribution were studied. Of these techniques, helium picnometry has been identified as the optimal technique for measuring porosity in an accurate and repeatable fashion.

From this analysis, it was found that the current scaffolds have a porosity of about 94%. A strong correlation between the porosity values and the starting concentration of the polymer solution was also determined. For example, the foams prepared from 5% (w/w) polymer solutions have porosity values around 94%, while the foams made from 10% (w/w) solutions have porosity values around 88%. This is understandable since the polymer solution is frozen during the lyophilization process and results in phase separation of the polymer and the solvent phases. The subsequent sublimation step and the drying step remove the solvent phase completely and therefore, leave an interconnected porous foam. This high correlation between solvent fraction and the porosity also confirms that the pores are not closed but open.

Pore size is a more difficult parameter to characterize due to the pore size distribution. To monitor the modal pore diameter mercury porosimetry was used. It has been reported that if a majority of the pores are greater than 10 μm, then they should support cellular in-growth and good nutrient transport. In the current studies, the modal pore size is well over 10 μm for all our scaffolds tested and hence should support cellular in-growth.

11.4.4 Conclusions

Making bioabsorbable elastomeric porous scaffolds for tissue engineering requires a careful selection of biocompatible polymers that exhibited the right mechanical properties and absorption profile. The two copolymers based on ε-caprolactone were expected to provide a "fast" (glycolide-based) and a "slow" (lactide-based) absorption profile. Furthermore, the crystalline glycolide and lactide domains form a network in the amorphous matrix that makes the copolymer elastomeric in its behavior.

A variety of porous foams were fabricated and the type of microstructure obtained in the scaffold depended on factors such as the polymer–solvent interaction, concentration, and kinetics of phase separation. Highly porous foams with a porosities ranging from 75 to 90% and pore size ranging from 50 to 150 μm were produced by this technique.

In vitro mechanical testing of these foams at 0, 7, 14, and 28 days was performed. The ε-caprolactone copolymers with glycolide or lactide had similar molecular weights and mechanical characteristics but differed in the resorption profile as expected. The high porosity, inherent interconnectivity of the pores, and the large surface area of this material provide an excellent scaffold for cellular invasion and attachment. Furthermore, due to the high surface area, the foam undergoes a rapid degradation that might provide the necessary space for tissue in-growth.

11.5 BUTTRESSING MATERIAL

11.5.1 Introduction

In patients who suffer from emphysema and other lung diseases, staple-line buttressing has been developed for lung volume reduction (LVR) and resulted in decreased patient morbidity and shorter hospital stays. Additionally, a growing need exists for a staple-line buttress material that provides hemostasis and/or pnemostasis in several surgical procedures involving soft tissues such as gastric bypass, gastric banding, and hysterectomy. Ideally, the buttress material for these applications needs to be supple, thin, compressible, and biocompatible with low force to fire and easy to cut using a stapler. Existing materials either lack one or more of these features or are difficult to attach to the staplers. Biologically derived products such as BioVascular's bovine pericardium have potential immunological issues and are difficult to use, while Gore's ePTFE Seamguard are permanent implants that become encapsulated [30]. To address these issues, a unique foam buttress material for surgical staplers from a bioabsorbable, elastomeric copolymer was developed.

11.5.2 LVR 35/65 Properties

As a thin film, the copolymer of 35% ε-caprolactone and 65% glycolide exhibits unique elastomeric properties with a tensile modulus of 2350 psi at 300% elongation and elongation to break greater than 600% [23, 24]. The elasticity of this copolymer and of foams made thereof is ideally suited for soft tissue repair. When used as a staple-line buttress, the foam will deform without tearing at the staple line.

A novel lyophilization process was developed to make thin foams (0.5–1 mm) from the copolymer solution using a freeze-dryer. The buttress material made by this process is very porous and has an overall porosity of 80–90% with interconnecting pore morphology (Figure 11.2) [23, 24]. The buttress material is attached to the cartridge and the anvil of a stapling device. When the surgical stapler is used, the staples pierce through the foam buttress material and are formed by the staple pockets on the anvil. The high porosity combined with the inherent elastomeric nature of the copolymer allows the buttress material to

compensate for tissue thickness variations. The skin on one surface of the foam buttress virtually ensures that there are no air or blood leaks at the staple legs. The in vitro degradation profile of the foam indicates that approximately one third of its strength is lost by day 3 and another third by day 7. This rapid loss of mechanical strength is suitable in many wound-healing situations where the natural tissue heals completely in 3 weeks. The breaking load when normalized for thickness indicated no inherent differences in the rate of degradation as a function of thickness (Fig. 11.3) [23, 24].

A long-term tissue reaction and absorption study showed that buttress material is absorbed in vivo in 120 days. The tissue reaction was the normal

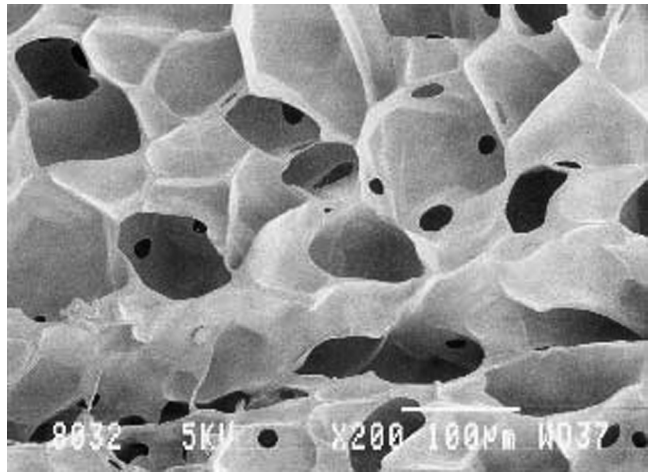

FIGURE 11.2 Scanning electron micrograph: Cross section of the foam buttress material. (From [23, 24].)

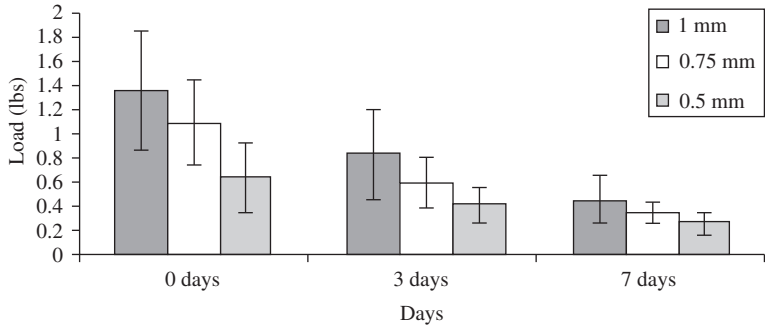

FIGURE 11.3 In vitro mechanical performance of foam buttress material (From [23, 24].)

foreign body type with granulomatous inflammation and with fibrous encapsulation that was usually less than 50 μm thick. This study established the biocompatibility of the foam buttress material and is consistent with the findings of other copolymers made from ε-caprolactone and glycolide [9–11].

11.6 SUMMARY

Poly(ε-caprolactone-*co*-glycolide) copolymers represent a unique class of absorbable copolyesters that with the proper composition and architecture have properties that provide useful elastomeric characteristics. Such copolymers have utility as sutures, suture coatings, and tissue engineering constructs given the elastomeric properties our research has found.

REFERENCES

1. Craig, P. H., J. A. Williams, K. W. Davis, et al. A biological comparison of polyglactin 910 and polyglycolic acid synthetic absorbable sutures. *Surg. Gyneco. Obstet*. 1975;**141**:1–10.
2. Chu, C. C., et al. *Wound Closure Biomaterials and Devices*. CRC Press: Boca Raton, FL, 1997.
3. Hang, Jong-Taek Hong, Nam-Sook Cho, Hye-Sung Yoon, Tae-Hun Kim, Myoung-Seok Koh, and Whan-Gi Kim. Biodegradable studies of poly(trimethylenecarbonate-ε-caprolactone)-*block*-poly(*p*-dioxanone), poly(dioxanone), and poly(glycolide-ε-caprolactone) (Monocryl®) monofilaments. *J. Appl. Poly. Sci*. 2006;**102**(1):737–743.
4. Wise, D. *Encyclopedic Handbook of Biomaterials and Bioengineering: Part A: Materials*. Dekker: New York, 1995.
5. Mangram, A., I. C Horan, M. L. Pearson, Donald, L. Wise, Debra J. Trantolo, David E. Altobelli, Michael J. Yaszernski, Joseph D. Gresser, and Edith R. Schwartz. Guideline for prevention of surgical site infection. *Infect. Control Hosp. Epidemiol*. 1999;**20**:247–280.
6. Alicia, J. Mangram, MD; Teresa C. Horan, MPH, CIC; Michele, L. Pearson, MD; Leah Christine Silver, BS; William, and R. Jarvis, MD. Joint Commission on Accreditation of E-Healthcare Organizations. Surveillance, Prevention, and Control of Infection [Hospital Infection Control Standards, 20051]. Joint Commission Perspectives2004; 24.
7. Ray, J. A., R. Doddi, D. Regula, J. A. Williams, and A. Melveger. Polydioxanone (PDS), a novel monofilament synthetic absorbable suture. *Surg. Gynecol. Obstet*. 1981;**153**:497–507.
8. Katz, A. R., D. P. Mukherjaa, A. L. Kaganov, and S. Gordon. A new synthetic monofilament absorbable suture made from polytrimethylane carbonate. *Surg. Gynecol. Obstet*. 1985;**181**:213–222.
9. Bezwada, R.S. and D.D Jamiolkowski. Segmented copolymers of ε-caprolactone and glycolide for new absorbable monofilament sutures. *Trans. Soc. Biomater*. (Annual Meeting, 28 April–2 May 1993), 1993;**XVI**:40.

10. Bezwada, R. et al. Monocryl® suture, a new ultra-pliable absorbable monofilament suture. *Biomaterials* 1995;**16**:1141.
11. Rao, S. Bezwada, Dennis D. Jamiolkowski, In-Young Lee, Vishvaroop Agarwal, Joseph Persivale, Susan Trenka-Benthin, Modesto Erneta, Jogendra Suryadevara, and Alan Yang and Sylvia Liu. Segment copolymers of ε-caprolactone and glycolide. U.S. Patent 5,133,739, July 1992.
12. Chuard, C., M. Herrmann, P. Vaudaux, F.A. Waldvogel, D.P. Lew, J.C. Lucet, and F. Rohner. Resistance of *Staphylococcus aureus* recovered from infective foreign body in vivo to killing by antimicrobiais. *J. Infect. Dis.* 1991;**163**:1369–1373.
13. Rothenburger, S., D. Spangler, S. Bhende, and D. Burkley. In vitro antimicrobial evaluation of Coated VICRYL* Plus antimicrobial suture (coated polyglactin 910 with triclosan) using zone of inhibition assays. *Surg. Infect.* 2002;**3** (Suppl):S79–S87.
14. Messier Kenneth A., and Joseph D. Rhum. U.S. Pat. 4,624,256, November 1986.
15. Jarret Peter K., Donald J. Casey and Leonard T. Lehmann. U.S. Pat. 4,791,929 December 1988.
16. Yannas, I. V. and J. F. Burke. Design of an artificial skin. I. Basic design principles. *J. Biomed. Mater. Res.* 1980;**14**:65–81.
17. Beumerm, G. I., C. A. van Blitterswijk, D. Baker, and M. Ponec. Cell-seeding and in vitro biocompatibility evaluation of polymeric matrices of PEO/PBT copolymers and PLLA. *Biomaterials* 1993;**14**:598–604.
18. Reis, R. L., et al. *Biodegradable Systems in Tissue Engineering and Regenerative Medicine*. CRC Press: Boca Raton, FL, 2005.
19. Jung, K., Rui L. Reis, Julio San Román. Influence of polyglecaprone 25 (Monocryl^{TM}) supplementation on the biocompatibility of a propylene mesh for hernia repair. *Hernia* 2005;**9**:212–217.
20. Eisenberg, M. Composite living skin equivalent. U.S. Patent 6,039,760, 2000.
21. Kerstein, M. and E. D. Reis. Surgical perspective in wound healing. *Wounds* 2001;**13**(2):53–58.
22. Hansbrough, J. F., M. L. Cooper, R. Cohen, R. L. Spievogel, G. Greenleaf, R. L. Nartel, and G. Naughton. Evaluation of a biodegradeable matrix containing cultured human fibroblasts as a dermal replacement beneath meshed skin grafts on athymic mice. *Surgery* 1992;**111**:438–46.
23. Maquet V., A. R. Boccaccini, L. Pravata, I. Notingher, R. Jerome. Preparation of macroporous biodegradable poly(L-lactide-*co*-ε-caprolactone) foams and characterization by mercury instrusion porosimetry, image analysis and impedence spectroscopy. *J. Biomed. Mater. Res. A* 2003;**66**(2):199–213.
24. Vyakarnam Murty N., Zimmerman; Mark C., Scopelianos; Angelo George, Chun; Iksoo, Melican; Mora C., Bazilio; Clairene A., Roller; Mark B., Gorky; V. David. U.S. Patent Nos. 7,112,417; 6,712,850; 6,534,084; 6,355,699; 6,365,149; 6,325,810; 6,333,029; 6,306,424; 6,273,897.
25. Chun, I. Mechanical testing of elastomeric copolymers and their blends—ATRM test results, May 2000.
26. Turck, P. A. Intramuscular and subcutaneous tissue reaction study of caprolactone/glycolide and caprolactone/lactide skin foams in Long Evans rats. *MPI Interim Report*#143-044, 2000.

27. Sherrell, A. L. Investigator Brochure for Project UK/GA/2000/0071, Johnson & Johnson Advanced Wound Care, 2001.
28. Vacanti, J. P., M. A. Morse, W. M. Saltzman, A. J. Domb, A. Perez-Atayde, and R. Langer. Selective cell transplantation using bioabsorbable artificial polymers as matrices. *J. Ped. Surg*. 1998;**23**:3–9.
29. Harris, I. R. et al. In vitro evaluation of biodegradable synthetic foam (Europa) for skin tissue engineering. Wound Healing Technology Resource Center Internal Report, 2001.
30. Cecil C. Vaughn; Paul L. Vaughn, Cecil C. Vaughn III, Paul Sawyer, Melanie Manning, Dena Anderson, Leonard Roseman, and Thomas J. Herbst. *Eur. J. Cardio-thorac. Surg*. 1998;**13**:259.

CHAPTER 12

MEDICINAL APPLICATIONS OF CYANOACRYLATE

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12.1 INTRODUCTION

Humans have attempted wound closure since 1100 BC, when abdominal incisions on mummies were closed with leather ligatures [1]. Sutures, staples, and clips are still used for wound closure and tissue reconstruction. Thus, the field of surgical sutures is expanding very much. Several new products have been developed to improve the results. An avoidance of bleeding during surgery is performed to minimize the morbidity and length of stay. As hospital administration and government regulators desire less invasive procedures, the challenges in surgery are increasing. Moreover, the operations are performed with minimum invasive incisions, using endoscopic, laparoscopic, and robotic approaches. In this process, tools that can reduced the bleeding by blood clotting, vessels sealing, or gluing tissues are gaining increasing importance. Traditionally, wound repair has been performed using sutures. Tissue adhesives were approved and used as alternative for wound closure in several countries. Numerous studies have reported that the use of tissue adhesives is faster, less painful, and more economical than suturing. However, some cases tissue adhesives result in more wound infection than with suturing. The cosmetic outcome of tissue adhesive is better than suturing. The technique of tissue adhesive in wound repairing is useful and skilled for several wound management [2].

Cyanoacrylate medical adhesives were developed as an important tool for the classical suture because of their good cosmetic effect. They also reduced pain and recovery period and hence preferred by patients. These techniques are more efficient, offer low surgery time-and, therefore, reduce cost. Still cyanoacrylate technology has some demerits. Lower homolog of the cyanoacrylate family form brittle adhesive layers in vivo, which may cause polymer biodegradation by adverse tissue response. Higher cyanoacrylates form flexible bonds, which are more biocompatible and expensive. Therefore, there is a need to get high-quality medical adhesives based on cyanoacrylates at an affordable price. For this, the alkoxycarbonyl group of the cyanoacrylate monomers are modified to different ester residue chain length compounds. Short-chain derivatives show a higher degree of toxicity. Inflammation and histological toxicity are related to the cyanoacrylates and alcohol degradation

by-products. As the concentrations of these breakdown products are directly proportional to the rate of degradation, slow degradation rate results in the decrease of toxicity to the tissues [3].

Recently, several cyanoacrylate tissue adhesives have been developed and reported [4]. Cyanoacrylate tissue adhesives are liquid, which polymerizes on contact with tissue surface in an exothermic reaction forming a strong flexible film that opposed the wound edges. As the cyanoacrylate tissue adhesives have been available outside the United States for several decades, their use has been restricted by the inferior mechanical properties of the butyl-cyanoacrylates, limiting their use to small low-tension lacerations and incisions. In 1998 the development and introduction of strong and more flexible 8-carbon 2-octylcyanoacrylate was the major achievement in the wound closure field. Food and Drug Administration (FDA) approval and multiple studies have consistently demonstrated that wound closure with 2-octylcyanoacrylate (OCA) is comparable with other wound closure devices and offers several additional benefits. The use of tissue adhesives might seem intuitive, but improper wound selection and technique may result in suboptimal results [4].

At present, commercially available cyanoacrylate tissue adhesive is only the *n*-butyl-2-cyanoacrylate. Although *n*-butyl-2-cyanoacrylate is an effective tissue adhesive, it also has certain limitations. It produces brittle adhesive film and increases the cytotoxicity. Recently, *n*-octyl-2-cyanoacrylate was formulated to correct these deficiencies. Slow degradation of cyanoacrylate polymers lowered the concentration of by-products and produced less inflammation. Additionally, plasticizers are added to increase tissue compatibility, which flexes with the skin and remains inherent for long duration. This strong and flexible bond may allow its use in longer incisions. This technology has proposed the production of several cyanoacrylate adhesives of different ester radical lengths. It is achieved by introducing a new degradation and stabilization system of cyanoacrylate oligomers, that is, depolymerization. As compared to other methods the cyanoacrylate technology is cleaner, faster, and simple. Various cyanoacrylate homologs with the C1−C8 range length are obtained using this technology for medical adhesives applications with diverse setting times, bond strengths, degradation rates, and biocompatibilities. The technology is also used in the synthesis of nanostructured homo- and copolymeric poly(cyanoacrylates). Its usefulness in drug delivery and targeting systems and in several other medicinal applications are discussed here.

The use of polyglycerol sebacate acrylate (PGSA) as a biodegradable and biocompatible tissue adhesive has been reported [5]. Clinical applications of "topical tissue adhesives" synthetic cyanoacrylate in various medical devices was approved by the U.S. FDA 1976. It includes cyanoacrylate liquid bandages, dental cements, and the like [6]. A series of lipophilic derivatives of gemcitabine poly(amino poly(ethylene glycol) cyanoacrlate-co-hexadecyl cyanoacrylate (poly (H2NPEGCA-*co*-HDCA)) nanospheres and nanocapsules has been synthesized. More lipophilic derivatives such as 4-(*N*)-stearoylgemcitabine, are incorporated to a high yield. A cytotoxicity study was done on two human cancer cell lines and compared to gemcitabine and free 4-(*N*)-stearoylgemcitabine [7].

A folic acid multifunctional nanodevice (MND) is used as ligand, which conjugated to the terminal amido group of polyamino polyethylene transfer and deoxyribonucleic acid (DNA) condenser. The experimental result shows optimum complexation of DNA, that is, about 97%. The MND loading polydiribose nucleic acid protamine sulphate (pDNA/PS) shows the luciferase activity over 0.5 ng luciferase/mg protein in KB cells. MND shows the highest transfection efficiency in KB cells as compared to A549 cells and other formulations such as LipofectAMINE or free pDNA/PS. Transfection efficiency of controlled multifunctional nano devices (CMNDs) is found greater than in lipid film coated with poly(HNPEGCA-*co*-HDCA) and dioleoyl phosphotidyl ethanolamine (DOPE). In addition, during encapsulation MND also shows low cytotoxicity. Overall, MND works as a more potential nonviral vector used for DNA delivery [8].

Microencapsulated transferrin-conjugated PEGylated polycyanoacrylate nanoparticles transferrin-polyethylene glycol nanoparticles (TF−PEG nanoparticles) have been developed to determine the stability of pDNA at various conditions in vitro targeting cell association. The open circular forms of pDNA increased on emulsifing pDNA with organic solvent. Poly(aminopoly(ethylene glycol) cyanoacrylate-*co*-hexadecyl cyanoacrylate) [poly(H2NPEGCA-co-HDCA)] shows a slight influence on pDNA in 0.1 M NaHCO3 at high concentration. K562 cell binding affinity of TF−PEG nanoparticles was found greater than of nontargeted PEG-nanoparticles. The results indicate that TF-PEG nanoparticles were useful in pDNA delivery to target cells [9].

PEG-coated biodegradable polycyanoacrylate nanoparticles (PEG nanoparticles), conjugated to transferrin for paclitaxel delivery have been developed. PEGylated paclitaxel-loaded nanoparticles were prepared using solvent a evaporation technique. Actively targeted nanoparticles (ATN) were prepared by coupling transferrin with PEG nanoparticles. The result shows an average encapsulation efficiency of these ATNs. A low-burst effect of paclitaxel-loaded ATN released only 16.2% of the drug within the first phase. Subsequently, paclitaxel release profiles display a sustained-release phase. The amount of cumulated paclitaxel release over 30 days was 81.6%. ATN exhibited a markedly delayed blood clearance in mice, and the paclitaxel level from ATN remained much higher at 24 h as compared to free paclitaxel injection. On intravenous (IV) administration, the distribution profiles of ATN in S-180 solid-tumor-bearing mice shows tumor accumulation of paclitaxel increases with time, and the paclitaxel concentration in the tumor was about 4.8 and 2.1 times higher than those from paclitaxel injection and PEG nanoparticles at 6 h after IV injection. Tumor burden on ATN-treated mice was found much smaller as compared with free paclitaxel. In addition, the life span of tumor-bearing mice was significantly increased with the treatment with ATN. Thus, PEG-coated biodegradable polycyanoacrylate nanoparticles conjugated with transferrin could be an effective carrier for paclitaxel delivery [10].

Nanoparticles of poly(isobutylcyanoacrylate) with dispersed insulin in a pluronic acid solution has been developed. The results show that particle diameter was reduced by increasing the Pluronic acid concentration. Nanoparticles

prepared in the presence of 2.5% Pluronic acid results in particles of 85-nm diameters and 59% intraparticular insulin load without the use of the oily core. Streptozocin-induced diabetic rats were used for in vivo study. Subcutaneous injection of insulin nanoparticles prolonged the duration of glycol (cyanoacrylate-*co*-hexadecyl cyanoacrylate) [poly(H2NPEGCA-*co*-HDCA)] to synthesize poly (polate-HNPEGCA-*co*-HDCA), protamine sulfate (PS) for nuclear hypoglycemic effect from 6 to 72 h. As compared to nonencapsulated insulin, oral absorption of the entrapped insulin was found significantly better [11].

Butylcyanoacrylate adhesive scaffold is used in a porcine model to enhance the tensile strength of tissue samples repaired ex vivo. The cyanoacrylate-doped scaffold repairs approximately 30% organ tissues and approximately 20% vascular tissues. These scaffold-enhanced adhesives offer a quick application with less skilled professionals, paraprofessionals, and bystanders in the emergency department [12].

A technique of latent fingerprints using controlled cyanoacrylate vapor exposure in fuming cabinets is used for artifacts recovered in investigations of a forensic crime scene. This technique involves the deposition of monomeric cyanoacrylate under warm, moist conditions on the object to polymerize and produce a white film that renders the fingerprint visible. Raman spectroscopic studies show several bands in the spectra of poly(ethyl-cyanoacrylate), indicating the presence of residual monomer and other species formed of the polymerizing material. The possible effect of unreacted monomer and other polycyanoacrylate film impurities on spectral analalysis are also highlighted [13].

Spray drying is the technique used to deliver particles to the lungs via a dry powder inhaler (DPI). Lactose was used as the excipient and spray-dried with gelatin and polybutylcyanoacrylate nanoparticles. Some of the carrier particles were found hollow, whereas others had a continuous matrix. Gelatin nanoparticles incorporated the matrix and sometimes accumulated at one end of lactose. Polycyanoacrylate nanoparticles formed clustered at different spots within the lactose carriers. Dispersion of the powder with passive inhaler and impact measurements showed that incorporation of the nanoparticles did not affect the fine particle fraction (FPF) or mass median aerodynamic diameter (MMAD). The nanoparticles were delivered to the lungs via carrier particles, which further dissolve in an aqueous environment of lung epithelium. This allows for new drug-targeting strategies using nanoparticles for pulmonary delivery of drugs and diagnostics [14].

Molecular simulation of doxorubicin with butyl-polycyanoacrylate has been used in brain drug delivery. Aggregation and desegregation mechanism of doxorubicin release can be summarized as follows: Oligomeric polyalkyl cyanoacrylates (PACAs) are lipophilic entities that facilitate scavenge of amphiphilic doxorubicin already during polymarization. The establishment of hydrogen bonds between the ammonium N−H function and the cyano groups is noteworthy. The cohesion in PACA nanoparticle comes, therefore, from a blend of dipole−charge interaction, H bonds, and hydrophobic forces [15].

Poly(bu-cyanoacrylate) (PBCA) nanoparticles coated with polysorbate 80 drug were also delivered to the brain. These carriers can penetrate the blood–brain barrier (BBB) and deliver various drugs, that is, proteins and peptides. In this process hydrophilic and lipophilic compounds were eliminated from the brain with glycoprotein. The suspension of polysorbate-coated PBCA nanoparticles is introduced into the blood. Apolipoproteins of the blood plasma adsorb on the particle surface and interact with low-concentrate receptors. Lipoproteins situate in endothelial cells of cerebral vessels, thus stimulating endocytosis [16].

Nanoparticles (NPs) of PBCA and poly(octyl-cyanoacrylate) (POCA) have been developed to study biodistribution in mice by loading radioiodine 125I-labeled radio iodine (IUDdR). Nanoparticulate injection to the brain increases the counts because of IUdR bioavailability in the brain when IUdR was loaded into NPs. The result shows nanoparticles crossed the blood–brain barrier and reached brain tissues [17].

Thermosensitive poly(cyanoacrylate) nanoparticle applications at high hydrostatic pressure (HHP) were also studied. Poly(cyanoacrylate) nanoparticles appeared to be extremely baroresistant. This process allowed the successful inactivation of vegetative bacteria, yeast, and fungi. HHP act as a new method for polymer drug carrier sterilization [18]. The combination of polycyanoacrylate with bone morphogenetic protein-2 (rhBMP-2) nanoparticle injection is prepared by an emulsion method. Their biological activities were tested in vivo as well as in vitro. The rhBMP-2 polycyanoacrylate nanoparticle was found to be homogeneous and stable. In vivo study showed 80% of bone morphogenetic protein (rhBMP) gets released in 10 days. The addition of the rhBMP nanoparticle to the marrow stromal cell (MSC) system increases ALP levels in MSC culture significantly [19].

It was found that the PEGylated polyalkylcyanoacrylate nanoparticles easily penetrate into the central nervous system to a larger extent than other formulations because of their long-circulating properties in blood. Thus, PEGylated polycyanoacrylate nanoparticles are used as a brain delivery reported system in neuroinflammatory diseases [20]. Pharmacokinetic study of poly(bu-cyanoacrylate) nanoparticles loaded with sulfonated aluminum phthalocyanine (Photosense) as a delivery system has been reported [21]. Poly(bu-cyanoacrylate) nanoparticles (PBCA nanoparticles) loaded with the hexapeptide dalargin has been studied. These nanoparticles were coated with apolipoproteins AII, B, CII, E, or J without or after precoating with polysorbate 80. The antinociceptive threshold was measured by the tail-flick test on I.V. injection to mice. The antinociceptive effect was found to be reduced in apolipoprotein-E-deficient mice. Transportation of drugs loaded to poly(bu-cyanoacrylate) nanoparticles with apolipoproteins B and E across the blood–brain barrier has been achieved [22].

Recently, biodegradable poly(ethyl-cyanoacrylate) (PECA) nanospheres were used to deliver insulin orally. Screen absorption enhancers are used to protect insulin loaded PECA in vivo after the oral administration to streptozotocin-induced diabetic rats. Orally administered insulin absorption was evaluated using

the hypoglycemic effect. Blood glucose level is significantly reduced on oral administrations of protease inhibitor with insulin-loaded PECA nanospheres [23].

Polycyanoacrylate adhesives were also used in inguinal hernia repair. Total extraperitoneal (TEP) repair is the most commonly employed technique by laparoscopic surgeons [24]. In this system biodegradable poly(iso-bucyanoacrylate) (PIBCA) collides with pilocarpine particles. Incorporating it in a Pluronic F127 (PF127), a gel delivery system has been developed. Its ability to prolong the release of pilocarpine is evaluated. The poly(isobutyl cyanoacrylate) nanocapsules (PIBCA-NC) of pilocarpine dispersed in the PF127MC gel delivery system showed significant potential to prolong a delivery of pilocarpine and other hydrophobic drugs [25].

Allopurinol-loaded poly(Et-cyanoacrylate) nanoparticles were tested against *Trypanosoma cruzi* using in vitro cultures of epimastigotes. High concentration of unloaded nanoparticles on zero-line cell cultures has been used for cytotoxicity study. The result shows that the poly (Et-cyanoacrylate) nanoparticles are good carriers of drugs against *T. cruzi*. Allopurinol-loaded nanoparticles significantly increased the trypanocidal activity in comparison to the free drugs [26].

12.2 CURRENT NEED IN MODERN SURGICAL APPROACHES

The modern practice of surgery is facing two problems. High-cost devices and an aging population. Both these factors push the technical frontiers of surgical practices to a more challenging level. These surgical conditions favor smaller and minimally invasive procedures. These procedures are tried with smallest incisions using laproscopic, endoscopic, and robotic technologies. In addition, it is assumed that reduction in blood transfusions may result in superior surgical outcomes [27–29]. It means that reducing the need for transfusions not only reduces the costs but also improves the results [30, 31]. In this process the devices used may decrease bleeding, reduce operative time, and improve the quality of surgical tissue. Such devices may successfully perform the modern surgical approaches with several improvements and reduced costs.

12.2.1 Current Challenges

Surgeons use modern procedures and devices for painless and fast surgery. Good skill and knowledge is necessary for excellent results. The modern devices are continuously in demand for improved outcome. So, there have been continuously increasing technical challenges for improved results. To improve the outcomes of these modern devices, certain effective hemostats, sealants, and adhesives should be developed and used [32].

12.2.2 Ideal Materials

The ideal material should possess important properties that enable its use by surgeons. Safety, efficacy, usability, cost, and approvability [33] are the five

important factors that should be taken into consideration when developing ideal devices. The material used must be safe and its metabolites should not be dangerous to patients. It should be free from infectious disease transmission. While using these materials and their metabolites, no carcinogenic or immunogenic response should be occurring. Second, the material must be effective when used for its labeled indication. Adjunctive materials cannot be used as substitutes for excellent surgical technique. Surgeons should not employ this product when suture is required. Some surgeon's don't apply such sealants and adhesives properly because of the lack of sufficient knowledge and experience. Efforts should be taken to setup standards for the efficacy of those devices that are not widely used. Third, the material must be easily usable and easily applied by the surgeons. It is also easily reconstituted by circulating in the operating room (OR). Fourth, cost and cost-effectiveness remain important considerations. If a product shortens the operative procedure and length of hospitalization, it will reduce costs [34]. Cost–benefit, however, remains an underutilized method of analysis in the clinical trials of these products. Such analysis gains an increasing importance in the future. Finally, the approval for this product is also an important aspect. FDA approval of these materials increases their clinical use by surgeons.

12.2.3 Education on the Use of Adhesives and Sealants

The education of surgeons is a long and challenging process. Surgical procedures are generally learned by observation, performance, and instruction. New materials must be presented by teachers in a clear manner so that surgeons in training get benefits. Established surgeons need to know about new materials. Their education should not be in a verbal or written form such as lectures, articles, books but by hands-on workshops or laboratory work. Also being aware of the use of new materials in medicine may be useful in facilitating new approaches. An important contribution of surgical success is the fast adoption of new and effective modalities. Several new materials are available to those willing to achieve proficiency in this area [32].

12.2.4 Cyanoacrylates

To date three cyanoacrylate products have been approved by U.S. FDA. The approved commercial cyanoacrylates are 2-octyl cyanoacrylate (Dermabond, J&J) and the two commercial forms of *n*-butyl-2-cyanoacrylates (Indermil, Covidien, Norwalk, CT; and Histoacryl and Histoacryl Blue, Tissueseal, Ann Arbor, MI). Dermabond, Indermil, and Histoacryl and Histoacryl Blue are used for surgical closure of skin incisions [35–37]. Additionally, Dermabond and Indermil are also approved as barries to bacterial skin penetration [35,

36]. These cyanoacrylate formulations are used for holding skin edges together. In the presence of hydroxyl groups, these adhesives polymerize within 30 s. The cyanoacrylate formulations are recommended for use with deep dermal sutures for skin closure. This is because dehiscence can occur if the cyanoacrylates are attached to the skin. Currently, cyanoacrylates are approved for topical use only and are not for internal use. When placed on the skin, the cyanoacrylate releases heat by an exothermic reaction and may cause discomfort in some patients. Only thin layers, applied externally to minimize heat and encourage wound healing effects, are recommended. They are not to be used in high-tension areas, across joints, on mucosal surfaces, at mucocutaneous junctions, or at dense hair areas. These cyanoacrylates sloughed during the process of skin exfoliation and do not require removal. Safety concerns of these materials include eye injury [35–37]. Specific precautions need to be taken to avoid dripping these products onto the eye or eyelids. If inadvertently applied onto the eyes, the eyes are flushed with saline and the ophthalmic treatment is applied. An ophthalmologist should be consulted and the eyes are kept moist. Debonding is likely to occur within 3 days, facilitated by periods of weeping. In this situation surgical removal may help. These materials should not be applied to patients who have hypersensitivity to cyanoacrylates, formaldehyde, or D&C violet No. 2. Their use in infected, gangrenous, decubitus, or poorly healing wounds should be avoided. Cyanoacrylates should not be employed below the skin because foreign body reactions may occur. Application to wet or alcohol-containing wounds should be avoided because moist wounds may increases the rate of polymerization. Cyanoacrylates are easy to remove with acetone, petroleum jelly, warm soapy water, or 5% sodium bicarbonate. Accidental spillage can be absorbed with talc. Several clinical trials for the efficacy of cyanoacrylates in skin closure have been performed and reported [38–41]. These materials are stored at room temperature [22°C (72°F)] for limited periods of time, although each one of them recommends long-term storage at a specific temperature range. Dermabond should be stored below 30°C (86°F) and Indermil and Histoacryl stored at 2°C (36°F) to 5°C (41°F). Multiple thin-layer application techniques are recommended to avoid heat discomfort. The material should not be scrubed at the sites of application. It sloughs in 5–10 days, and patients show its effect after 48 h.

All cyanoacrylates are marketed in 0.5-mL ampoules of various forms. Dermabond is also sold as an applicator pen in a high-viscosity formulation designed for easy application. Indermil ampoules are attached to an intravenous access catheter to facilitate application. A significant use of cyanoacrylates for esophageal and gastric varices [42] and clinical trials of internally usable cyanoacrylates have been performed [43, 44]. Cyanoacrylates are relatively inexpensive as compared to other tissues. (See Figs. 12.1–12.3.)

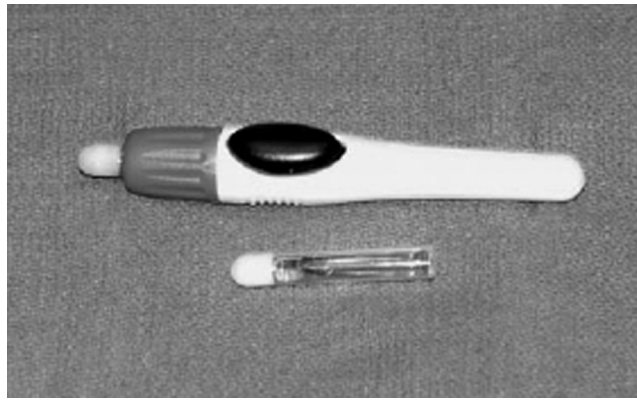

FIGURE 12.1 Octyl cyanoacrylate (Dermabond, Johnson & Johnson) (From [32].)

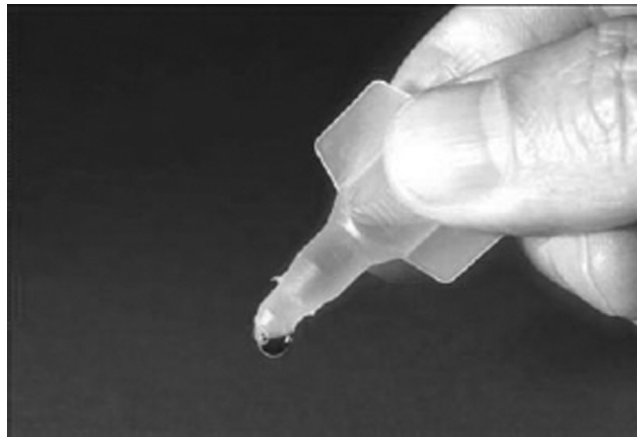

FIGURE 12.2 *n*-Butyl-2-cyanoacrylate (Indermil, Covidien). (From [32].)

12.3 IMPORTANT APPLICATIONS OF CYANOACRYLATES

Certain important applications of cyanoacrylates have been studied and reported recently. Some of them are discussed in the following sections.

12.3.1 Topical Skin Adhesives

Every year about 7 million traumatic lacerations [45] and 26 to 90 million surgical incisions are performed by surgeons, emergency physicians, and primary care practitioners [46]. Traditionally, these wounds and incisions are closed with

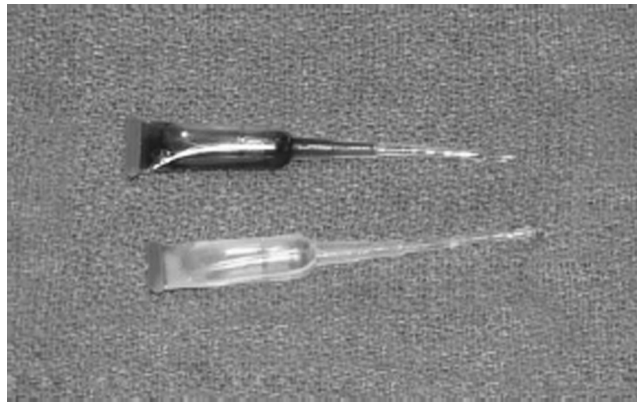

FIGURE 12.3 *n*-Butyl-2-cyanoacrylate (Histoacryl and Histoacryl Blue, Tissueseal). (From [32].)

sutures, staples, or surgical tape. Recently, this can be done using tissue adhesives. A wound closure device should be easily used, painless, and faster, should result in excellent cosmesis, be nonremovable, and be cheaper [47]. Although, none of the available devices meet all of these needs, the topical cyanoacrylate tissue adhesives offers an ideal wound closure device. The cyanoacrylates were synthesized first in 1949 by a German chemist [48]. After 10 years wound closure with a cyanoacrylate was reported [49]. At first, short-chain cyanoacrylates were crudely manufactured and found to be toxic in pharmacological doses [50]. Long-chain derivatives are comparatively nontoxic, thus more sophisticated manufacturing techniques have been developed of pure nontoxic monomers and used as adhesives clinical practice.

Preparation, Structure, and Mechanical Properties. The cyanoacrylates are obtained by condensing formaldehyde and cyanacetate [51]. The resultant cyanoacrylate monomer was distilled to get a pure form and to remove toxic by-products formed in the synthesis [52]. Furthermore, the monomer was formulated with stabilizers, plasticers, and additives to improve its biocompatibility, stability, and clinical performance [52]. The basic cyanoacrylate monomer is a low-viscous liquid. On contact with anionic substances, such as blood, it polymerizes to long chains forming a solid film that bridges the wounds and holds the apposed wound edges together [52]. The adhesive film generally sloughs off within 5–10 days as the epidermis regenerates. Although the monomers are formulated to improve their performance, their properties are based on the number of carbons in their side chain (Fig. 12.4). In general, the strength and several other physical properties of cyanoacrylate adhesives are directly related to the length and complexity of their alkyl side chain. Short, straight-chain derivatives (ethyl or butyl cyanoacrylate) form tight and stronger bonds as compared with complex or long-chain derivatives (propoxypropyl

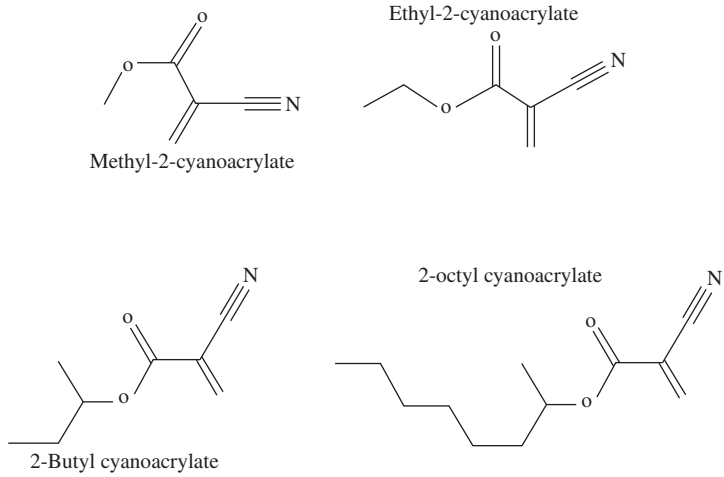

FIGURE 12.4 Structure of the cyanoacrylate tissue adhesives. (From [53].)

cyanoacrylate and octylcyanoacrylate). Although these tight bonds are used as an interface bond, they tend to form brittle bonds that fracture prematurely when used as a topical bridge leading to lower clinical tensile strength than the longer chain derivatives [53].

Overall, the cyanoacrylate topical skin adhesives are important wound closure devices. They also work as a microbial barrier and as an occlusive dressing. Their mechanical properties, advantages, and disadvantages helps the clinician to determine their proper use. Although they are easy to use and offer many potential advantages to both patients and practitioners, they are not suitable for all type of wounds. Careful and meticulous wound evaluation, preparation, and closure are required regardless of wound closure device to achieve optimal results [53].

Advantages and Disadvantages of the Cyanoacrylate Adhesives. Cyanoacrylate topical adhesives possess several advantages over traditional devices. Application of the adhesives is comparatively rapid and painless; hence no local anesthetics are necessary. In addition, as the cyanoacrylate adhesives slough off within 5–10 days, removal of devices is not required. This procedure may be painful and threatening, especially in children. Although the cyanoacrylate adhesives are expensive, when all other expenses are included, the other sutures are more expensive [54]. The use of such adhesives reduces the risks of needle sticks [55] and prevents the suture mark formation on either side of the wound. They save the operative time, especially with longer incisions and lacerations. Most of the surgical patients prefer topical adhesive to sutures or staples [56, 57]. In a study of emergency patients it was found that two thirds of patients had a prior or current laceration and preferred sutures to adhesives [58].

In the early 1960s it was demonstrated that a moist wound environment was optimal for wound healing [59, 60]. Both the butyl-cyanoacrylates and octylcyanoacrylates create an occlusive wound healing environment, a barrier to microbial penetration [61, 62], and decreased the wound infection rates in comparison with other sutures [63]. Its resulted in the use of cyanoacrylate adhesives as consumer liquid bandages [64]. Commercially available octylcyanoacrylate liquid bandage does not have enough tensile strength for wound closure. The cyanoacrylates also show antimicrobial properties both in vitro and in animal models [65–67].

Even though the topical skin adhesives have several advantages, they also have certain disadvantages compared to the other wound closure devices. On exposure to moisturem, they result in adhesive failure; hence cyanoacrylate adhesives are not used in mucous membranes such as the mouth. The adhesives are also not to be used over hair-bearing areas.

12.3.2 Tissue Glues

Tissue glues are used in surgery to promote adhesion, hemostasis, and wound healing. Broadly, these tissue glues may be synthetic or biologic. Synthetic tissue glues are mainly derived from cyanoacrylate and used for skin approximation.

Principles of Wound Healing. The wound-healing foundation involves mainly three phases: inflammatory, proliferative, and remodeling. The inflammatory phase is initiated because of tissue injury with extravasations of blood and leads to hematoma and platelet aggregation. Activated platelets play an important role by initiating the clotting cascade by releasing growth factors and cytokines. The coagulation cascade leads to the formation of a stable crosslinked fibrin mesh. The matrix formed by the fibrin mesh provides the regenerative space and scaffolding for cell migration and proliferation [68].

First, Neutrophilic cells migrate to the wound region, providing both protection from infection and removal of debris. This is followed by monocytes that differentiate into macrophages and T lymphocytes whose role is still unclear [69–71]. The beginning of the proliferative phase starts with the proliferation of blood vessel endothelial cells at the site of injury. In this process the migration of fibroblasts, which form the extracellular matrix and undifferentiated mesenchymal stem cells provide the basis of regeneration. Remodeling is the third and final phase of wound healing. It is characterized by the reshaping and reorganization of the newly formed duplicate tissue of the original preinjured one. The process of remodeling is lengthy and may take up to 2 years before reaching its original state. Several groups of compounds have been used for the purposes of clotting, adhesion, and ultimately wound healing [68].

Cyanoacrylates. Cyanoacrylates are synthetic adhesives used topically for skin closure since 1959. Only one cyanoacrylate derivative, 2-octylcyanoacrylate (Dermabond; Ethicon Inc., Somerville, NJ)] is approved by the (FDA).

2-Octylcyanoacrylate has been used for skin closure in hand surgery, oral surgery, head and neck surgery, and cosmetic surgery [72, 73]. These tissue adhesives are used for long incisions with minimal tension [74]. On comparison with sutures, Dermabond shows no difference in cosmoses at 3 months with reduced time for closure and less pain [75]. The available class of adhesive is recommended for topical use only because permeation into human tissue may be toxic and generate an inflammatory response. The preclusion of use within a healing wound is the major limitation for soft-tissue surgical applications [76].

12.3.3 Glue for Gastric Varices

Bleeding gastric varices treatment is one of the frontiers of endoscopy. Hemostatic methods used for esophageal varices are not suitable for gastric varices. This is because of their large size and extensive distribution. Tissue necrosis results in endoscopic interventions that cause significant complications. Consequently, patients with gastric variceal bleeding are referred for a transjugular intrahepatic portosystemic stent shunt (TIPS) or surgical shunt procedure.

Cyanoacrylate glue was found to be ideal for endoscopic treatment of gastric varices. Native cyanoacrylate is a liquid similar to water, hence it lends itself to intravariceal injection. Cyanoacrylates on addition to a physiologic medium blood polymerizes quickly, forming a hard substance. This results not only in rapid hemostasis in cases of active bleeding, but it also prevents the recurrence of bleeding from the treated varix. Because of their efficacy and safety, cyanoacrylates are used in specific clinical situations such as pregnancy and in children [77].

Indication. Most of the cyanoacrylate studies for gastric varices fail to provide subset analyses on the basis of location or morphology. Gastric varices treated were fairly homogenous with a fundal location in 94% and a nodular or tumorous type in 94% [78]. In another study 50% of varices included in the endosonography arm were gastroesophageal in location, and a stratification of results according to location or morphology was not provided [79]. On the basis of currently available data, cyanoacrylate injection is better compared to TIPS at a significantly lower cost. Factors that increase the risk associated with TIPS, including advanced liver disease, jaundice, and renal failure, favor the use of cyanoacrylate therapy. It is not clear whether eradication of esophageal varices increases the risk of bleeding from gastric varices.

Equipment and Injection Technique. At present, no standard equipment or injection technique is available. The injector used has a liver-lock metal fitting because, since histoacryl is caustic in nature, it may crack a plastic hub. The dilution ratio of Histoacryl to Lipiodol has varied among studies. The rationale for diluting Histoacryl with Lipiodol is delayed to avoid an instant polymerization reaction. A series of new cyanoacrylate homolog with prolonged polymerization time are available that are suitable for undiluted injection [80].

Treatment Protocol. It is necessary to standardize the protocol for the endpoint of treatment. The purpose of cyanoacrylate injection is the obliteration of visible varices. The term "obliteration" is more accurately described as "eradication." Complete obliteration needs the cyanoacrylates to induce mucosal necrosis at the site of injection. During the second period the rates of late-onset recurrent bleeding were significantly lower. The investigators give endosonography credit for the improved results. Although it is not proved, this modal is superior in the determination of variceal obliteration. Fluoroscopy is rarely used to monitor the intravariceal injection of cyanoacrylate. To provide real-time monitoring of cyanoacrylate injection technically the use of a convex array echoendoscope is possible [80].

Safety. Cyanoacrylate injection has been found amazingly safe. However, cases of severe complications including cerebral stroke, pulmonary embolism, portal vein embolism, splenic infarction, and retrogastric abscess have been reported. Moreover, further investigations are essential to minimize and eliminate the risk of embolization. In one of the investigation, the patient, who had a cough, was found to have small pulmonary emboli on the chest radiograph [79]. Similarly, after histoacryl injection, embolic deposits were found on the chest radiograph of one patient who developed a cough. Two cases of visceral fistulae after Histoacryl injection were also reported with some new unreported complications [81]. One gastric varices patient on cyanoacrylate treatment showed the complication of empyema of the left pleural cavity after 6 months. A fistulous association between the gastric fundus and pleural cavity was also documented. Operative investigation showed the gastric fundus to be adherent to the diaphragm with inflammation. This suggests that an injection of Histoacryl into the stomach wall may caused this complication, as histoacryl is known to be ulcerogenic to the tissues. In a second case, Histoacryl injection to a bleeding esophageal varix patient was complicated by a fistulous tract to the right para-mediastinal region, which resulted in fatal pulmonary abscesses.

The issue of safety also extends to equipment and personnel. A clogging case of the endoscope accessory channel requires repairing. Clogging occurs even though precautionary lubrication with silicone oil can be done to prevent adherence of Histoacryl. All personnel should wear protective goggles to avoid serious eye injury as a result of cyanoacrylate spraying [80].

Future Studies. One study shows the efficacy and overall safety of glue injection of cyanoacrylate for gastric varices. However, several questions regarding the indications, equipment, technique, treatment protocol, and safety remain unsolved. The hemostatic efficacy of glue injection coupled with high mortality associated with nonendoscopic treatments is in favor of glue injection. Optimal management of the stable patient without active bleeding needs further study. Results of endoscopic treatment using sclerotherapy or band ligation are different for the subsets of gastric varices. A random trial by comparing cyanoacrylate injection to sclerotherapy or band ligation is warranted for junctional varices.

Future studies must consider the control for varix obliteration as the endpoint of treatment. Finally, future studies must address the risk of embolization. Although the risk is small, embolization can be fatal. Certain modifications in injection technique and new cyanoacrylate compounds may reduce or eliminate this risk [80].

12.3.4 Endoscopic Therapy of Peptic Ulcer Bleeding

Gastroduodenal ulcer is the root of upper gastrointestinal bleeding. Even complete eradication of *Helicobacter pylori* (HP), the rate of ulcer bleeding remains unchanged [82, 83]. However, in western countries, the epidemiology is changing with an increasing rate of HP-negative ulcers. To a certain extent the rate of drug-induced ulcers with a malignant clinical course is increasing. In gastroduodenal ulcerations, the rate of complicated ulcers (bleedings and perforations) mostly remains unchanged. Consequently, the mortality rate remains constant at between and 7 and 10% [84]. Surgery was frequently required in ulcer patients with ongoing bleeding or high-risk features to solve the situation [85]. However, the endoscopic therapy has been recognized for the treatment of these ulcers [86, 87]. It is important to acknowledge the role of endoscopy in acute upper gastrointestinal (GI) bleeding situations. General care, endoscopy, surgery, and radiological service with selective embolization needs a multidisciplinary panel of expertise to offer the optimal treatment.

Indications. Endoscopy plays an important role along with the initial monitoring of patients with cute upper GI bleeding. This comprises partly the determination of subsequent care (out-patient, general ward, intensive care), partly a precise diagnosis, and partly endoscopic therapy. All these are very important elements in endoscopy, usually integrated in a single endoscopic procedure. On the basis of endoscopic findings and results of therapy, the subsequent handling is precisely planned, and the prognosis is more precisely conveyed to the patient and any caretakers. A low-risk endoscopic stigmata patient was associated with a significant lowering of hospital cost, without any adverse effects. Even if discharge is not possible, triage helps for care within the hospital. However, such triage benefit requires strict adherence of the recommendations [88]. Early endoscopy increases the chances of an intermittent hemorrhage from an obscure focus. Sometimes, a stable situation may be acutely aggravated by manipulation of the ulcer in the duodenal bulb. In such an emergency the operating theater is required, in addition to abundant intravenous access and cross-matched blood. Analysis and comparison of the different modalities is very difficult. Several available comparative studies are flawed by expertise, and/or preferences. Local preference, expertise, and availability will be major determinants in the choice of method.

Injection Therapy. Injection therapy is used to create a hydrostatic pressure, tissue edema, vasoconstriction, and inflammatory changes in ulcer region, with

acute and long-lasting effects. Technically, it is the easiest method to learn and implement, although injections are more precise. Acute angulation of the endoscope tip does not affect the efficacy of the injection. One injection rarely reduces the viability to make another. More importantly, the injections must be placed at the anticipated location of the feeding vessel going into the ulcer [89].

Cyanoacrylates Used. Histoacryl (*N*-butyl-2-cyanoacrylate) is a well-known tissue adhesive possessing a variety of medical applications. It is regularly used in gastric varices treatment in Europe, while its role in bleeding peptic ulcers is more undetermined. A mixture of 0.5 mL of Histoacryl glue with 0.8 mL of Lipiodol is injected in small boluses directly into the bleeding vessel. After injecting the glue, the sterile water boluses are used to empty the residual glue in the needle. The substance rapidly hardens with an inherent risk of endoscope damage. Moreover, the glue/lipiodol mixture is viscous and offers significant resistance to injection. To avoid premature solidification of the glue, care must be taken to do the procedure quickly [89].

Comparing the Histoacryl study with saline/epinephrine injections, 126 patients with bleeding or nonbleeding visible vessels were randomized. Initial hemostasis was achieved in 92 and 95% of patients, with slightly more rebleeding in the epinephrine group. In active bleeding ulcers, the benefit of Histoacryl was more assured. Because of the rare but feared complication, most doctors use this substance only as a last resort before surgery. The substance is unavailable in several countries, including the United States. [90].

12.3.5 Endoscopic Surgery in Pediatric Practice

In open and endoscopic surgical practice, tissue adhesives or glues are classified in three categories on the basis of their origin that is, biological, synthetic, and genetically engineered polymer protein glues [91]. All cyanoacrylate (CA) and noncyanoacrylate polymeric sealants are animal-derived hemostatic agents considered to be of biological origin. Cyanoacrylates are introduced in surgical procedures and used as embolic agents by interventional radiologists and endoscopists. Several studies shows CAs are easy to use as compared to the other tissue adhesives [92].

Discussion. Perfect hemostasis in open surgery is one of the main problems in pediatric laparoscopic surgery. Several tools (i.e., titanium or reasorbable clips, ultrasonic scalpel, and bipolar and tripolar forceps) have been developed to solve this problem. Tissue adhesives employed in pediatric surgery reduced the risk of postoperative complications, especially in cases of parenchymal resection or vascular anastomoses. Glues are found to be good for controlling bleeding in the case of intervention on the spleen or liver. These advantages are of greater applicability for pediatric laparoscopic surgery. The use of adhesives proved to be of great interest and efficacy. In particular, in esophageal or

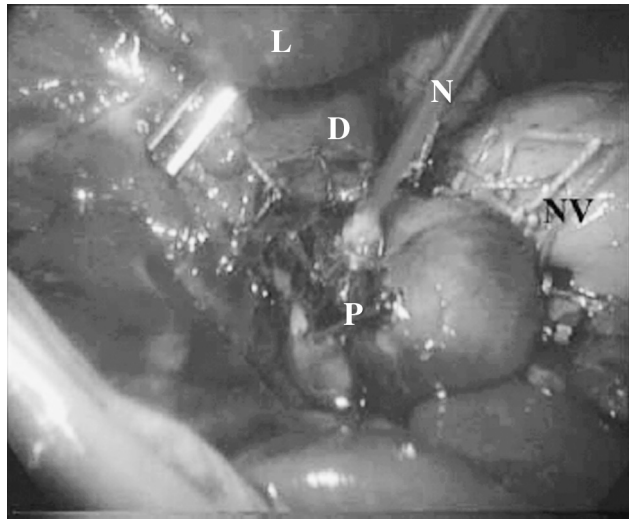

FIGURE 12.5 Glue is extremely useful in cases of complications, such as in this perforation of the right side of the valve during a Nissen fundoplication. NV, Nissen valve; N, needle; L, liver; D, diaphragm; P, perforation. (From [93].)

gastric perforation Glubran has two advantage, sealing and consolidation. It acts within a short time with an application on sutures and anastomoses; thus rapid consolidation and sealing of the suture is possible. Glubran is easy to use, does not polymerize in the needle, acts rapidly, and does not give foreign body reactions. This glue is extremely useful in organ perforation to consolidate the suture or control the hemostasis in case of bleeding. In addition, not only are tissue adhesives useful in abdominal surgery, they are also used in thoracic surgery and urology. As per reports from medical teams during the Vietnam War and unpublished literature, CAs may be associated with the occurrence of malignant tumors on the liver or spleen. Initial experimental study on the carcinogenicity of CAs have failed to find any association with malignant transformation (Figs. 12.5 and 12.6.) [93].

12.3.6 Colorectal Variceal Bleeding

Colorectal variceal bleeding is rare but may be massive and fatal. Its initial appearance includes severe, painless rectal bleeding in most cases. Bleeding from esophagogastric varices is the major complication of portal hypertension. In contrast, ectopic varices may also develop in the duodenum, ileum, cecum, ascending, descending, and rectosigmoid colon, and abdominal stomas. Most common sites for colorectal varices are the rectum and cecum. No significant difference in the prevalence of lower gastrointestinal (GI) varices in patients with cirrhosis, noncirrhotic portal fibrosis, or extrahepatic portal venous

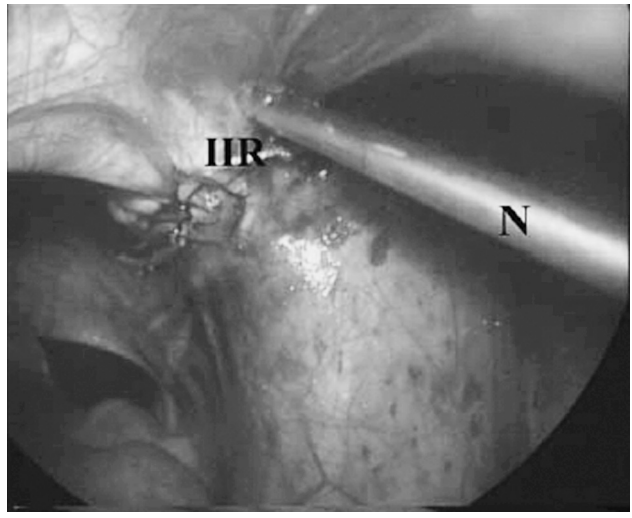

FIGURE 12.6 Glue was adopted to close a patent processes vaginalis in a congenital inguinal hernia. N, needle; IIR, internal inguinal ring. (From [93].)

obstruction was observed. Theoretically, by repeated endoscopic variceal sclerotherapy (EVS) obliteration, esophageal varices may increase the flow through other portosystemic collaterals and subsequent varices formation and bleeding. Hepatic venous pressure and the severity of cirrhosis are similar in cirrhotic patients with or without anorectal varices. Sustained reduction of portal venous pressure is achieved. Substantial perioperative complications restrict the use of surgical procedures in high-risk and advanced cirrhotic patients. Ligation of the inferior mesenteric vein is used as an alternative to the portocaval shunt, although it increases the chances of esophageal varices development. Under certain conditions, colectomy is lifesaving, although it carries postoperative morbidity and mortality resulting from hepatic failure and sepsis.

Once bleeding is recognized, endoscopic sclerotherapy with different sclerosants has been used. Although active bleeding may be arrested, rebleeding remains a problem. Major complications reported with sclerotherapy of colorectal varices have been necrosis and perforation. A massive hemorrhage from rectosigmoid varices were treated by endoscopic injection of *N*-butyl-2-cyanoacrylate tissue adhesive, achieving temporary hemostasis. The cyanoacrylate glue injection may offer an approach to controlling active bleeding from colorectal varices, allowing time to stabilize the patient and to plan definite therapy (Fig. 12.7) [94].

12.3.7 Tissue Adhesives in Corneal Cataract Incisions

Introduction of clear corneal incisions manifest a new era in cataract surgery. The beginning of clear corneal wounds, phacoemulsification experienced a new

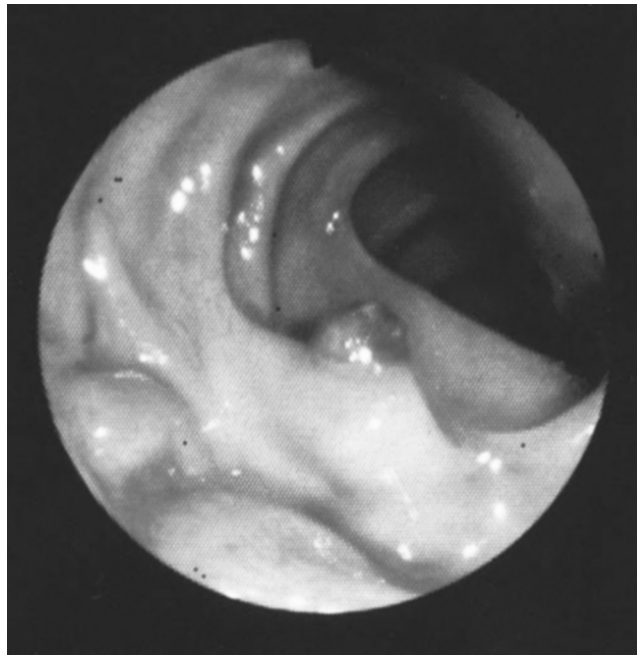

FIGURE 12.7 Rectosigmoid varix with stigmata of recent hemorrhage, protruding thrombus, at one of the bleeding sites (From [94].)

breakthrough, with reduced surgical time, faster postoperative recovery, reduced rates of astigmatism, and less complications associated with scleral tunnels such as conjunctival manipulation and hyphemas [95].

Clear Corneal Incisions and Endophthalmitis. Several ophthalmic surgeons have recognized advantages of corneal incisions. Use of these incisions now far exceeds and may dominate the field of cataract extraction. The American Society of Cataract and Refractive Surgery (ASCRS) performed a survey in July 2003 that demonstrated that 72% of the respondents used clear corneal incisions for phacoemulsification. No-suture closure was the preferred technique for 92% of the responding physicians [96]. Although clear corneal wounds are the favored incision in cataract surgery, recent scrutiny increased the risk of postoperative endophthalmitis with these wounds. Recently, a multinational prospective study conducted by the European Society of Cataract and Refractive Surgery (ESCRS) demonstrated that the risk for contracting endophthalmitis following phacoemulsification cataract surgery was 0.38% [97]. Several studies have been conducted to compare the incidence of postoperative endophthalmitis in clear corneal incisions compared with the traditional scleral-tunnel incisions.

Tissue Adhesives and Clear Corneal Incisions. Synthetic and biological tissue adhesives have been utilized for various purposes in ophthalmology, including corneal perforations, wound repair, amniotic membrane transplantation, and even as sealants for glaucoma and plastics procedures. Cyanoacrylate-based glues have been most widely used as adhesives in the treatment of corneal perforations. Several studies have been published on the efficacy of cyanoacrylate-based glue as a substitute for sutures in clear corneal incisions. A series of prospective interventional cases in which 2-octyl cyanoacrylate with parabens was used as a wound sealant after clear corneal cataract surgery has been studied [98]. In each case, the wound was sealed with one or two drops of 2-octyl cyanoacrylate with parabens. All 51 eyes had watertight wounds after the application of 2-octyl cyanoacrylate with parabens. On the first day of postoperation, 45 eyes (88%) had tissue adhesive still completely covering the wound, two eyes (4%) had tissue adhesive over portions of their wounds, and four eyes (8%) had no adhesive noted on their wounds. Frequently, the complaint was a transient foreign body sensation, found in 31 patients (61%). Other side effects included focal or diffuse bulbar conjunctival hyperemia and retention of glue on the second postoperative visit [98].

Novel Tissue Adhesives. Many new novel adhesives have been developed as an alternate modality to sealing corneal wounds. A modified chondroitin sulfate aldehyde adhesive was compared with standard sutures for sealing corneal incisions in rabbit eyes. This study measured the tensile strength of the wounds and the leakage through the wounds with intraocular pressure variation. Results showed the adhesive was an effective sealant for corneal incisions and may be superior to sutures for this purpose [99]. The efficacy and clinical tolerance of a new acrylic copolymer tissue adhesive, adhesives of alicanate (ADAL), in corneal surgery has been evaluated [6]. In this study, corneal incisions in rabbit eyes were sealed with ADAL bioadhesive, hydrated with a balanced salt solution, or closed with 10-0 nylon sutures. The incisions undergo weekly tensile strength testing and clinical, histopathologic, and confocal microscopic evaluations. Clinical evaluation showed no incision leakage or anterior chamber reaction in any of the groups. The patients in the ADAL group showed a mild increase in wound neovascularization and opacification with slight greater inflammatory reaction as compared with the hydrated group. Tensile strength during the first week was significantly greater in the ADAL group than in the other groups. Thereafter, all groups had similar tensile strength [100].

12.3.8 Adhesives for Tissue Reconstruction

Sutures, staples, and clips are considered the gold standard for wound closure and tissue reconstruction. These devices have the same function as employed in ancient times to surgical tools [101]. Use of sutures is essential for closure of a wide variety of simple wounds. However, in many cases the suture is either

unable to effect repair or the repair interferes with the functional rehabilitation of the site [102]. An example of this includes closure of dura mater [103], urethral defects [104], lung tissue [105], nerve repair [106], hernia repair [107], blood vessel anastomosis [108], and traumatic injuries [109]. In these cases, various surgical "glues" have been used, including synthetic polymer glues such as cyanoacrylate or polyethylene glycol (PEG) laser-activated albumin solders, and chitosan adhesives [110].

Cyanoacrylate Glues. Cyanoacrylates are the esters of cyanoacrylic acid [(C5H5NO2)CH2═C(CN)COOCH3]. Acrylate double bond plays an important role in the polymerization (hardening) of this glue. Cyanoacrylate monomers polymerizes on contact with water or a weak base, such as cell membranes and tissue. The process of hydroxylation takes place through the elimination of oxygen from the substances being bonded [111]. Modification of the alkyl side chain has been done to produce cyanoacrylates with different bonding properties. The compound becomes more biocompatible as the ester chain increases from one carbon to higher numbers. On the contrary, short-chain esters (<4C) or their degradation products are toxic [111]. Cyanoacrylate derivatives with short side chains degraded rapidly into cyanoacetate and formaldehyde. These degradation products accumulate in tissues and produce significant histotoxicity confirmed by both acute and chronic inflammation. The longer alkyl chains of glues (e.g., *N*-butyl-2-cyanoacrylate) lower the degradation significantly, limiting accumulation of products to be effectively eliminated by tissues.

Cyanoacrylate glues have been successfully applied in ophthalmology to repair corneal perforations. A small skin patch is usually glued to the cornea, which has suffered perforation. The procedure improved visual outcomes without re-epitheliazation into the damaged zone [111, 112]. Cyanoacrylate glues also prevent collagenase production, which leads to stromal melting, and possess bacteriostatic activity against gram-positive organisms [113]. The major concern of these glues is toxicity of cyanoacrylate because of direct contact with the corneal endothelium and lens. The efficacy of *N*-butyl-2-cyanoacrylate in corneal perforations was also compared with fibrin glue in a randomized clinical trial [114]. Both glues were effective in the closure of corneal perforations up to 3 mm in diameter, although fibrin glue provides faster healing and significantly induced less corneal vascularization. It required a significant longer time for the formation of the adhesive plug. Cyanoacrylate glues are also used as conventional suturing for wound closure. A random trial was conducted to compare 2-octylcyanoacrylate with standard subcuticular suture for surgical incision closure. The glue was found easy to use without any complications. Despite these positive results, intracutaneous absorbable sutures are still preferred for skin closure as they usually guarantee no tissue dehiscence and a better wound cosmesis than cyanoacrylate glue [115–117].

12.4 SURGICAL APPLICATIONS OF CYANOACRYLATE ADHESIVES

12.4.1 Biochemistry of Cyanoacrylate

Adhesive cyanoacrylate is a compound synthesized by the condensation of cyanoacetate and formaldehyde in the presence of a catalyst. Cyanoacrylate adhesive film develops by rapid polymerization, triggered by an hydroxyl group on the surface being glued. In this process, water acts as a catalyst to activate the anionic polymerization [118]. The good wetting potential of proteins makes CA compounds extremely adhesive to biological tissue, even being sufficient adhesive used in tissue grafting. This phenomenon takes place because CA compounds retain their adhesive qualities in the presence of moisture. Furthermore, in biological applications also they show bacteriostatic and hemostatic activity. Diluted CA surgical glues allow the antimicrobial activity against cultures of *Bacillus subtilis* when used over a 3-week period clinical study [119].

12.4.2 Surgical Applications

Several surgical and medical applications of CA and their homologs are studied. CA has been primarily used as a tissue adhesive for surgical and traumatic wound repair. A randomized controlled trial showed no detectable differences in the cosmetic result or complication rate at 3 months, when wound closure was carried out either mechanically or chemically [120]. Wound closure study incurred an additional benefit of much faster than traditional suturing [120]. Instead of mechanical suturing CA compounds may also help to reduce the incidence of keloid formation, an important cosmetic consideration for certain racial groups where this complication often occurs. Widespread replacement of sutures with CA compounds has significant benefits, beyond the cosmetic improvements experienced by patients. From an occupational health and patient safety perspective, there would be much to gain, as the risk of percutaneous exposure incidents (PEI) could be largely lessened by a reduced necessity for sharp instruments, such as suture needles. The risk of PEI is known to be high in surgical settings. In one study, 87% of surgeons reported experiencing a percutaneous injury at some stage during their career, whereas the risks posed by PEI related to blood-borne pathogens, such as the human immunodeficiency virus (HIV), hepatitis B virus, and hepatitis C virus. Significant psychological and economic costs are also associated with this particular hazard [121]. To avoid these problems, at least one recent study has found that the possibility of successfully integrating CA and its homologs into non-"sharps"-based operations [121]. The less sharps-intensive procedures would be a positive step in reducing PEI hazards within the operating room, hospital emergency department, and elsewhere.

12.4.3 Toxicity in Clinical Use

The toxicity of medical-grade CA is lower in domestic compound due to the lower proportion of impurities. It does not mean that surgical-grade CA

compounds have no toxicity. When used as a tissue adhesive, CA may cause inflammation and tissue necrosis in vivo. Thrombotic events have been described with CA-associated necrosis. An other study describes that the toxicity of CA adhesive was an important contributor to postoperative arterial occlusive lesions in several patients who had undergone surgery for non-ruptured cerebral aneurysms, using CA and 100% cellulose cotton for repair. More recently, intimal thickening occurred due to inflammatory reactions, results in successive narrowing, or occlusion of the intracranial arteries [122].

In addition, due to CA compounds calcification has been observed in an earlier animal experiment using human cadaveric dura. In certain studies, CA derivatives have been found to be histotoxic to tissues. The CA polymer degrades to formaldehyde and cyanoacetate compounds, and accumulated within treated areas. Certain evidence suggests that formaldehyde released from CA may be toxic to cells both in vitro and in vivo. This degradation rate can be reduced by increasing the alkyl chain length from methyl cyanoacrylate (MCA), ethyl cyanoacrylate (ECA), isobutyl-CA, to isohexyl-CA radicals. Lowering the degradation process reduced the number of releasing toxic by-products. Improvements in CA tissue adhesive delivery systems reduced the amount of CA being used with high accuracy of its application. Aside from clinical toxicity, the actual metabolic processes in vivo and the overall metabolism of CA remain unknown. However, some radiolabel has been detected in rat urine following dermal application and oral administration of MCA, as well as in the feces of rats following oral administration of MCA. Despite this, no toxicokinetic information is available for ECA. Furthermore, clinical and experimental research into the metabolic fate of CA is clearly needed [118].

12.4.4 Cytotoxicity

Numerous clinical and experimental studies have investigated the cytotoxicity of CA and their derivatives. CA found to be cytotoxic to cells in vitro both with direct contact and in extract dilution assays of cell culture [123], even though a 1 : 10 dilution of surgical CA glue was shown to be nontoxic to L929 cells following polymerization [119]. The polymerization or setting reaction of CA is exothermic, causing cell damage in cell cultures. When filter paper is used as the vehicle to minimize direct cell contact with the adhesive, this particular form of CA was found to be cytotoxic to human oral fibroblast cell culture. Earlier work proposed that CA adhesives may form lipid hydroperoxides, which activate prostaglandin and thromboxane biosynthesis. This may explain the existence of certain thrombosis, associated with necrosis on the use of CA adhesives in vivo. The use of prostaglandin H synthetase inhibitors, such as acetylsalicylic acid (aspirin) and indomethacin reduce the cytotoxicity of CA up to eightfold in vitro [118].

12.4.5 Toxicity Resulting from Occupational Exposure

Cyanoacrylates are also widely used in various industries because of their adhesive properties and easy handling. However, their volatility and chemical

reactivity may produce a hazardous atmospheric environment for workers. Several conditions are recognized for increasing CA toxicity in the workplace, including preexisting dermatological, allergic, respiratory, and neurological conditions, as well as accidental applications and skin contact. Experience of skin patch testing for acrylate allergies, including allergies to ECA, has been recently reported. However, in this study no allergic reactions of ECA compounds were reported. The precise allergic mechanism among humans requires further clinical investigation of CA, believed to be of the Th2 type. Apart from cutaneous reactions, CA was also associated with pulmonary symptoms. Despite this caveat, some researchers have reported peripheral neuropathy associated with CA exposure. Accidental application to eyes, ears, and mouth, having the potential to compromise respiratory function, appeared in the scientific literature. The toxic effect of CA therefore should not be underestimated in the working environment [118].

12.4.6 Genotoxicity and Carcinogenic Potential of Cyanoacrylates

Although, as previously noted, the accurate in vivo metabolic processes of CA are not still clearly understood, there is the possibility of some long-term risk of these compounds. For example, methyl-2-CA adhesives were found to have direct mutagenic effects in standard in vitro *Salmonella* microsome mutagenicity assay. In certain studies, volatile compounds produced from methyl-2-CA shown to be mutagenic to a strain of *S. typhimurium* (strain TA100). Additional concerns have been raised that methyl-2-CA may pose a potential carcinogenic hazard. At present, no published reports with clinical data on the long-term toxicity/carcinogenicity effects of either MCA or ECA are available [121], whereas the possibility of CA-induced neoplasia raises some concerns in toxicology and occupational health. Further studies will be needed to provide conclusive data on the genotoxicity and carcinogenic potential of CA compounds [118].

12.5 CONCLUSION

In recent years CA and their homologs are being used in surgical and medical practices, posing a certain risk to medical staff and patients. As a result, various adverse effects are known to be occurring by exposure to CA. Although CA adhesives show cytotoxicity in vitro, only commercial CA adhesives release cytotoxic substances for a prolonged period. At present insufficient evidence to prove CA carcinogenic to humans is available, although it may show toxicological effects on the neurological and respiratory systems in addition to contact dermatitis and urticaria. These effects are harmful to surgical and other medical staff, and they should avoid direct contact with CA compounds. Healthcare management also considers the adoption of appropriate environmental control measures, such as acid carbon filters. At a molecular level, increasing the alkyl chain length of CA reduces the toxicity by slowing the degradation rate of the

molecule and thereby reducing the toxic substances released. Even though the metabolism and the harmful effects of this material and its metabolites still require further investigation, CA and its compounds have been widely used for surgical and medical practice in recent years.

REFERENCES

1. Majno, G. *The Healing Hand. Man and Wound in the Ancient World.* Harvard University Press: Cambridge, MA, 1975.
2. King, M. E. and A. Y. Kinney. Tissue adhesives: A new method of wound repair, *Nurse Pract*. 1999;**24**(10):66.
3. Pawar, R. P., S. R. Sarda, R. M. Borade, A. Jadhav, S. A. Dake and A. J. Domb. Cyano acrylate polymers in medical applications. *Recent Pat. Mater. Sci.* 2008;**1**:186–199.
4. Singer, A. J. and H. C. Thode, Jr. A review of the literature on octylcyanoacrylate tissue adhesive. *Am. J. Surg*. 2004;**187**:238–248.
5. Mahdavi A., L. Ferreira, C. Sunback, J. W. Nichol, E. P. Chan, D. J. D. Carter, C. J. Bettinger, S. Patanavanich, L. Chignozha, E. Ben-Joseph, A. Galakatos, H. Pryor, I. Pomerantseva, P. T. Masiakos, W. Faquin, A. Zumbuehl, S. Hong, J. Borenstein, J. Vacanti, R. Langer, and J. M. Karp. A biodegradable and biocompatible gecko inspired tissue adhesive. *PNAS* 2008;**105**(7):2307–2312.
6. George M. J., US FDA perspective on the regulations of medical-grade polymers: Cyanoacrylate polymer medical device tissue adhesives. *Expert Review of Medical Devices* 2008;**5**(1):41–49.
7. Stella B., Arpicco, S., Rocco, F., et al. Encapsulation of gemcitabine lipophilic derivatives into polycyanoacrylate nanospheres and nanocapsules. *Int. J. Pharm.* 2007;**344**(1–2):71–77.
8. Gao, Y., W. Gu, L. Chen, Z. Xu, and Y. Li. A multifunctional nano device as non-viral vector for gene delivery: In vitro characteristics and transfection. *J. Control. Release* 2007;**118**(3):381–388.
9. Li, Y., M. Ogris, E. Wagner, J. Pelisek, and M. Ruffer. Nanoparticles bearing polyethylene glycol coupled transferrin as gene carriers: Preparation and in vitro evaluation. *Int. J. Pharm.* 2003;**259**(1–2), 93–101.
10. Xu, Z., W. n. Gu, J. Huang, H. Sui, Z. Zhou, Y. Yang, Z. Yan, Y. Li. In vitro and in vivo evaluation of actively targetable nanoparticles for paclitaxel delivery. *Int. J. Pharm.* 2005;**288**(2):361–368.
11. Mesiha, M. S., M. B. Sidhom, and B. Fasipe. Oral and subcutaneous absorption of insulin poly(isobutylcyanoacrylate) nanoparticles. *Int. J. Pharm.* 2005;**288**(2): 289–293.
12. McNally, H., M. Karen, J. N. Riley, and D.L. Heintzelman. Scaffold enhanced albumin and n-butyl cyanoacrylate adhesives for tissue repair: Ex vivo evaluation in a porcine model. *Biomed. Sci. Instrum.* 2003;**39**:312–317.
13. Edwards, H. G. M. and J. S. Day. Anomalies in polycyanoacrylate formation studied by Raman spectroscopy: Implications for the forensic enhancement of latent fingerprints for spectral analysis. *Vibrat. Spectro*. 2006;**41**(2):155–159.

14. Sham, J. O. H., Y. Zhang, W. H. Finlay, W. H. Roa, and R. Lobenberg. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. *Int. J. Pharm.* 2004;**269**(2):457–467.
15. Poupaert, J. H. and P. Couvreur. A computationally derived structural model of doxorubicin interacting with oligomeric polyalkyl cyanoacrylate in nanoparticle. *J. Control. Release* 2003;**92**(1–2):19–26.
16. Alyautdin, R.N., J. Kreuter, and D. A. Kharkevich. Nanoparticles for drug delivery to brain. *Eksp. Klin. Farmakol.* 2003;**66**(2):65–68.
17. Aminabhavi, T.M., A. R. Kulkarni and P. V. Kulkarni. Polymers in drug delivery: Radioactive iodine 125I loaded polymeric nanoparticles for biodistribution study in mice. *Polymer News* 2003:**8**(3):83–86.
18. Brigger I., L. L. Armand, P. Chaminade, M., Besnard, Y. Rigaldie, A. Largeteau, A. Andremont, L. Grislain, G. Demazeau, and P. Couvreur. The stenlying effect of high hydrostatic pressure on thermally and hydrolytically labile nanosized carrie. *Pharm. Res.* 2003;**20**(4):674–683.
19. Wu, D.C., M. Wan, S. Zhang, H. Wu, and Y. Hu. Preparation and property of bone morphogenetic protein-2 polycyanoacrylate nanoparticle for injection. *Yingyong Huaxue* 2002;**19**(7):613–616.
20. Calvo, P., B. Gouritin, H. Villarroya, F. Eclancher, C. Giannavola, C. Klein, J. P. Andreux, and P. Couvreur. Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat. *Eur. J. Neurosci.* 2002;**15**(8):1317–1326.
21. Steiniger, S., S. E. Gelperina, I. N. Skidan, A. I. Bobruskin, S. E. Severin, and J. Kreuter. Optimization of photosensitizer pharmacokinetics with biodegradable nanoparticles. Proceedings—28th International Symposium on Controlled Release of Bioactive Materials and 4th Consumer & Diversified Products Conference, San Diego, CA. 2001;**2**:1273–1274.
22. Kreuter, J., D. Shamenkov, V. Petrovet al. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. Proceedings—28th International Symposium on Controlled Release of Bioactive Materials and 4th Consumer & Diversified Products Conference, San Diego, CA. 2001;**1**:587–588.
23. Radwan, M. A., and H.Y. Aboul-Enein. The effect of oral absorption enhancers on the in vivo performance of insulin-loaded poly(ethyl cyanoacrylate) nanospheres in diabetic rats. *J. Microencapsul.* 2002;**19**(2):225–235.
24. Beattie, G. C., S. Kumar, and S. J. Nixon. Laparoscopic total extraperitoneal hernia repair: Mesh fixation is unnecessary. *J. Laparoendosc. Adv. Surg. Tech. A* 2000;**10**(2):71–73.
25. Desai, S. D., and J. Blanchard. Pluronic F127-based ocular delivery system containing biodegradable poly(isobutyl cyanoacrylate) nanocapsules of pilocarpine. *Drug Deliv.* 2000;**7**(4):201–207.
26. Gonzalez-Martin, G., C. Figueroa, I. Merino, and A. Osuna. Allopurinol encapsulated in polycyanoacrylate nanoparticles as potentiallysosomotropic carrier: Preparation and trypanocidal activity. *Eur. J. Pharm. Biopharm.* 2000;**49**(2):137–142.
27. Klein, M., C. B. Mahoney, C. Probst, H. D. Schulte, and E. Gams. Blood product use during routine open heart surgery: The impact of the centrifugal pump. *Artif. Organs* 2001;**25**:300–305.

28. Earley, A.S., V.H. Gracias, E. Haut, C.P. Sicoutris, D. J. Wiebe, P. M. Reilly, and C. W. Schwab. Anemia management program reduces transfusion volumes, incidence of ventilatorassociated pneumonia, and cost in trauma patients. *J. Trauma* 2006;**61**:1–5.
29. Rana, R., B. Afessa, M. T. Keegan, F. X. Whalen, Jr., G. A. Nuttall, L. K. Evenson, S. G. Peters, J. L. Winters, R. D. Hubmayr, S. B. Moore, and O. Gajic. Transfusion in the ICU Interest Group. Evidence-based red cell transfusion in the critically ill: Quality improvement using computerized physician order entry. *Crit. Care Med.* 2006;**34**:1892–1897.
30. Martinez, V., A. Monsaingeon-Lion, K. Cherif, T. Judet, M. Chauvin, and D. Fletcher. Transfusion strategy for primary knee and hip arthroplasty: Impact of an algorithm to lower transfusion rates and hospital costs. *Br. J. Anaesth.* 2007;**99**:794–800.
31. Murphy, G. J., B. C., Reeves, C. A. Rogers, S. I. Rizvi, L. Culliford, and G. D. Angelini. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. *Circulation* 2007;**27**:2523–2524.
32. Spotnitz, W. D. and S. Burks. Hemostats, sealants, and adhesives: components of the surgical toolbox. *Transfusion* 2008;**48**:1502–1516.
33. Spotnitz, W.D. Tissue adhesives: Science, products and clinical use. In W. S. Pietrzak, and B. L. Eppley, (Ed.). Musculoskeletal Tissue Regeneration: Biological Materials and Methods. Humana Press: Totowa, NJ, 2008;pp. 531–546.
34. Meraney, A. M., and I. S. Gill. Financial analysis of open versus laparoscopic radical nephrectomy and nephroureterectomy. *J. Urol.* 2002;**167**:1757–1762.
35. Package Insert. Dermabond, Johnson & Johnson, New Brunswick, NJ, 2003.
36. Spotnitz W. D., and S. Burks, Hemostats, sealants, and adhesives: components of the surgical toolbox, *Transfusion*, 2008;**48**(7):1502–1516.
37. Jay G., FDA Issues Draft Guidance on Labor Monitoring Systems, Herpes Virus Assays, *Journal of Clinical Engineering*, 2007;**32**(3):88–89.
38. Quinn, J., A. Drzewiecki, M. Li, I. Stiell, T. Sutcliffe, T. Elmslie, and W. Wood. A randomized, controlled trial comparing a tissue adhesive with suturing in the repair of pediatric facial lacerations. *Ann. Emerg. Med.* 1993;**22**:1130–5.
39. Sinha, S., M. Naik, V. Wright, J. Timmons, and A. C. Campbell. A single blind, prospective, randomized trial comparing n-butyl 2-cyanoacrylate tissue adhesive (Indermil) and sutures for skin closure in hand surgery. *J. Hand Surg. [Br]* 2001;**26**:264–265.
40. Simon, H. K., D. J. McLario, T. B. Bruns, W. T. Zempsky, R. J. Wood, and K. M. Sullivan. Long-term appearance of lacerations repaired using a tissue adhesive. *Pediatrics* 1997;**99**:193–195.
41. Greenwald, B. D., S. H. Caldwell, E. E. Hespenheide, J. T. Patrie, J. Williams, K. F. Binmoeller, L. Woodall, and O. Haluszka. N-2-butyl-cyanoacrylate for bleeding gastric varices: A United States pilot study and cost analysis. *Am. J. Gastroenterol.* 2003;**98**:1982–1988.
42. Schenk, W. G. 3rd, W. D. Spotnitz, S. G. Burks, P. H. Lin, R. L. Bush, and A. B. Lumsden. Absorbable cyanoacrylate as a vascular hemostatic sealant: A preliminary trial. *Am. Surg.* 2005;**71**:658–661.

43. Lumsden, A. B., and E. R. Heyman, Closure Medical Surgical Sealant Study Group. Prospective randomized study evaluating an absorbable cyanoacrylate for use in vascular reconstructions. *J. Vasc. Surg*. 2006;**44**:1002–1009.
44. Spotnitz, W. D. and S. Burks. Hemostats, sealants, and adhesives: Components of the surgical toolbox. *Transfusion* 2008;**48**:1502–1516.
45. Singer, A. J., H. C. Thode, Jr., and J. E. Hollander. National trends in emergency department lacerations between 1992–2002. *Am. J. Emerg. Med*. 2006;**24**: 183–188.
46. U.S. Markets for Current and Emerging Wound Closure Technologies, 2001–2011. Medtech Insight: Tuscan, CA, 2002.
47. Singer, A. J., J. E. Hollander, and J. V. Quinn Evaluation and management of traumatic lacerations. *N. Engl. J. Med*. 1997;**337**:1142–1148.
48. Ardis, A. E. U.S. Patent nos. 2467926 and 2467927, 1949.
49. Coover, H. W., F. B. Joyner, N. H. Shearer, and T. H. Wicker. Chemistry and Performance of cyanoacrylate adhesives. *J. Soc. Plast. Eng*. 1959;**15**:413–417.
50. Leonard, E. The N-alkaalpha cyanoacrylate tissue adhesives. *Ann. N. Y. Acad. Sci*. 1968;**146**:203–213.
51. Mattamal, G. J. History and background. In *Tissue Adhesives in Clinical Medicine*, 2nd ed. J. V. Quinn, BC Decker: Hamilton, Canada, 2005, pp. 15–26.
52. Quinn, J. V. Clinical approaches to the use of cyanoacrylate tissue adhesives. In *Tissue Adhesives in Clinical Medicine*, 2nd ed. J. V. Quinn, (Ed.). BC Decker: Hamilton, Canada, 2005, pp. 27–76.
53. Singer, A. J., J. V. Quinn, and J. E. Hollander. The cyanoacrylate topical skin adhesives. *Am. J. Emerg. Med*. 2008;**26**:490–496.
54. Osmond, M. H., T. P. Klassen, and J. V. Quinn. Economic comparison of a tissue adhesive and suturing in the repair of pediatric facial lacerations. *J. Pediatr*. 1995;**126**(6):892–895.
55. Gordon, C. A. Reducing needle-stick injuries with the use of 2-octyl cyanoacrylates for laceration repair. *J. Am. Acad. Nurse Pract*. 2001;**13**:10–12.
56. Spauwen, P. H., W. A. de Laat, and E. H. Hartman. Octyl-2-cyanoacrylate tissue glue (Dermabond) versus Monocryl 6×0 sutures in lip closure. *Cleft Palate Craniofac. J*. 2006;**43**:625–627.
57. Roberts, A. C. The tissue adhesive Indermil and its use in surgery. *Acta Chir. Plast*. 1998;**40**:22–25.
58. Singer, A. J., C. Mach, H. C. Thode, S. Hemachandra, F. S. Shofer, and J. E. Hollander. Patient priorities with traumatic lacerations. *Am. J. Emerg. Med*. 2000;**18**:683–686.
59. Winter, G. D. Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature* 1962;**193**:293–294.
60. Hinman, C. D, H. Maibach, and G. D. Winter. Effect of air exposure and occlusion on experimental human skin wounds. *Nature* 1963;**200**:377–378.
61. Mertz, P.M., S. C. Davis, A. L. Cazzaniga, A. Drosou, and W. H. Eaglstein. Barrier and antibacterial properties of 2-octyl cyanoacrylate-derived wound treatment films. *J. Cutan. Med. Surg*. 2003;**7**:1–6.
62. Singer, A. J., M. Nable, P. Cameau, D. D. Singer, and S. A. McClain. Evaluation of a new LOD for excisional wounds. *Wound Repair Regen*. 2003;**11**:181–187.

63. Silvestri, A., C. Brandi, L. Grimaldi, G. Nisi, A. Brafa, M. Calabrò, and C. D'Aniello. Octyl-2-cyanoacrylate adhesive for skin closure and prevention of infection in plastic surgery. *Aesthetic Plast Surg*. 2006;**30**(6):695–699.

64. Eaglstein, W. H., T. P. Sullivan, P. A. Giordano, and B. M. Miskin. A liquid adhesive bandage for the treatment of minor cuts and abrasions. *Dermatol. Surg*. 2002;**28**:263–277.

65. Quinn, J. V., J. L. Maw, K. Ramotar, G. Wenckebach, and G. A. Wells. Octylcyanoacrylate tissue adhesive wound repair versus suture wound repair in a contaminated wound model. *Surgery*, 1997;**122**:69–72.

66. Quinn, J.V., M. H. Osmond, J. A. Yurack, and P. J. Moir. N-2butylcyanoacrylate: risk of bacterial contamination and its antimicrobial effects. *J. Emerg. Med*. 1995;**13**:581–585.

67. Singer, A. J., M. Mohammad, G. Tortora, H. C. Thode, Jr., and S. A. McClain. Octylcyanoacrylate for the treatment of contaminated partial-thickness burns in swine: A randomized controlled experiment. *Acad. Emerg. Med* 2000;**7**:222–227.

68. Yoo, J., S. Chandarana, and R. Cosby. Clinical application of tissue adhesives in soft-tissue surgery of the head and neck. *Curr. Opin. Otolaryngol. Head Neck Surg*. 2008;**16**:312–317.

69. Anderson, J. M. The cellular cascades of wound healing. In *Bone engineering*. J. E. Davies, (Ed.). em squared: Toronto, 2000, pp. 81–93.

70. Szpaderska, A. M., E. I. Egozi, R. L. Gamelli, and L. A. DiPietro. The effect of thrombocytopenia on dermal wound healing. *J. Invest. Dermatol*. 2003;**120**: 1130–1137.

71. Bhanot, S. and J. C. Alex. Current applications of platelet gels in facial plastic surgery. *Facial Plast. Surg*. 2002;**18**:27–33.

72. Sinha, S., M. Naik, V. Wright, J. Timmons, and A. C. Campbell. A single blind, prospective, randomized trial comparing n-butyl 2-cyanoacrylate tissue adhesive (Indermil) and sutures for skin closure in hand surgery. *J. Hand Surg [Br]*. 2001;**26**: 264–265.

73. Perez, M., I. Fernandez, D. Marquez, and R. M. Bretana. Use of N-butyl-2-cyanoacrylate in oral surgery: Biological and clinical evaluation. *Artif. Organs* 2000;**24**:241–243.

74. Blondeel, P. N., J. W. Murphy, D. Debrosse, J. C. Nix, L. E. Puls, N. Theodore, and P. Coulthard. Closure of long surgical incisions with a new formulation of 2-octylcyanoacrylate tissue adhesive versus commercially available methods. *Am. J. Surg*. 2004;**188**(3):307–313.

75. Quinn, J., G. Wells, T. Sutcliffe, M. Jarmuske, J. Maw, I. Stiell, and P. Johns. A randomized trial comparing octylcyanoacrylate tissue adhesive and sutures in the management of lacerations. *JAMA* 1997;**277**(19):1527–1530.

76. Gosain, A. K. and V. B. Lyon. The current status of tissue glues. Part II. For adhesion of soft tissues. *Plast. Reconstr. Surg*. 2002;**110**:1581–1584.

77. Dhiman, R. K., R. Biswas, N. Aggarwal, H. Sawhney, and Y. Chawla. Management of variceal bleeding in pregnancy with endoscopic variceal ligation and N-butyl-2-cyanoacrylate: report of three cases. *Gastrointest. Endosc*. 2000;**51**:91–93.

78. Huang, Y. H., H. Z. Yeh, G. H. Chen, C. S. Chang, C. Y. Wu, S. K. Poon, H. C. Lien, and S. S. Yang. Endoscopic treatment of bleeding gastric varices

by N-butyl-2-cyanoacrylate injections: long-term efficacy and safety. *Gastrointest. Endosc.* 2000;**52**:160–167.

79. Lee, Y. T., F. K. L. Chan, K. W. Ng, V. K. S. Leung, K. B. Law, M. Y. Yung, S. C. Sydney, J. Chung, and J. Y. Sung. EUS-guided injection of cyanoacrylate for bleeding gastricvarices. *Gastrointest. Endosc.* 2000;**52**:168–174.
80. Binmoeller, K. F. Glue for gastric varices: Some sticky issues. *Gastrointest. Endosc.* 2000;**52**(2):298–301.
81. Battaglia, G., T. Morbin, E. Patarnello, E. Ancona, C. Merkel, and M. Corona. Visceral fistulae as a complication of sclerotherapy for oesophageal and gastric varices using isobutyl-2-cyanoacrylate. *Gastrointest. Endosc.* 2000;**52**:267–270.
82. Silverstein, F. E., D. A. Gilbert, F. J. Tedesco, N. K. Buenger, and J. Persing. The national ASGE survey on upper gastrointestinal bleeding, II. Clinical prognostic factors. *Gastrointest. Endosc.* 1981;**27**:80–93.
83. Czernichow, P., P. Hochain, J. B. Nousbaum, J. M. Raymond, A. Rudelli, J. L. Dupas, M. Amouretti, H. Gouérou, M. H. Capron, H. Herman, R. Colin. Epidemiology and course of acute upper gastro-intestinal haemorrhage in four French geographical areas *Eur. J. Gastroenterol Hepatol.* 2000;**12**(2):175–81.
84. Rockall, T. A., R. F. Logan, H. B. Devlin and T. C. Northfield. Incidence of and mortality from acute upper gastrointestinal haemorrhage in the United Kingdom. Steering committee and members of the National Audit of Acute Upper Gastrointestinal Haemorrhage. *BMJ*, 1995;**38**:222–226.
85. Laine, L. and W. L. Peterson. Bleeding peptic ulcer. *N. Engl. J. Med.* 1994;**331**: 717–727.
86. Cook, D. J., B. Salena, G. H. Guyatt and L. Laine. Endoscopic therapy for acute non-variceal upper gastrointestinal hemorrhage—a meta-analysis. *Gastroenterology*. 1992;**102**:130–148.
87. Sacks, H. S., T. C. Chalmers, A. L. Blum, J. Berrier, and D. Pagano. Endoscopic hemostasis: An effective therapy for bleeding peptic ulcers. *JAMA* 1990;**264**:494–499.
88. Bjorkman, D. J., A. Zaman, M. B. Fennerty, D. Lieberman, J. M. DiSario, and G. Guest-Warnick. Urgent vs. elective endoscopy for acute non-variceal upper-GI bleeding: An effectiveness study. *Gastrointest. Endosc.* 2004;**60**(1):1–8.
89. Aabakken, L. Current endoscopic and pharmacological therapy of peptic ulcer bleeding. *Best Pract. Res. Clin. Gastroenterol.* 2008;**22**(2):243–259.
90. Lee, K. J., J. H. Kim, K. B. Hahm, S. W. Cho and Y. S. Park. Randomized trial of N-butyl-2-cyanoacrylate compared with injection of hypertonic saline-epinephrine in the endoscopic treatment of bleeding peptic ulcers. *Endoscopy* 2000;**32**:505–511.
91. Bardari, F., L. D'Urso, and G. Muto. Conservative treatment of iatrogenic urinary fistulae: the value of cyanoacrylic glue. *Urology*, 2001;**58**:1046–1048.
92. Cuschieri, A. Tissue adhesives in endosurgery. *Semin. Laparosc. Surg.* 2001;**8**: 63–68.
93. Esposito, C., R. Damiano, A. Settimi, M. De Marco, P. Maglio and A. Centonze. Experience with the use of tissue adhesives in pediatric endoscopic surgery. *Surg. Endosc.* 2004;**18**:290–292.
94. Chen, W. -C., M. -C. Hou, H.-C. Lin, F.-Y. Chang, and S. -D. Lee. An endoscopic injection with N-butyl-2-cyanoacrylate used for colonic variceal bleeding: A case report and review of the literature. *Am. J. Gastroenterol.* 2000;**95**(2):540–542.

95. Kim, T. and B. V. Kharod. Tissue adhesives in corneal cataract incisions. *Curr. Opin. Ophthalmol.* 2007;**18**:39–43.

96. Leaming, D. V. Practice styles and preferences of ASCRS members—2003 survey. *J. Cataract Refract. Surg.* 2004;**30**:892–900.

97. Seal, D. V., P. Barry, G. Gettinby, F. Lees, and M. Peterson. C.W. ESCRS study of prophylaxis of postoperative endophthalmitis after cataract surgery: Case for a European multicenter. *J Cataract Refract Surg.* 2006;**32**(3):407–410.

98. Meskin, S.W., D. C. Ritterband, D. E. Shapiro, J. Kusmierczyk, J. A. Seedor, R. S. Koplin. Liquid bandage (2-octyl cyanoacrylate) as a temporary wound barrier in clear corneal cataract surgery. *Am. J. Ophthalmology.* 2005;**112**: 2015–2021.

99. Reyes, J. M., S. Herretes, A. Pirouzmanesh, D. A. Wang, J. H. Elisseeff, A. Jun, P. J. McDonnell, R. S. Chuck, and A. Behrens. A modified chondroitin sulfate aldehyde adhesive for sealing corneal incisions. *Invest. Ophthalmol. Vis. Sci.* 2005; **46**:1247–1250.

100. Alio, J. L., M. E. Mulet, D. Cotlear, Y. Molina, I. Kremer, and J. M. Martin. Evaluation of a new bioadhesive copolymer (ADAL) to seal corneal incisions. *Cornea,* 2004;**23**:180–189.

101. Breasted, J. H. *The Edwin Smith Surgical Papyrus: Hieroglyphic Transliteration, Translation and Commentary*. Kessinger Publishing: Oriental Institute Of *The University Of Chicago*, US; 2006.

102. Pecha, R. E., T. Prindiville, R. Kotfila, B. Ruebner, A. T. Cheung and W. Trudeau. Gastrointestinal hemorrhage consequent to foreign body reaction to silk sutures: case series and review. *Gastrointest. Endosc* 1998;**48**:299–301.

103. Hida, K., S. Yamaguchi, T. Seki, S. Yano, M. Akino, S. Terasaka, T. Uchida, and Y. Iwasaki. Nonsuture dural repair using polyglycolic acid mesh and fibrin glue. *Surg. Neurol*, 2006;**65**:136–142.

104. Cimador, M., M. Castagnetti, M. Milazzo, and M. Sergio, and E. De Grazia. Suture materials: Do they affect fistula and stricture rates in flap urethroplasties? *Urol. Int.* 2004;**73**:320–324.

105. Murray, K. D., C. H. Ho, J. Y. Hsia, and A. G. Little. The influence of pulmonary staple line reinforcement on air leaks. *Chest.* 2002;**122**:2146–2149.

106. Millesi, H., Peripheral nerve injuries. Nerve sutures and nerve grafting. *Scand. J. Plast. Reconstr. Surg. Suppl.* 1982;**19**:25–37.

107. Chelala, E., M. Thoma, B. Tatete, A. C. Lemye, M. Dessily, and J. L. Alle. The suturing concept for laparoscopic mesh fixation in ventral and incisional hernia repair: Mid-term analysis of 400 cases. *Surg. Endosc.* 2007;**21**:391–395.

108. Lumsden, A. B. and E. R. Heyman. Closure Medical Surgical Sealant Study Group, Prospective randomized study evaluating an absorbable cyanoacrylate for use in vascular reconstructions. *J. Vasc. Surg.*, 2006;**44**:1002–1009.

109. Sheffy, N., Y. Mintz, A. I. Rivkind, and S. C. Shapira. Terror-related injuries: A comparison of gunshot wounds versus secondaryfragments-induced injuries from explosives. *J. Am. Coll. Surg.* 2006;**203**:297–303.

110. Lauto, A. D. Mawad, and L. J. R. Foster. Adhesive biomaterials for tissue reconstruction. *J. Chem. Technol. Biotechnol.* 2008;**83**:464–472.

111. Vote, J. T. and M. J. Elder, Cyanoacrylate glue for corneal perforations: A description of surgical technique and a review of the literature. *Clin. Exper. Ophthalmol.* 2000;**28**(6):437–442.

112. de Almeida Manzano, R. P., S. C. Naufal, R. Y. Hida, L. O. Guarnieri, and M. C. Nishiwaki-Dantas, Antibacterial analysis *in vitro* of ethyl-cyano-acrylate against ocular pathogens. *Cornea* 2006;**25**(3):350–351.

113. Eiferman, R. A. and J. W. Snyder, Antibacterial effect of cyanoacrylate glue. *Arch. Ophthalmol.* 1983;**101**(6):958–960.

114. Sharma, A.R., S. Kaur, P. Kumar, S. Gupta, B. Pandav, A. Patnaik, and A. Gupta. Fibrin glue versus N-butyl-2-cyanoacrylate in corneal perforations. *Clin. Exper-Ophthalmology* 2003;**110**(2):291–298.

115. van den Ende, E. D., P. W. Vriens, J. H. Allema, and P. J. Breslau, Adhesive bonds or percutaneous absorbable suture for closure of surgical wounds in children. Results of a prospective randomized trial, *J. Pediatr. Surg.* 2004;**39**(8):1249–1251.

116. Marcovich, R., A. L. Williams, M. A. Rubin and J. S. Wolf, Jr. Comparison of 2-octyl cyanoacrylate adhesive, fibrin glue, and suturing for wound closure in the porcine urinary tract. *Urology* 2001;**57**(4):806–810.

117. Garcia Paez, J. M., E. Jorge Herrero, A. Rocha, M. Maestro, J. L. Castillo-Olivares, I. Millan, and S. A. Carrera. Comparative study of the mechanical behaviour of a cyanoacrylate and a bioadhesive. *J. Mater. Sci. Mater. Med.* 2004;**15**(2):109–115.

118. Leggat, P. A., D. R. Smith, and U. Kedjarune. Surgical applications of cyanoacrylate adhesives: A review of toxicity. *ANZ J. Surg.* 2007;**77**:209–213.

119. Montanaro, L., C. R. Arciola, E. Cenni, G. Ciapetti, F. Savioli, F. Filippini, and L. A. Barsanti. Cytotoxicity, blood compatibility and antimicrobial activity of two cyanoacrylate glues for surgical use. *Biomaterials* 2001;**22**:59–66.

120. Quinn, J., G. Wells, T. Sutcliffe, M. Jarmuske, J. Maw, I. Stiell, and P. Johns. A randomized trial comparing octylcyanoacrylate tissue adhesive and sutures in the management of lacerations. *JAMA* 1997;**277**:1527–30.

121. Makary, M. A., P. J. Pronovost, E. S. Weiss, D. Chang, S. P. Baker, E. E. Cornwell, D. Syin, E. A. Millman, and J. A. Freischlag. Sharpless surgery: A prospective study of the feasibility of performing operations using non-sharp techniques in an urban, university-based surgical practice. *World J. Surg.* 2006;**30**:1224–1229.

122. Yasuda, H., S. Kuroda, R. Nanba, T. Ishikawa, N. Shinya, S. Terasaka, Y. Iwasaki, and K. Nagashima. A novel coating biomaterial for intracranial aneurysms: Effects and safety in extra- and intracranial carotid artery. *Neuropathology*, 2005;**25**:66–76.

123. Kaplan, M. and K. Baysal. In vitro toxicity test of ethyl 2-cyanoacrylate, a tissue adhesive used in cardiovascular surgery, by fibroblast cell culture method. *Heart Surg: Forum* 2005;**8**:E169–72.

CHAPTER 13

POLYETHYLENE GLYCOL IN CLINICAL APPLICATION AND PEGYLATED DRUGS

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CONTENTS

Biodegradable Polymers in Clinical Use and Clinical Development, First Edition. Edited by Abraham J. Domb, Neeraj Kumar, and Aviva Ezra
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13.1 INTRODUCTION

Poly(ethylene glycol) (PEG) is a simple polymer. It is one of the most frequently used polymers in biomedical applications. PEG is useful in medical application because of its high solubility in water. It is important to note that solubility characteristics are dependent on the molecular weight of the polymer. These properties led to a number of useful application: (i) the addition of PEG to aqueous solutions of proteins frequently induces crystallizations, (ii) the addition of high concentrations of PEG induces cell fusion, (iii) the immobilization of PEG on polymer surfaces greatly reduces protein adhesion, and (iv) the covalent coupling of PEG to proteins decreases their immunogenicity and increases their half-life in plasma.

The biotechnology revolution has produced novel peptides and proteins that have become important new drugs. More than 80 polypeptide drugs are marketed in the United States, and 350 more are undergoing clinical trials. About one third of drug candidates in clinical trials today are polypeptides. It has been forecast that the protein engineering market will rise to $118 billion (12% of pharma sales) in 2011 compared to $67 billion in 2006 (10% of pharma sales) [1].

Recombinant DNA (deoxyribonucleic acid) techniques using *Escherichia coli* and other organisms also allow for the production of polypeptide drugs in large quantities. Despite such tremendous advances, polypeptide drugs possess several shortcomings that limit their usefulness. These disadvantages include susceptibility to destruction by proteolytic enzymes, a short circulating half-life, short shelf life, low solubility, rapid kidney clearance, and their propensity to generate neutralizing antibodies [2]. In addition, most polypeptide drugs must be delivered by injection, either subcutaneously or intravenously [2].

Many scientists have experimented to overcome the problems associated with polypeptides as drugs. Various research workers have attempted to improve the clinical properties of polypeptides by altering their amino acid sequences to reduce degradation by enzymes and their antigenicity potential, by fusing them to immunoglobulins or albumin to improve half-life, and by incorporating them into drug delivery vehicles such as liposomes [3–5]. Although sometimes successful, these methods have limitations, as illustrated by the studies on liposomes. Liposomes not only deliver drugs to diseased tissues, such as tumors, but also rapidly enter the liver, spleen, kidneys, and reticuloendothelial systems, and drugs leak from them while in circulation. In addition, liposomes activate

complement, and this causes pseudoallergic reactions that damage heart and liver cells [6]. PEGylation is an alternative method that can overcome some of these deficiencies [7, 8] by attaching PEG chains to polypeptides or other candidate molecules. In fact, liposomes are now PEGylated to improve the delivery of encapsulated drugs, such as the anticancer agent doxorubicin [9].

Many different approaches have been adopted in attempts to utilize drugs more effectively; in particular, PEGylation provides benefits that are derived from their covalent attachment to bioactive species. Theoretically, PEGylation can be used to target any active molecule, but it is usually applied to macromolecules such as peptides, proteins, oligonucleotides, and small organic molecules. Proteins are the most actively studied targets for PEGylation. When PEG is attached to the surface of a protein, the resulting increase in size reduces renal clearance and increases stability by enhancing resistance to proteolysis and prolongs protein circulating times [10]. PEGylation also reduces drug immunogenicity, antigenicity, and toxicity and provides additional benefits by minimizing injection volume wastage due to adsorption at injection sites and by enhancing solubilities [11]. In addition to these pharmacological advantages, PEGylation offers commercial advantages because it transforms molecules into PEGylated equivalents and, thus, reduces one threat posed by biosimilars.

PEG is biocompatible, relatively nontoxic, and soluble in aqueous and many organic solvents [12]. PEG has been approved by the U.S. Food and Drug Administration (FDA), and its safety is assured by its established usage in drugs, foods, and cosmetics. A PEGylated drug has at least 600-fold lower toxicity associated with PEG-related adverse events in man. When PEG is associated with proteins or other biological molecules, it is unlikely to induce toxicity such as renal tubular necrosis [11]. No critical renal toxicity of PEGylated drugs have been reported. Initially, PEGylation with low molecular weights (< 12 kDa) had substantial levels of PEG diol impurities. However, PEGs available today have monodispersity. Diol impurities have been eliminated during chemical synthesis. As a result, current research is directed toward the selectivity of protein PEGylation [12].

Despite the fact that protein drugs are capable of exerting potent effects, their usages are restricted by application problems. In most cases, parenteral administration routes are required, and even when they are optimally delivered, proteins are rapidly degraded within hours or even minutes by enzymes or are rapidly excreted. Immunogenicity and antigenicity are also problems presented by protein drugs, and solubility becomes a crucial issue when preparing medications for injection because low solubility can cause precipitates to form at physiological pH values, which may substantially reduce their bioavailability and cause inflammation. Furthermore, even though protein drugs are generally administered parenterally, their bioavailabilities are poor, and their developments are challenging. Moreover, because they must be administered frequently, they tend to be associated with poor patient compliance. Recent developments have increased the proportion of biopharmaceuticals in the pharmaceutical market,

and available biopharmaceuticals now include enzymes, peptides, proteins, antibodies, and oligonucleotides. Thus, means of improving the bioavailabilities of protein drugs are being actively researched.

Oligonucleotides (e.g., antisense oligonucleotides and aptamers) are obvious PEGylation targets because they are rapidly degraded by nuclease enzymes *in vivo* and are rapidly eliminated from blood. Hence, PEGylation technology can enhance stabilities and prolong half-lives. It was also applied to small organic molecules, such as cytotoxic drugs, and some of these are on clinical trials.

PEGylation could also be used to improve the characteristics of commonly used anticancer drugs, such as docetaxel, paclitaxel, and camptothecin, which have poor solubilities and high toxicities and require frequent administration. In addition, the vasculatures of cancer tissues have heterogeneous endothelial linings, which can encourage the accumulations of supramolecular entities such as polymers and liposomes [13]. Thus, it is hoped that PEGylated high-molecular-weight anticancer drugs will selectively accumulate in cancer tissues and that this will lead to sustained drug release.

13.2 CHEMISTRY OF PEGYLATION

PEGylation is now a well-recognized tool that can improve the properties of protein and peptide drugs. PEG is formed from repeating units of ethylene glycol, and the polymer can have linear or branched chains of different molecular weights. PEG is synthesized by the anionic ring-opening polymerization of ethylene oxide using anhydrous alkanols such as methanol, methoxyethoxy

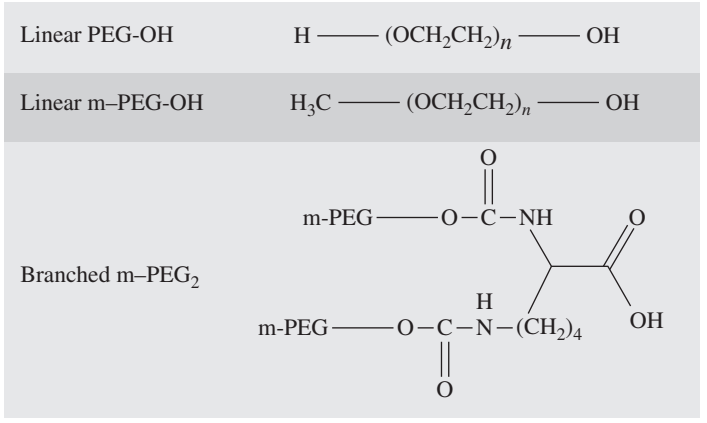

FIGURE 13.1 Structures of polyethylene glycol (PEG) molecules, monomethoxy–PEG (m-PEG) and branched chain m-PEG. (Redrawn from [15].)

ethanol as initiator to yield diol PEG, or monoalkyl-capped PEG such as m-PEG, respectively (Fig. 13.1). m-PEG is useful for protein modification as it does not give rise to crosslinking [14]. The presence of trace amounts of water during polymerization resulted in a number of diol PEGs as an impurity. The amount of the diol PEG can exceed 10%, which will increase heterogenicity of the conjugate synthesis. An extensive variety if conjugation chemistry has been used to prepare PEGylated protein. The PEG must first be functionalized and then binding to the protein can be accomplished by a hydroxyl, amino, or carboxyl group present in the protein [2, 15]. A summary of the PEG derivatives and their properties is shown in Table 13.1.

Some linking chemistry used to synthesize the first generation of PEG conjugates involving PEG–dichlorotriazine, –benzotriazole carbonate, –succinimidyl carbonate, and –tresylate had poor selectivity. They preferentially reacted with lysine and also displayed side reactions with the hydroxyl groups of serine and tyrosine and the imidazole side chain of histidine. This formed unstable sulfamate and imidazole carbamate linkages, respectively. The product was a heterogeneous mixture of conjugates with undesired linkages that can be easily degraded [2]. The first-generation PEGylation often involved a random reaction resulting in either steric hindrance or direct blocking of the protein active site. In this way, PEGylation leads to a reduction in bioactivity [16].

13.2.1 PEG–Dichlorotriazine

The cyanuric chloride method was the most frequently used first-generation method to activate PEG. PEG proteins were prepared by modification of bovine serum albumin (BSA) with PEG–dichlorotriazine [Fig. 13.2(a)]. This resulted in a conjugate with reduced immunogenicity and antigenicity [10, 17]. The classical approach couples one triazine ring per PEG molecule, despite the

TABLE 13.1 PEG Derivatives and Their Properties

PEG Derivatives	Properties
First generation	
PEG–dichlorotriazine	Crosslinking due to remaining chloride
PEG_2–chlorotriazine	Crosslinking due to remaining chloride
PEG–succinimidyl succinate	Succinate left attached to protein
PEG–carbonylimidazole	Low reactivity
PEG-*p*-nitrophenyl carbonate	Toxic by-product of 4-nitrophenol
PEG–trichlorophenyl carbonate	Toxic by-product of 2,4,5-trichlorophenol
Second generation	
PEG–aldehyde	It labels only α-amino group
PEG-*N*-hydroxysuccinimidyl ester	Highly reactive toward amino groups
m-PEG orthopyridyl disulfide (OPSS)	Attach to free cysteine on peptides
Heterobifunctional PEGs	Need water exclusion to prevent diol formation

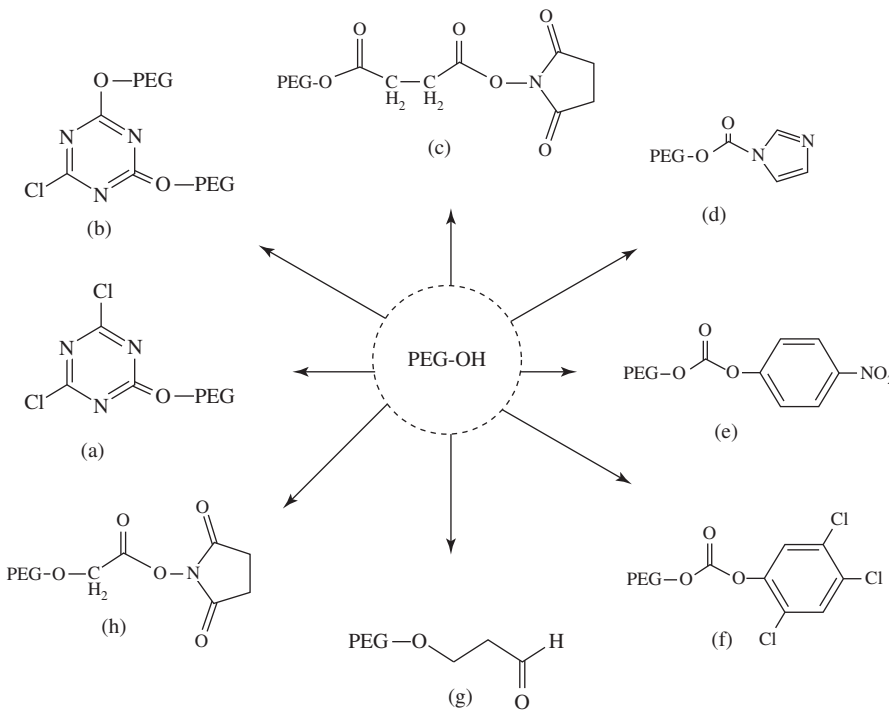

FIGURE 13.2 Method for the activation of PEG molecules: (a) PEG–dichlorotriazine, (b) PEG_2–chlorotriazine, (c) PEG–succinimidyl succinate, (d) PEG–carbonylimidazoyl formate, (e) PEG-*p*-nitrophenyl carbonate, (f) PEG–trichlorophenyl carbonate, (g) PEG–aldehyde, and (h) PEG–succinimidyl active ester. (Adapted from [15].)

fact that cyanuryl chloride derivatives are known as some of the least selective protein modifiers. Cyanuric chloride can react not only with amino groups but also with Sulfhydryl groups, and this is most likely responsible for the loss of activity of many proteins [18].

13.2.2 PEG_2–Chlorotriazine

PEG_2–chlorotriazine was employed for conjugation with proteins to avoid crosslinking [Fig. 13.2(b)]. The lower reactivity of the remaining chlorine translates into a more selective modification of lysine and cysteine residues without further side reaction [2]. Since this reagent is derived from trichloro-*s*-triazine, it reacts by replacement of its two most reactive chlorides with PEG-*O* residues and leaving the least reactive third chloride [8]. The PEG_2–chlorotriazine is more effective than PEG–dichlorotriazine in rendering proteins nonimmunogenic without compromising biological activity. The

m-PEG–dichlorotriazine is easy to prepare and produces stable products with both amino and thiol groups [17].

13.2.3 PEG–Succinimidyl Succinate

PEG–succinimidyl succinate [Fig. 13.2(c)] is synthesized by conjugation of m-PEG with succinic anhydride and links the carboxylic acid to the succinimidyl ester. The ester linkage is highly susceptible to hydrolysis after the polymer has been attached to the protein. Therefore, this drawback leads to loss of the benefits of PEG attachment [2].

13.2.4 PEG–Carbonylimidazole

Using PEG–carbonylimidazole derivatives to react with amino groups, carbamate linkages can be generated between the protein and PEG [18]. PEG–carbonylimidazole [Fig. 13.2(d)] gives a slower reaction rate compared to chloroformates and requires a longer reaction time of up to 72 h [17].

13.2.5 PEG–*p*-Nitrophenyl Carbonate and PEG–trichlorophenyl Carbonate

When the hydroxyl groups of PEG are activated by *p*-nitrophenyl chloroformate or 2,4,5-trichlorophenyl chloroformate, these activated PEGs can be used for conjugation. This conjugation method requires a very high pH (8.5–9.2) for the coupling step of PEG-*p*-nitrophenyl carbonate and PEG–trichlorophenyl carbonate. The reaction can be monitored by a colorimetric analysis for the phenolate ion leaving group. Both reagents exhibit lower reaction rates than *N*-hydroxysuccinimide-activated PEGs [Figs. 13.2(e) and (f)]. This enables exploitation of the different amino group reactivities in the protein and to stop the reaction at a desired degree of modification. A carbamate derivative is obtained. There is an interesting difference between these two carbonate-activated PEGs. PEG–nitrophenyl carbonate has a pale yellow color, whose intensity increases with the conjugation reaction due to the release of *p*-nitrophenol. PEG–trichlorophenyl carbonate is a colorless product. The extent of amino group modification can be followed directly by colorimetric analysis, which quantifies the residual amino groups in the reaction mixture without the need for removing excess reagent or reaction by-products [17].

A second-generation PEGylation chemistry rapidly developed to avoid a randomly modified protein surface, unstable linkages, side reactions, and lack of selectivity in substitution. m-PEG–propionaldehyde (Fig. 13.3), PEG–carboxylic acid and PEG-N-hydroxysuccinimide ester were used for amine conjugation to give a stable secondary amine or amide likages [2]. High-molecular-weight branched PEGs were also used to enhance pharmacokinetic and pharmacodynamic properties of the protein conjugates [19]. Table 13.2

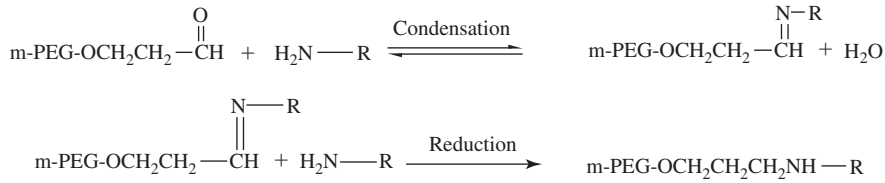

FIGURE 13.3 Reductive amination using PEG–propionaldehyde. In the first reaction, m-PEG–aldehyde is covalently linked to an amino group on the protein. The linkage formed in the first step, a so-called Schiffs base linkage, can be reversed by hydrolysis. This linkage, however, is rapidly stabilized to a stable, nonhydrolyzable amine linkage in a second step by sodium borohydride reduction. (Redrawn from [15])

TABLE 13.2 Influence of PEGylation on Pharmacokinetics and Pharmacodynamics[a]

Pharmacokinetic Effect	Pharmacodynamic Effect
Interferon-α2a	
Sustained absorption	In vivo antiviral activity increased 12–135 times
Increased half-life (from 3–8 h to 65 h)	Antitumor activity increased 18-fold
Decreased volume of distribution (from 31–73 L to 8–12 L)	Improved sustained response to chronic hepatitis C
Decreased systemic clearance (from 6.6–29.2 to 0.06–0.10 L/h)	
Interleukin-6	
Increased half-life (from 2.1 to 206 min)	Thrombopoietic potency increased 500 times
Tumor Necrosis Factor	
Increased half-life (from 3 to 45–136 min)	Antitumor potency increased four times

[a]Influence of PEGylation on pharmacokinetics and pharmacodynamics of some therapeutic proteins, compared with corresponding native proteins.

Source: Adapted from [15].

summarizes the influence of PEGylation on the pharmacokinetic and pharmacodynamic properties of several therapeutic polypeptides.

13.2.6 PEG–Aldehyde

This reaction gives a highly stable amine linkage between PEG and protein after Schiff base formation followed by cyanoborohydride reduction [17] [Fig. 13.2 (g)]. A positive charge is critical for retention of biological activity. The reaction rate of the Schiff base formation is relatively slow, sometimes up to a day is

necessary for complete reaction leading to inactivation of labile molecules. It has been found that the best reaction takes place with α- and ε-amino groups of protein at neutral or mildly alkaline pHs, while good selectivity for the α-amino terminal of amino acid occurs at pH 5–6.

13.2.7 PEG–*N*-Hydroxysuccinimidyl Ester

To react PEG–*N*-hydroxysuccinimidyl ester [Fig. 13.2(h)] with a protein, an anhydride such as succinic anhydride is added followed by activation with *N*-hydroxysuccinimide in the presence of carbodiimide [18]. This reaction is highly reactive toward amino groups. The kinetic rate of conjugation depends on the number of $-CH_2$ groups linked to the carboxyl group. However, it is important to note that changing the distance between the active ester and the PEG backbone by the addition of methylene units has a profound influence on the reactivity toward amino groups and water [2]. For example, the succinimidyl ester of carboxymethylated PEG (PEG-O-CH_2-COOH) is extremely reactive with a half-life of hydrolysis rate of 0.75 min. This makes it impossible to use in the conjugation process [20]. In contrast, the half-life of hydrolysis for the propanoic acid (PEG-O-CH_2-CH_2-COOH) derivatives of PEG is 17 min [21]. Reactivity of the PEG active esters toward amino groups and water can be decreased by introducing an α-branching moiety to the carboxylic acid [2].

13.2.8 m-PEG Orthopyridyl Disulfide (OPSS)

Although there are far fewer cysteine residues than lysine groups on polypeptides, the thiol groups of cysteine are ideal for specific modifications. Moreover, cysteines can now be added to polypeptides precisely where they are desired by genetic engineering [22]. The preparation of a highly active, long-circulating and stable conjugate of interferon-β (IFN-β) illustrates how such structural manipulation can lead to the development of a PEGylated drug. In this case, a two-step method with m-PEG orthopyridyl disulfide (OPSS) was used [23]. In the folded structure of the native IFN-β, the free cysteine residue at position 17 is proximal to the surface but is hidden [24]. Consequently, a PEG derivative of high molecular mass cannot be directly attached to this residue. Instead, one active group of the smaller, low-molecular-mass (2 kDa) difunctional reagent di-OPSS–PEG is first attached to the hidden cysteine of IFN-β, followed by coupling of a high-molecular-mass m-PEG thiol to the other active group of OPSS–PEG. Although many proteins might not benefit from site-specific PEGylation, in others, such as antibody fragments, it is crucial that the PEG be attached at a site distant from the binding site.

13.2.9 Heterobifunctional PEGs

Heterobifunctional PEGs contain two different terminal groups. They have advantages in immunoassays as well as drug targeting, liposomes, or viruses to

specific tissues [25]. Heterobifunctional PEGylation is expected to release drugs from PEGs in a mild condition within cells [26]. PEG polymers in second generation have branched structures. Branched PEGs are increased in molecular weights up to 60 kDa or more [27]. Branched PEGs protect attached polypeptide drugs from the immune system and proteolytic enzymes [28].

Another improvement in the second-generation PEG polymers is the use of branched structures, in contrast to the solely linear structures found in the first-generation PEGs. Branched PEGs of greatly increased molecular masses—up to 60 kDa or more, compared with the 12 kDa or less found in the first-generation PEGs—have been prepared. A branched PEG "acts" as if it is much larger than a corresponding linear PEG of the same molecular mass [27]. Branched PEGs are also better at cloaking the attached polypeptide drug from the immune system and proteolytic enzymes, thereby reducing its antigenicity and likelihood of destruction [28].

13.3 PEGYLATION TECHNOLOGY AND PEGYLATED DRUGS

Peptide, protein, and small organic molecule-based drugs are generally removed from the bloodstream via renal ultrafiltration. The maximum size of native globular protein in terms of renal elimination is around 70 kDa [29], and because the majority of drugs have molecular weights of substantially <70 kDa, they are likely to be removed by the kidneys. Peptides and proteins bind weakly to plasma proteins and are rapidly removed from the systemic circulation when administered intravenously. PEGylated drugs have increased in molecular weight [29]. This ability of PEG to increase molecular dimensions provides the theoretical basis for reduced renal ultrafiltration, extended half-life, and increased bioavailability associated with PEGylation [Fig. 13.4(a)].

Steric hindrance caused by the hydrodynamic volume of PEG molecules also prevents proteolytic enzymes, antibodies, and the macrophages of liver reticuloendothelial cells from attacking the drug. As a result of this "shielding effect" [Fig. 13.4(b)], enzymes fail to recognize protein substrates, and antibodies and macrophages fail to recognize proteins. As a result, PEGylation not only reduces immunogenicity and antigenicity by suppressing proteolysis and immune system recognition but also prolongs half-life and increases bioavailability.

In addition, the hydrophilic PEG molecule, with repeating units of ethylene oxide, can bind to two or three water molecules, can be utilized to enhance the solubilities of essentially insoluble small organic molecules such as docetaxel, paclitaxel, or camptothecin, and provides advantages during manufacture. Furthermore, the enhanced solubility conferred by PEG can be useful for the development of therapeutic antibodies that must be solubilized [Fig. 13.4(c)].

Specific PEGylation method is an essential part of PEGylation technology. It is important because if conjugation is nonspecific, then PEG bonding may mask the active sites of peptides or small drug molecules and prevent receptor binding, which would substantially reduce their biological activities. It can be envisaged

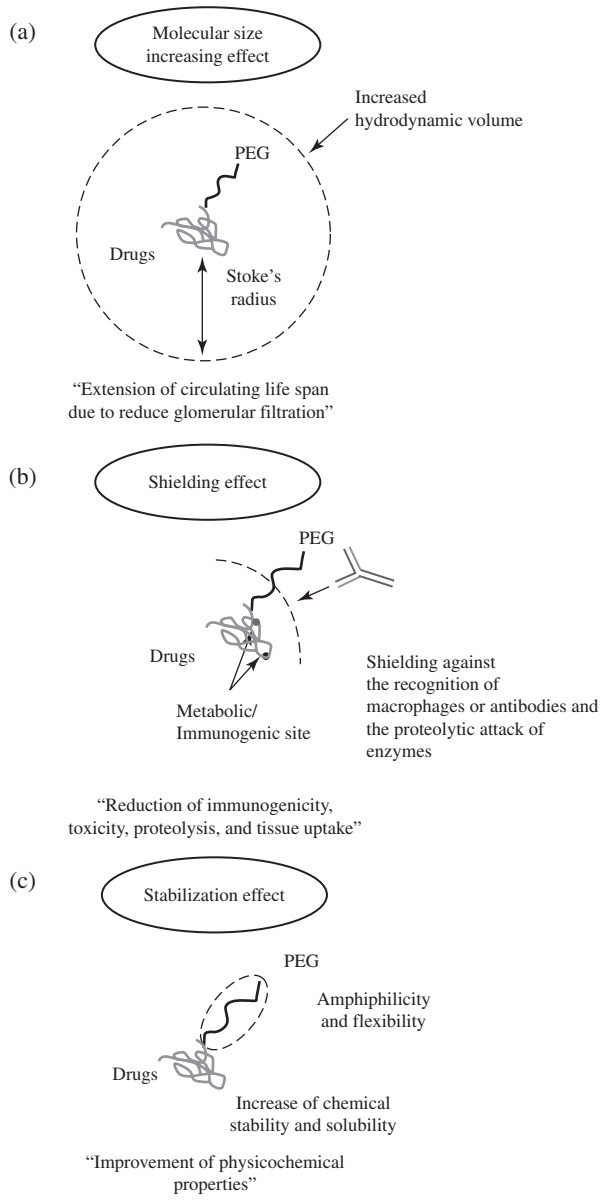

FIGURE 13.4 Benefits of PEGylation technology: (a) prolong plasma resident time, (b) decrease immunogenicity, and (c) increase stability. (Adapted from [30].)

that the negative effect is greatest when PEG attachment sites are located near active sites. On the other hand, increases in the molecular size of conjugates by increasing the molecular weight of PEG, improves pharmacokinetic properties.

Finally, biological half-life and bioavailability of PEGylated drugs outweigh the disadvantage of reduced biological activity. Thus, PEGylated drugs are more superior than their non-PEGylated counterparts.

For the application of biopharmaceuticals in human PEGylation therapy, the covalent coupling of PEG chains to drugs, has been the important innovation of the last few years. Pioneering work in this field was performed by Davis and Abuchowski, who laid the cornerstone for the commercial success of this technology [31]. PEGylation increases the hydrodynamic radius of a biopharmaceutical product and shields its peripheral surface. Thus the stability of these conjugates against proteases is increased, their immunogenicity is reduced, and their renal excretion is decelerated. Consequently, PEGylation secures a prolonged half-life of the biopharmaceutical, reduces side effects, and finally increases efficiency of the therapy.

Even though many attempts have been undertaken to develop new polymers with improved properties, none of these new substances has been able to compete with PEG for this application. This can be explained by the biocompatibility of PEG and the good experience with PEG as a low-cost additive for the pharmaceutical product and for the cosmetic industry over the last decades. Since PEGylation is a permanent modification of the biopharmaceutical product, the relevant national and international authorities for drug approval make high demands on the PEG reagents and the final PEGylated product. Major requirements are the specification of the degree of PEGylation, analysis of the dispersity index, and determination of the PEGylation sites. Thus, an ideal PEG reagent fulfills at least the following criteria:

- Monodispersity or at least a dispersity index close to 1, in order to assure a reproducible high quality
- Availability of one single terminal reactive group for the coupling reaction, in order to avoid crosslinking between drug molecules
- A nontoxic and nonimmunogenic, biochemically stable linker
- Branching for optimal surface protection [32]
- Options for site-specific PEGylation

13.3.1 Problem of PEGylated Drugs

The continuing increase in the polydispersity of PEG becomes a quality problem for PEGylated drugs. As a consequence of the production process, long and linear PEG chains used for PEGylation today are only available as a mixture of PEG chains with different chain lengths. But now efforts are under way to solve this problem by using monodisperse starting materials.

Many efforts have been undertaken to achieve an efficient and stable coupling of PEG chains to the biopharmaceutical product. Very successful developments have been achieved with regard to the variability of the coupling chemistry and the availability of specialized linkers [2].

Site-specific mono-PEGylation is of significant relevance in order to provide highly reproducible products that maintain maximum activity. In the majority of cases, high-molecular-weight PEG chains (10–40kDa) are used for the mono-PEGylation of proteins. At best, it is possible to attach one single PEG chain to the *N*-terminal amino group of a protein by reductive amination. Especially with small proteins, such as cytokines, it is possible to apply genetic methods to introduce rare amino acids, which then can be used for the coupling of PEG [33]. Preferred for this purpose is a cysteine residue that can be specifically PEGylated at the thiol group by maleimide coupling.

Some examples have also been published in which the site-directed PEGylation has been achieved by an enzyme-catalyzed reaction with a transglutaminase [34]. Branched PEG reagents show superior protecting properties compared with linear molecules. Today there are only a few commercial examples of such branched PEG molecules because very often their synthesis is complex and time consuming. However, some important progress in this field is expected in the next few years.

After two decades of experience, PEGylation technology can be regarded as a proven and tested method for the drug delivery of biopharmaceuticals, especially proteins. Already today, biopharmaceuticals are a multibillion-dollar market that is expected to grow rapidly within the next decade. The sales of several PEGylated drugs such as PEG-Intron have reached $3 billion, comparable to the sales of conventional synthetic blockbusters. However, the development of PEG technology is by no means finished. Recent publications reveal that some questions still remain to be resolved. In particular, there is a requirement for new synthetic routes to provide functionally expanded PEG reagents of reproducible high quality. Site-specific conjugation will be one of the major topics for the coming years, especially with regard to technically applicable and commercially reasonable solutions.

Many investigations are under way to explore new fields for the application of PEGylation. These are manifold and not limited to protein drugs or biopharmaceuticals. In the area of oncology, for example, the coupling of conventional synthetic drugs (so-called "small molecules"), such as Ara-C [35] or camptothecin, to polymers resulted in a significant reduction of toxicity and an increased selectivity for tumor tissue. The latter phenomenon is based on a nonspecific accumulation of large molecules in malignant tissue. This effect is called the EPR effect: enhanced permeability and retention. Other promising applications for PEGylation are PEGylation of liposomes, nonviral gene shuttles, nanoparticles, oligonucleotides, and surfaces for technical or medical devices [14].

Due to the increasing relevance of biopharmaceuticals and the high regulatory demands for their approval, innovative and specialized drug delivery systems have gained considerable importance. Thus, intelligent drug delivery systems such as PEGylation will determine the commercial success of the pharmaceutical industry in the future. With its broad applicability, high efficiency and comparably low costs, PEGylation will maintain a leading position. As a result, PEGylation, alone or in combination with other drug delivery technologies, will

be an essential part of a successful strategy for the development of biopharmaceutical drugs.

A polydisperse drug conjugate is produced when PEG is conjugated to a low-molecular-weight drug because of the intrinsic polydispersity of PEG. This influences product consistency and clinical effects [36]. Therefore, research is continuing in producing homogeneous products with consistent activity. Initial efforts made to minimize the polydispersity of PEG focused on its synthesis and purification. Also, improvement in separation and purification technology would help. Other efforts are conjugating PEG at specific target sites. Furthermore, enzymes are utilized to improve the specificity of conjugation [34, 37]. PEGylation may occur at thiol groups, at α and ε amino groups. However, most proteins have few thiol groups, and even when present these groups are likely to be associated with active sites. However, carboxylic groups can cause problems because they activate amino groups [38]. Nevertheless, due to improvements in genetic engineering techniques, we now can make sequence-specific modifications using thiol-reactive PEGs [39]. A recent technology is based on protein sequences with PEG-like properties that are genetically fused to biopharmaceuticals, thus avoiding the need for chemical conjugation [40]. This technology increases hydrodynamic radius and thus increases the apparent molecular weights of pharmaceutical proteins by approximately 15-fold [41].

PEGylation utilizes high-molecular-weight PEG with various shapes prompts us to be aware of the possibility of hepatotoxicity and altered clearance of the PEGylated drug. PEGylation technologies are now targeting an ever-increasing range of medications. Research on small organic molecules is considered to be somewhat more complicated than the development of PEGylated protein drugs. Generally, PEGs have only one or two reactive groups, and the numbers of functional groups present on small molecules, such as taxol and camptothecin, are relatively small compared to peptides and proteins, which means that the drug payloads of PEGylated small molecules are much lower than those of peptides or proteins. From the comparatively large dimensions of PEGs, conjugation may prevent small drug molecules reaching therapeutic concentrations at target sites. Finally, the PEGylation of small organic antitumor agents could increase their toxicities by prolonging their half-lives *in vivo*.

To alleviate the problems associated with the permanent PEGylation of small organic molecules, extensive research is being conducted on the temporary PEGylation of proteins ("releasable PEGylation"), especially of PEG prodrugs for the delivery of antitumor agents [42, 43]. A prodrug is a biologically inactive entity that requires enzymatic transformation in vivo to release the active principle [44]. In addition to greater solubility, PEG prodrugs of small organic molecules provide advantage over parent drugs, such as improved efficacy and reduced toxicity due to site-specific delivery. Paclitaxel prodrugs, conjugated with high-molecular-weight PEG, show EPR and low toxicity due to increased accumulations in tumors with a relatively slow rate of cleavage of the ester bond or a more controlled ester bond hydrolysis [45,46]. However, the PEG prodrugs of antitumor agents utilize hydrolytically unstable bonds or

enzymatically labile spacers to obtain optimal drug release and control activity and toxicity. An excessively rapid prodrug breakdown may lead to a spike in drug levels and possible toxicity, whereas too slow a rate of release might compromise efficacy [14]. For example, Enzon dropped pegamotecan (PEG-camptothecin) during phase IIb trials in 2005 due to a lack of efficacy [47].

13.3.2 Novel Applications of PEGylation

PEG can be crosslinked to form polymer networks that swell in water. These swollen materials are hydrogels and suitable for medical applications. The biocompatibility of hydrogels makes them ideal for wound-healing applications [48]. In 2000, the FDA approved a surgical sealant FocalSeal to prevent air leaks in the lungs. FocalSeal employs PEG that is applied as a liquid and is then transformed into a waterproof hydrogel seal by irradiation. The sealant protects wound sites from leaking during tissue healing [49, 50]. Another example is SprayGel, which prevents postoperative adhesion formation [51]. It is sprayed onto the wounds and acts as a protective barrier during healing. This material degrades and dissolves at a programmed rate. Other PEG-based hydrogels deliver encapsulated drugs as implants. Degradable linkages between hydrogels and incorporated drugs allow drugs to be slowly released in the body [48].

These are just a few of the biomedical applications of PEGylation either approved by the FDA or undergoing investigations. Although proteins and peptides have been the main targets for PEGylation, other molecules, including small-molecule drugs, cofactors, oligonucleotides, lipids, saccharides, and biomaterials, can be PEGylated as well. Other candidates include PEGylated insulin with a lengthened circulation time and reduced immunogenicity [52]; PEGylated antibody fragments for immunotherapy or tumor targeting [53]; and PEGylated superoxide dismutase for the treatment of ischemia reperfusion injury or burns [54]. The benefits of PEGylated catalase, uricase, honeybee venom, hemoglobin, pyrolidone, and dextran are also under investigation [55]. Other research workers are designing PEGylated nanoparticles to cross the blood–brain barrier [56] or use PEGylated DNA-containing liposomes with tethered antibodies to provide targeted gene therapy [57].

13.4 SAFETY AND TOXICITY DATA OF PEGYLATED DRUGS

Toxicology studies with PEGylated drugs are normally carried out in animals before testing in humans. These provide excellent evidence for the safety of PEG-containing molecules. Such safety studies are normally accompanied with corresponding metabolism and biological fate of the molecule in humans. These studies are very difficult to conduct on the PEGylated material, and this leaves possible concern about the PEGylated portion of the molecule and its impact on human safety.

Preclinical toxicology studies performed with PEGylated proteins have also not revealed any PEG-specific toxic findings. For example, with PEG interferon, the toxicity profile was evaluated in cynomolgus monkeys dosed subcutaneously for 4 weeks either twice weekly (dose up to 562.5 μg/kg) or daily (doses up to 600 μg/kg) and for 13 weeks administered twice weekly (doses up to 150 μg/kg). PEG interferon was well tolerated. The characteristic pattern of interferon-α toxicity was observed with PEG interferon. These effects included suppressive effects on the hematopoietic system and increased in liver enzymes (Pegasys). With Peg-Intron, repeated-dose toxicity studies were performed in cynomolgus monkeys using subcutaneous doses administered every other day for 1 month. Important findings included decrease in all types of blood cells, serum proteins, calcium, phosphorus, and potassium. The findings observed in PEG-Intron-dosed monkeys were similar to those produced by Intron A. There was no unique toxicity due to the PEGylation. Greater incidence and/or severity of the findings were noted in the high-dosed monkeys given PEG-Intron compared with those given Intron A. This is in accordance with the prolonged exposure and higher AUC values obtained using PEG-Intron. Treatments with PEGylated proteins such as PEG interferon-2-α (40 kDa; Pegasys) and PEGylated asparaginase do not reveal any specific adverse events linked to the PEG moiety [58, 59]. Overall, the acute or chronic administration of PEG with a range of molecular weights by a range of routes has not led to any major toxicities, and signs of toxicity that do occur are only apparent at high doses. In light of this information, PEG can be considered to have a toxicological profile of very low concern in animals.

The FDA has approved PEG for use as a vehicle or base in foods, cosmetics, and pharmaceuticals, including injectable, topical, rectal, and nasal formulations. PEG shows little toxicity and is eliminated from the body intact by either the kidneys (for PEGs <30 kDa) or in the feces (for PEGs >20 kDa) [60]. PEG lacks immunogenicity [61], and antibodies to PEG are generated in rabbits only if PEG is combined with highly immunogenic proteins [62]. No one has ever reported the generation of antibodies to PEG under routine clinical administration of PEGylated proteins. However, under extreme experimental conditions, antibodies to PEG have been generated in animals as a result of the injection of PEG–protein conjugates [63].

13.5 CLINICAL APPLICATION OF PEG

PEGylated drugs in the market or currently under development can be categorized by native drug type, that is, as enzyme, peptide, protein (except enzymes, antibodies, and antibody fragments), antibody, antibody fragment, oligonucleotide, or small-organic-molecule-based entities. At present, there are nine products on the market that utilize enzymes, proteins, oligonucleotides, or antibody fragments. The development of PEGylated pharmaceuticals originates from pegademase (Adagen, Enzon Pharmaceuticals), a PEG–bovine adenosine

deaminase, which was approved by the FDA in March 1990. This approval was followed by the approvals of pegaspargase (Oncaspar, Enzon), peginterferon-α (PEG-Intron, Schering-Plough; Pegasys, Hoffman-La Roche), pegfilgrastim (Neulasta, Amgen), and pegvisomant (Somavert, Pharmacia & Upjohn). More recently, certolizumab pegol (Cimzia, UCB), a PEGylated antibody fragment product, and pegaptanib sodium (Macugen, Pfizer), a PEGylated aptamer (an oligonucleotide), were also commercialized (Table 13.3). PEGylated versions of adenosine deiminase, asparaginase, IFN-α, G-CSF, and EPO, have also been commercialized with improved bioavailabilities, whereas PEGylation was found to be essential for the therapeutic applications of human growth hormone (hGH) analogs, pegaptanib, and anti-TNF-α Fab′. Research on the PEGylations of small organic molecules is ongoing, but no approved products have been released to date.

The PEG polymer, along with its associated water molecules, acts like a shield to protect the attached drug from enzyme degradation, rapid renal clearance, and interactions with cell surface proteins, thereby limiting adverse immunological effects. PEGylated drugs are also more stable over a range of pH and temperature changes [64] compared with their unPEGylated counterparts. Consequently, PEGylation confers on drugs a number of properties that are likely to result in a number of clinical benefits, such as sustained blood levels that enhance effectiveness, fewer adverse reactions, longer shelf life, and improved patient convenience [65]. However, PEGylation can produce a decrease in the in vitro activity of proteins, but generally this negative effect is offset in biological systems by an increased half-life. PEGylation can influence the binding affinity of therapeutic proteins to cellular receptors, which results in changes in the bioactivity of polypeptides [13].

Anticancer agents have been most actively pursued and can be categorized as: (i) PEG derivatives of previously marketed anticancer agents such as irinotecan, (ii) derivatives of therapeutically beneficial drugs awaiting suitable application methods, such as arginine deiminase, arginase, and glutaminase, and (iii) PEGylated monoclonal antibody medications, such as alacizumab. PEGylation has also being applied to drugs other than anticancer agents, such as drugs for gout, thrombosis, opioid-induced bowel dysfunction, and diabetes.

In addition to the nine PEGylated products on the market, several are in clinical trials. In particular, many PEGylated drugs developed to treat cancer and chronic disorders are in the pipeline (Table 13.4). These PEGylated drugs are best classified according to the native drug, that is, as protein, enzyme, peptide, antibody fragment, and small organic molecule based.

13.5.1 Marketed PEG Conjugates

Pegademase Bovine. The FDA has approved several PEGylated polypeptides as therapeutics and more are undergoing clinical investigation. In 1990, pegademase (Adagen) received approval for the treatment of severe combined immunodeficiency (SCID), a disease associated with an inherited deficiency of

TABLE 13.3 Marketed PEGylated Drugs (adapted from [30])

Brand name	PEG Conjugates	Company	PEGylation	Indication	Dosing Information	Year Approved
Adagen	Pegademase bovine	Enzon	Random, multiple linear 5 kDa PEGs, amine pegylation	Adenosine deaminase deficiency in patients with severe combined immune deficiency disease who are not suitable candidates for, or who have failed, bone marrow transplantation	Initial dosing: IM once a week, 10 units/kg first dose, 15 units/kg for second dose, 20 units/kg for third dose. Maintenance dosing: 20 units/kg/week IM; increase by 5 units/kg/week if necessary. Maximal single dose 30 units/kg	1990 (US)
Oncaspar	Pegaspargase (PEG-L-asparaginase)	Enzon	Random, multiple linear 5 kDa PEGs, amine pegylation	First-line acute lymphoblastic leukemia, acute lymphoblastic leukemia, and hypersensitivity to asparginase	2500 IU/m^2 IM/IV every 14 days	1994 (US, EU)
Doxil	PEGylated liposomal doxorubicin hydrochloride	Ortho Biotech	Random, linear 20 kDa PEGs, amine pegylation	Ovarian cancer, AIDS-related Kaposi's sarcoma and multiple myeloma	Ovarian cancer: 50 mg/m^2 IV every 4 weeks for 4 courses minimum, AIDS-related Kaposi's sarcoma: 20 mg/m^2 IV every 3 weeks,	1995 (US)

					multiple myeloma: 30 mg/m^2 IV on day 4 following bortezomib, which is administered at 1.3 mg/m^2 bolus on days 1, 4, 8, and 11, every 3 weeks	
Pegintron	Peginterferon α-2b	Schering-Plough	Random, linear 12-kDa PEGs, amine pegylation	Alone or in combination with ribavirin for the treatment of chronic hepatitis C in patients with compensated liver disease who have not been previously treated with interferon-α and are aged ≥ 18 years	Monotherapy: 1 μg/kg/week SC for 1 year Combination therapy: 1.5 μg/kg/week SC for 1 year with oral ribavirin 800 mg/day	2000 (EU) 2001 (US)
Pegasys	Peginterferon α-2a	Hoffmanm-La Roche	Random, branched 40-kDa PEG with two 20-kDa linear PEGs, amine pegylation	Alone or in combination with Copegus, indicated for the treatment of adults with chronic hepatitis C virus infection who have compensated liver disease and have not been previously treated with interferon-α,	Monotherapy: 180 μg SC once a week for 48 weeks. Combination therapy: 180 μg SC once a week with oral ribavirin 800 mg/day in two divided doses for 24 weeks (chronic HCV genotypes 2,3) or with oral ribavirin 1200 mg/day in two	2002 (US, EU)

(*Continued*)

TABLE 13.3 (Continued)

Brand name	PEG Conjugates	Company	PEGylation	Indication	Dosing Information	Year Approved
				treatment of adult patients with HBeAg positive and −negative chronic hepatitis B who have compensated liver disease and evidence of viral replication and liver inflammation	divided doses for 48 weeks (chronic HCV genotypes 1,4) or with oral ribavirin 800 mg daily in two divided doses (chronic HCV HIV co-infection)	
Somavert	Pegvisomant	Pharmacia & Upjohn	Random, 4−6 linear 5-kDa PEGS, amine pegylation	Acromegaly in patients who have had an inadequate response to surgery and/or radiation therapy and/or other medical therapies, or for whom these therapies are not appropriate	Loading dose: 40 mg SC Maintenance dose: 10 mg SC daily	2002 (EU) 2003 (US)
Neulasta	Pegfilgrastim	Amgen	Selective, linear 20-kDa PEG, *N*-terminal pegylation	Decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving	Adult: 6 mg SC once per chemotherapy cycle Pediatric: 100 μg/kg SC once per chemotherapy cycle	2002 (US) 2003 (EU)

				myelosuppressive anticancer drugs associated with a clinically significant incidence of febrile neutropenia		
Macugen	Pegaptanib sodium	OSI/Pfizer	Selective, branched 40 kDa PEG with two 20kDa linear PEGs, amine pegylation	Neovascular (wet) age related macular degeneration	0.3 mg IV once every 6 weeks	2004 (US) 2006 (EU)
Micera	mPEG-epoetin β CERA	Roche	Random, linear 30 kDa PEG, amine pegylation	Anemia associated with chronic renal failure, including patients on dialysis and patients not on dialysis	Initial EPO treatment: 0.6 μg/kg IV or SC twice a month Conversion from another EPO: 120–360 μg IV/SC once a month or once every 2 weeks based on total weekly EPO dose at time of conversion reduce the dose of Micera by approximately 25% if rate of rise in hemoglobin is > 1 g/dL in 2 weeks if hemoglobin is	2007 (US, EU)

(*Continued*)

TABLE 13.3 (Continued)

Brand name	PEG Conjugates	Company	PEGylation	Indication	Dosing Information	Year Approved
					increasing and approaching 12 g/dL	
Cimzia	Certolizumab pegol	UCB	Selective, branched 40-kDa PEG, thiol pegylation	Reducing signs and symptoms of Crohn's disease and maintaining clinical response in adult patients with moderately to severely active disease who have had an inadequate response to conventional therapy	400 mg SC initially and at weeks 2 and 4; if response occurs, follow with 400 mg SC every 4 weeks	2008 (US)

TABLE 13.4 Clinical PEG Conjugate Drugs

PEG Conjugated	Company	Indication	Stage of Development	Route of Administration	Therapeutic class
PEG-hGH/ARX-201	Ambrx/Merck Serono	Growth disorder	Phase II	IV	Growth hormone receptor agonist
PEG-G-CSF/DA-3031	Dong-A	Neutropenia	Phase I	IV	G-CSF receptor agonist
PEG-IFN alfa-2a/DA-3021	Dong-A	Hepatitis C virus	Phase I	IV	Immune modulator
Pegloticase	Savient	Gout	Preregistration	IV infusion	Uricase stimulator
ADI-PEG-20/PEG-arginine deiminase	Phoenix Pharmacologics	Hepatocellular carcinoma, melanoma	Phase II	IM	Arginine deiminase stimulator
PEG-arginase/BCT-100	Bio-Cancer Treatment International	Cancer	Phase II	IV	Arginine stimulator
PEG-glutaminase + DON/ GlutaDON	Medical Enzyme	Solid tumor	Phase II	IV	Apoptosis stimulator
PEG-Sak	Bharat Biotech	Thrombosis	Phase II		Fibrinolysis stimulator
Uricase-PEG-20	Phoenix Pharmacologics	Gout	Phase I		Uricase stimulator
Abciximab pegol/CDP-791	UCB	NSCLC	Phase II		VEGF-2 receptor antagonist
PEG-naloxol/NKTR-118	Nektar	Opioid-induced constipation	Phase II	Oral	μ-Opioid receptor modulator
PEG-irinotecan/NKTR-102	Nektar	Solid tumor	Phase II	IV	Topoisomerase inhibitor
PEG−SN38	Enzon	Solid tumor	Phase I	IV	Topoisomerase inhibitor
PEG−camptothecin	Enzon	Solid Tumor	Phase II	IV	Topoisomerase inhibitor

(*Continued*)

TABLE 13.4 (Continued)

PEG Conjugated	Company	Indication	Stage of Development	Route of Administration	Therapeutic class
PEG–docetaxel/NKTR-105	Nektar	Lung and colon cancer	Phase I	IV	Antimitotic
PEG–paclitaxel	Enzon	Anticancer	Phase I	IV	Antimitotic
PEG–methioninase	Anticancer, Inc.	Anticancer	Preclinical		Amino acid depleting
PEG–rHuMGDF	Kirin Brewery Company	Neutropenia	Phase II	IV	G-CSF receptor agonist
PEG-rFVIII Liposome/ BAY 79-4980	Bayer Schering Pharma	Hemophilia	Phase II	IV	Recombinant Factor VIII
PEG–Lactoferrin	NRL Pharma	Cancer and gangrene	Preclinical	Oral	Iron-binding glycoprotein

adenosine deaminase [66]. Before the availability of pegademase, SCID patients were transfused with red blood cells containing adenosine deaminase. However, blood transfusions raised the risks of iron overload and transfusion-associated viral infections [67, 68]. Pegademase has a better pharmacokinetic profile than its non-PEGylated counterpart. Because pegademase carries about 1800 times more adenosine deaminase activity per milliliter than red blood cells, the drug achieves higher blood levels of the missing enzyme [66]. However, pegademase has been reported to cause immune function problems and the benefits of its long-term treatment have yet to be elucidated. Furthermore, the high cost of treatment ($200,000–$300,000 per annum per patient) is an obvious disadvantage [69, 70].

Pegaspargase (PEG-L-Asparaginase). A further established PEGylated enzyme drug is, for example, PEG-asparaginase (Oncaspar) (5-kDa linear PEG attached at multiple sites) for the treatment of acute lymphoblastic leukemia in children. The major advantage of Oncaspar is a prolonged plasma half-life and a strongly reduced immunogenicity compared to the non-PEGylated asparaginase. Other PEGylated enzymes to be mentioned are uricase, glutaminase, arginine deiminase, and glucocerebrosidase. These drugs are still under clinical development.

Oncaspar contains the PEGylated enzyme L-asparaginase, used clinically in combination with chemotherapy for the treatment of acute lymphocytic leukemia, acute lymphoblastic leukemia, and chronic myelogenous leukemia. Leukemic cells cannot synthesize asparagine and depend on outside sources of this amino acid; asparaginase, therefore, kills leukemic cells by rapidly depleting them of asparagine [71]. Un-PEGylated asparaginase causes allergic reactions and generates neutralizing antibodies that shorten its half-life [72]; PEGylation, however, extends the half-life from the 20 h observed for un-PEGylated asparaginase to 357 h for pegaspargase. In addition, pegaspargase reduces adverse immune responses, thus improving patient compliance [73].

Peginterferon-α-2b. Cytokines are commercially the most important biopharmaceuticals today. Even their efficiency has been improved by PEGylation. Blockbuster products are PEG-α-Interferon-2b (PegIntron) and PEG-α-Interferon-2a (Pegasys) [74], which are used for the treatment of infections with hepatitis C virus. Both proteins have a very similar amino acid sequence; however, they may be distinguished by the kind of PEG chains used for PEGylation. PegIntron has been conjugated with a linear PEG molecule with a molecular weight of 10 kDa, while Pegasys has been modified with a branched reagent consisting of two 20-kDa PEG chains. Pegasys is superior to PegIntron because of a higher specific activity and a prolonged plasma half-life.

In 2001, peginterferon-α-2b (PegIntron) became available as a once-a-week treatment for hepatitis C. Chronic hepatitis C virus (HCV) infection is the leading cause of liver cirrhosis and liver cancer [73] and the principal reason for liver transplants in the United States. An estimated 2.7 million Americans are

chronically ill, with the virus, and about 35,000 new infections arise each year. Hepatitis C causes 8000–10,000 deaths yearly, and the number of deaths is estimated to increase to 38,000 by 2010 [75]. The molecular mass of IFN-α-2b is 19 kDa, whereas the molecular mass of PegIntron is 31 kDa. Peginterferon-α-2b consists of 14 positional isomers of a 12-kDa linear PEG and IFN-α-2b. Forty-seven percent of the isomers of peginterferon-α-2b involve unexpected conjugations at His(34), which makes peginterferon-α-2b more heterogeneous than peginterferon-α-2a (45–50% of peginterferon-α-2a is monosubstituted) [76–78]. Clinical studies show PEG-Intron to be superior to unPEGylated IFN-α-2b (Intron-A). PegIntron gives significantly higher virological responses and allows the reduction of dosages from three times a week to once weekly. PegIntron has a sevenfold lower clearance rate and a fivefold greater in vivo half-life than Intron-A. A standard three-times-weekly administration of Intron-A causes peaks and troughs in blood levels of interferon. In comparison, once-weekly administration of PegIntron produces more constant and longer-lasting blood levels of interferon, which results in the better suppression of viral replication [79]. In a pivotal phase III monotherapy trial of 1219 chronic hepatitis C adult patients, subjects received PEG-Intron once weekly at doses of 0.5, 1.0, or 1.5 μg per kilogram body weight, or a standard dose of Intron-A given three times a week. All patients had chronic hepatitis C with compensated liver disease and were positive for hepatitis C viral RNA (ribonucleic acid). The treatments lasted 48 weeks, and the efficacy endpoint was the sustained elimination of detectable hepatitis C viral RNA. At the end of therapy, 33, 41, and 49% of the respective PEG-Intron treatment groups showed detectable loss of viral RNA, compared with 24% of Intron-A recipients. The investigators concluded that all three PEG-Intron doses were as safe as, but superior to, Intron-A [80]. The IDEAL trial (Individual Dosing Efficacy Versus Flat Dosing to Access Optimal Peginterferon Therapy), which was the first large, randomized, clinical head-to-head comparative study, detected no significant differences between combination therapies based on peginterferon-α-2b plus ribavirin (Rebetol, Schering-Plough) and peginterferon-α-2a plus ribavirin (Copegus, Hoffman-La Roche) in terms of primary endpoints (sustained viral response and viral levels 6 months after cessation of treatment). Furthermore, their safety and tolerabilities were also comparable [81]. However, peginterferon-α-2a has a higher market share because peginterferon-α-2b is dosed on a body weight basis, whereas peginterferon-α-2a is not. As a result, peginterferon-α-2a is more frequently utilized to treat hepatitis C [82]. Nevertheless, some reports have suggested that peginterferon-α–ribavirin combination therapy has higher risks of neutropenia and thrombocytopenia than interferon-α–ribavirin combination therapy [83], although both therapies have been reported to have similar side-effect profiles [84, 85].

Meanwhile, there are other successful commercial examples of PEGylated drugs. Many others are under clinical development or close to approval. The first ever PEGylated drug on the market was Adagen (PEGylated adenosine deaminase), produced by Enzon, which is used for the treatment of a severe

combined immune deficiency in children and adolescents (SCID disease), caused mainly by the lack of the body's own adenosine deaminase. Adagen was approved under the orphan drug act in 1990 on the basis of a clinical trial with 6 patients. This method of therapy, called enzyme replacement therapy, is the starting point for the treatment of many genetically caused diseases of metabolism. The market success of such an enzyme drug for human therapy very often heavily depends on an efficient PEGylation.

Peginterferon-α-2a. Second-generation clinical PEG conjugates started to appear in the early 1990s. A competing treatment for chronic hepatitis C utilizes IFN-α-2a coupled to PEG. The first formulation in 1999 used a first-generation linear PEG of 5 kDa. In the first clinical trials, this PEGylated drug was administered to patients with chronic hepatitis C once weekly and compared with its un-PEGylated counterpart administered three times a week. The PEGylated IFN-α-2a produced no clinical advantages [86]. A second-generation, branched PEG of 40 kDa was then coupled to IFN-α-2a [87]. Peginterferon-α-2a consists of four major positional isomers formed by branched succinimidyl PEG and two 20-kDa linear PEG chains, which are attached at one site (PEG2-NHS) to Lys(31), Lys(121), Lys(131), or Lys(134) of IFN-α-2a. Moreover, due to the different PEG molecular weights used and the number of isomers formed, the pharmacokinetic profiles of these two products are dissimilar [88–90]; for example, the half-life of peginterferon-α-2a is approximately twice that of peginterferon-α-2b. However, their pharmacodynamic profiles are comparable [91]. This version (Pegasys) was subjected to clinical investigation by Hoffmann-La Roche. Once weekly injections of Pegasys produced nearly constant blood concentrations of IFN-α-2a and renal clearance is reduced 100-fold relative to un-PEGylated IFN-α-2a. PEGylation increases the half-life of IFN-α-2a from 9 to 77 h [92]. A phase III trial of 531 patients with hepatitis C compared Pegasys administered once weekly with un-PEGylated IFN-α-2a administered three times a week for 48 weeks. Undetectable levels of hepatitis C viral RNA occurred more in the Pegasys group (68%) than in the other group (28%). Compliance was also better in the Pegasys group, with 84% of patients completing the study, compared with 60% of patients receiving the un-PEGylated drug. The reduction of dosing injections for Pegasys can result in improved patient compliance. The investigators concluded that "a regimen of peginterferon α2a given once weekly is more effective than a regimen of interferon α2a given three times weekly" [93] (Page 1666).

Another randomized study compared Pegasys with un-PEGylated IFN-α-2a in 271 patients with chronic hepatitis C and liver cirrhosis. Two doses of Pegasys (90 or 180 μg injected once weekly) were compared with un-PEGylated IFN-α-2a injected three times a week.

At the end of 72 weeks, hepatitis C viral RNA was undetectable in 8, 15, and 30% of the patients treated with un-PEGylated IFN-α-2a, 90 μg of Pegasys and 180 μg of Pegasys, respectively. In addition, liver biopsy samples showed histological improvements of 31, 44, and 54% for the same respective drugs and

doses [94]. Before the development of PEGylated interferons, the optimal treatment for chronic hepatitis C combined interferon and the antiviral agent ribavirin, which results in enhanced clinical outcomes over interferon alone [73, 95, 96]. Studies were undertaken to assess whether combining PEGylated interferons with ribavirin also offers clinical advantages. A small pilot study of Pegasys plus ribavirin in 20 patients with chronic hepatitis C found that sustained virological and biochemical responses occur in 9 out of 20 patients. The genotype of hepatitis C appears to affect the outcome: Just 5 of 16 patients infected with genotype 1 achieved sustained responses, whereas all 4 patients infected with other genotypes responded [81].

A phase III study investigated the response of 1530 patients with hepatitis C, who received PEG-Intron plus ribavirin or Intron-A plus ribavirin for 48 weeks. A 54% sustained virological response was observed in those receiving PEG-Intron plus ribavirin, compared with 47% in those receiving Intron-A plus ribavirin.

This response rate can be improved when the HCV genotype is taken into account. The response rate for patients with HCV genotypes 2 and 3 reached 80% on both treatments, but only 42% for HCV genotype 1 [84]. Another trial compared Pegasys plus ribavirin with IFN-α-2b plus ribavirin in 1121 patients with chronic hepatitis C. A significantly higher proportion of patients who received Pegasys plus ribavirin showed sustained elimination of detectable virus than patients who received IFN-α-2b plus ribavirin (56 versus 44%, respectively). However, the proportions of patients with HCV genotype 1 who had a sustained virologic response were only 46 and 35%, respectively [97]. The competing PEGylated forms of IFN-α (PegIntron and Pegasys) show superior efficacy compared with their un-PEGylated counterparts. Our knowledge of PEGylation chemistry predicts that the higher-molecular-mass branched formulation of Pegasys should show a superior pharmacokinetic profile. However, only head-to-head clinical trials can confirm whether one drug is superior to the other under different clinical circumstances. In the treatment of a chronic illness such as hepatitis C, clinical efficacy will be determined by the therapy that induces the highest, sustained blood levels of the drug with the fewest injections. Pegasys was approved by the FDA for the treatment of chronic hepatitis C in October 2002, and the combination Pegasys and ribavirin therapy was approved in December 2002. Several other PEGylated polypeptides are undergoing clinical trials.

Pegvisomant. A PEGylated form of human growth hormone antagonist called pegvisomant (Somavert) is being developed for the treatment of acromegaly. Human growth hormone receptor has two binding sites: GH first binds to site 1 and then to site 2, which induces the functional dimerization of the hGH receptor. Acromegaly results when the pituitary gland produces excess GH after epiphyseal plate closure, and pegvisomant acts to inhibit the dimerization of the hGH receptor. More specifically, pegvisomant has increased affinity for site 1 of the hGH receptor, with eight amino acid mutations at the site, and by the substitution

of position 120 glycine to arginine, inhibits hGH receptor dimerization. Furthermore, the 4–6 PEG-5000 moieties added to pegvisomant prolong its half-life and allow once-daily administration [98]. Patients with acromegaly experience extremely high serum levels of insulinlike growth factor-1 (IGF1) that contributes to soft-tissue enlargement. A phase III trial of pegvisomant showed that daily treatment normalized levels of IGF1 and improved soft-tissue enlargement in 131 patients [99, 100].

Pegvisomant has been approved in Europe and is awaiting FDA approval in the United States. The cytokine tumor necrosis factor-α (TNF-α) plays a key role in mediating inflammation. A cloned soluble recombinant form of a natural inhibitor of TNF-α—sTNF receptor type I (TNF-RI)—has been attached to a high-molecular-mass PEG to improve its pharmacokinetics, and preclinical studies in rodent models of rheumatoid arthritis (RA) and Crohn's disease demonstrate its potential efficacy [101]. A 12-week trial of 194 patients with RA compared two doses of PEGTNF-RI with a placebo. Patients receiving the drug reported improvement in physical function, pain, general health, vitality, social function, and mental health in a dose-dependent manner [102]. A competing PEGylated anti-TNF-α antibody fragment (CDP870) also had encouraging clinical results [103]. Current treatments for acromegaly include adenomectomy, radiotherapy, and therapy with dopamine agonists or somatostatin analogs. Pegvisomant, a genetically engineered GH-receptor antagonist, could be added to this list of treatments; however, because pegvisomant can increase glucose tolerance, care must be taken with diabetes mellitus patients [104].

Pegfilgrastim. Pegfilgrastim (Neulasta, Amgen), produced from filgrastim (Neupogen, Amgen) using Nektar (formerly Shearwater) PEGylation technology, is formed by conjugating a 20-kDa linear monomethoxy–PEG aldehyde with granulocyte colony-stimulating factor (G-CSF) in an acidic buffer solution (pH 5). This minimizes problems associated with nonselective conjugation, in the presence of sodium cyanoborohydride. This process leads to site-specific PEGylation at the *N*-terminal methionyl residue of G-CSF, and improves the biological activity of filgrastim [105]. Filgrastim is administered by injection daily for 2 weeks, whereas pegfilgrastim requires only one injection per chemotherapy cycle. In addition, the side effects of pegfilgrastim are smaller than those of filgrastim; they include splenic rupture, sickle cell crises, allergic reactions, and anaphylaxis. Nevertheless, mild to moderate bone pain has been reported for pegfilgrastim therapy [106, 107]. Data from two pivotal phase III trials in breast cancer patients indicated that a single dose of pegfilgrastim provides as much protection from infection as 11 daily injections of filgrastim [108, 109].

PEG–Doxorubicin. PEGylated liposomal doxorubicin, a topoisomerase II inhibitor (Doxil) is a doxorubicin liposome coated with PEG that prevents opsonization and reduces clearance by the reticulo-endothelial system, leading to a significant prolongation in the liposome circulation time. It has excellent

activity in the treatment of metastatic breast cancer as a single agent or in combination with taxane, gemcitabine, cyclophosphamide, or trastuzumab [110].

The phase II clinical trial examined the efficacy and safety of Doxil given at 35 mg/m^2 plus cyclophosphamide at 600 mg/m^2 as the first-line therapy, delivered every 3 weeks, to 70 patients who developed metastatic disease more than 12 months after completion of an adjuvant anthracycline-containing regimen. After a median of six cycles, 33% of patients achieved stable disease lasting more than 6 months, for an overall clinical benefit rate of 71%. Treatment was well tolerated. The most common grade 3 to 4 toxicities were palmar-plantar erythrodysesthesia (PPE; 10%), dyspnea (9%), and neutropenia (9%). No symptomatic cardiac events were observed [111].

Pegaptanib Sodium. Pegaptanib sodium (Macugen, Pfizer) is a 28-nucleotide aptamer (RNA aptamers consisting of short strands of oligonucleotides) with affinity for vascular endothelial growth factor (VEGF), and was approved by the FDA in 2004 as an orphan drug to treat age-related macular degeneration. In order to improve its stability against nuclease digestion, deoxythymidine was bound to the 3′-terminus of pegaptanib sodium by a′3 -3′ linkage, and a branched PEG molecule with two 20-kDa linear units was linked to the lysine 5′-terminus residue [112].

Mono-m-PEG-epoetin-β. Mono-m-PEG-epoetin-β (Mircera, Hoffman-La Roche), also known as continuous erythropoietin receptor activator (CERA), is a long-acting erythropoiesis stimulating agent (ESA), and is used to normalize or maintain hemoglobin levels in chronic kidney disease (CKD) patients. Mono-m-PEG-epoetin-β is synthesized by reacting epoetin-β with methoxy polyethylene glycol-succinimidyl butanoic acid (m-PEG-SBA) at the *N*-terminal amino group of epoetin-β or the ε-amino group of lysine at positions 52 and 45, to form an amide bond with 30-kDa PEG. These features prolong its half-life to approximately 130 h [113, 114]. Because erythropoietin (EPO, a glycosylated protein hormone) regulates erythropoiesis homeostasis, recombinant human EPO (rhEPO) is used to treat CKD or anemia in cancer patients. However, rhEPO must be injected three times a week due to its short half-life. Darbepoetin-α (Aranesp, Amgen), a second-generation EPO, has a higher glycosylation rate due to the inclusion of an amino acid mutation and requires only weekly or biweekly injections. Furthermore, third-generation EPO (CERA) improves quality of life markedly because it requires only monthly administration. Nevertheless, like other ESAs, it has been reported to have negligible effects on morbidity or mortality [113]. In the United States, no indications for the treatment of anemia caused by cancer chemotherapy have been approved, and because most patients with anemia associated with chronic renal failure are inpatients, the prolonged circulating times of m-PEG-epoetin-β may not be a crucial advantage.

Certolizumab Pegol. Unlike other antibody drugs, which are produced in mammalian cell cultures, the base drug in certolizumab pegol (Cimzia, UCB) is

produced in *E. coli*. Certolizumab pegol is designed such that 40 kDa PEG-maleimide is covalently bound to the Fab fragment, directed against TNF-α, through the *C*-terminal cysteine at position 227 of the heavy chain. The Fab fragment has a hinge structure at the *C*-terminus of the heavy chain, which includes a single cysteine residue [115], and specific PEGylation between the thiol group of the *C*-terminal cysteine and the maleimide group of the branched 40-kDa PEG forms a stable thioether linkage. Due to the monosite attachment of the branched PEG, certolizumab pegol is more homogeneous, maintains full binding activities of the antibody fragment [33], and lacks the Fc portion, which has been reported to induce adverse effects by other TNF-α inhibitors [116]. In addition, because it is produced in *E. coli*, certolizumab pegol is priced competitively versus infliximab (Remicade, Centocor, Inc.) or adalimumab (Humira, Abbott), which are also biological TNF-α inhibitors that are used to treat rheumatoid arthritis or inflammatory bowel disease. Certolizumab pegol could be used to treat Crohn's disease or rheumatoid arthritis because it inhibits TNF-α, but it has only been approved for the treatment of Crohn's disease. When PEGylated drugs with enhanced bioavailabilities enter the market after their native proteins, they tend to have an additive effect; in other words, they do not reduce use of the native protein. According to IMS Health, during the 12 months in 2008 world sales of pegfilgrastim were $3745 million, which represented 61.8% of the CSF market ($6059 million). In contrast, total sales of filgrastim were just $1490 million. In the case of IFN-α-2a, the high market shares of PEGylated drugs are more evident; for example, the sales of Pegasys and Roferon-A (interferon-α-2a, Hoffman-La Roche) in 2008 were $1014 million and $68 million, respectively and whereas the sales and growth of peginterferon-α-2a were maintained, the sales of IFN-α-2a remained at a constant level.

13.5.2 Clinical PEG Conjugates

PEG–Recombinant Factor VIII. Factor VIII was extensively used in hemophilia patients who suffered from abnormal bleeding. However, patients have to periodically inject factor VIII because it will be eliminated from the body over time. The PEG–recombinant factor VIII (rFVIII) liposome provided longer acting factor VIII that can sustain its activity for several months. A phase I clinical trial has shown that the mean number of bleeding free days using PEG-rFVIII liposome was significantly improved over the conventional rFVIII treatment [117].

PEG–Arginase. Arginase, an endogenous protein with arginine-depleting activity, has been shown to have potent anticancer properties. However, arginase alone is not suitable for development as a medication because of its short half-life and low affinity at a physiological pH. BCT-100 is a PEGylated product of arginase (produced using *Bacillus subtilis*) and m-PEG–succinamide propionic acid (m-PEG-SPA, with molecular weight 5 kDa). Unlike other

PEGylated enzymes, BCT-100 is as active as arginase and has a prolonged *in vivo* half-life of 3 days with acceptable activity at physiological pH values. Moreover, BCT-100 does not produce ammonia as a by-product.

PEG–Abciximab. Abciximab is a platelet aggregation inhibitor preventing platelets from sticking together and causing thrombus formation. PEGylated abciximab did not change the intrinsic affinity to the target but did decrease the IC50 prolonged inhibition of platelet aggregation.

Pegloticase. Rasburicase (uricase) is used to prevent the chemotherapy-induced hyperuricemia and acute uric acid nephropathy even though it has a short half-life and shows immunogenicity. A PEGylated form of uricase is pegloticase that prevents inflammation and pain due to urate crystal formation in the plasma of gout patients [118]. However, pegloticase has been reported to be immunogenic. Subcutaneous injections of pegloticase in a phase I clinical trial and intravenous injections of pegloticase in a phase II clinical trial both resulted in the production of antibodies. It was later shown that these antibodies were produced against PEG rather than uricase.

PEG–Lactoferrin. Lactoferrin (LF) is an iron carrier glycoprotien, with a molecular weight of about 80 kDa. It exhibits several functions such as antimicrobial, antiviral, immunomodulation, antioxidation, anti-inflammation, antistress, and analgesic effects, as well as enhancement of lipid metabolism. The conventional oral lactoferrin has the drawback that it is unstable in gastric juice and has a low absorption [119]. The PEGylated lactoferin diminished immunogenicity and improved the pharmacokinetic behavior. A preclinical study of a 20-kDa PEG conjugated bovine lactoferin (bLF) produced a high serum concentration of unmodified bLF (10 mg/kg) that decreased rapidly, while the serum concentration of the 20k-PEGbLF (1 mg/kg) was detected 10 min postadministration, then decreased very slowly, and continued to be detected up to 240 min after intravenous administration. Moreover, the oral administration of 20k-PEGbLF showed better activity that unmodified bLF [120]. Therefore, PEGylated bLF is a promising alternative for the improved oral delivery of bLF.

PEG–Camptothecin. PEG–camptothecin exhibits several good characteristics in tumor treatment and it is currently in various phases of clinical trials. The approach to the synthesis of PEG–camptothecin includes producing a carbamate derivative of 10-hydroxy-7-ethylcamptothecin and amino acid esters at the 20-OH group [121]. Derivatization can be done with mono-conjugated or di conjugated PEG (Fig. 13.5) [122].

The clinical phase I and pharmacokinetics study of PEG-CPT (Pegamotecan, Enzon, USA) has indicated that patients were well tolerant to repetitive treatment at 7000 mg/m^2. Adverse drug reactions such as cystitis, nausea, and vomiting were also observed but these were rarely severe. Free CPT in the

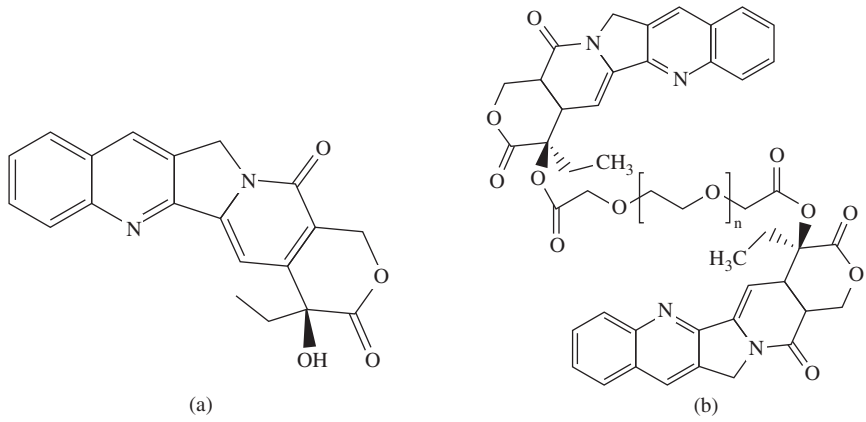

FIGURE 13.5 (a) Camptothecin and (b) camptothecin–PEG conjugate. Adapted from [122].

plasma was prolonged [123]. However, the efficacy during a phase IIb trial was not satisfactory and was dropped from further development [124].

PEG–Paclitaxel. PEG–paclitaxel with a molecular weight ≥30 kDa could prevent rapid elimination by the kidneys. Although a PEG-paclitaxel derivative have entered phase I clinical trials Enzon has discontinued the development of this product [125].

PEG–Docetaxel. A PEG–docetaxel derivative has shown good preclinical efficacy in colon and lung cancer xenograft models. The phase I clinical studies were conducted in refractory solid tumor patients [126].

PEG–Irinotecan. PEG–irinotecan was developed using new multiarm PEGs architectures. The drug was covalently bound to four PEG arms. In preclinical studies plasma half-life was evaluated in a mouse model. The conjugate showed prolonged pharmacokinetic profiles with a half-life of 15 days when compared to 4 h with free irinotecan. Thereafter, 57 patients with advanced solid tumors whose tumors had failed prior treatment options were enrolled in a phase I clinical trial to test the safety, pharmacokinetic, and antitumor activity. The patients were divided into three single agent administration schedules: weekly administration for 3 weeks with the fourth week drug free ($n = 32$; doses ranged from 58 to 230 mg/m^2), every two weeks ($n = 10$; doses ranged from 145 mg/m^2 to 220 mg/m^2) and every 3 weeks ($n = 15$; doses range from 145 mg/m^2 to 245 mg/m^2). Each patient received a 90-min. infusion of PEG–irinotecan. Seven patients showed a reduction of tumor size that ranged from 40 to 58% while 6 patients showed only a minor response. Cumulative SN-38, the active metabolite of irinotecan was 1.2- to 6.5-fold higher than that predicted for irinotecan [127].

Toxicity effects such as diarrhea were manageable by limiting the dose. Multiple phase II studies are ongoing alone or in combination with cetuximab for the treatment of ovarian, breast, colorectal, and cervical cancer.

PEG–Insulin. The conventional method to administer insulin is by injection, and patients suffer from the pain and inconvenience. Therefore, many pharmaceutical companies have worked hard to develop an oral insulin delivery. However, one of the most difficult obstacles to oral insulin delivery is protecting the protein against the unsuitable environments in the gastrointestinal (GI) tract such as enzymatic degradation and strong acid/base condition [52]. Hydrogels composed of PEG and its derivatives have shown potential for oral insulin delivery. For example, poly(methacrylic acid-*g*-ethylene glycol) [P(MAA-*g*-EG)] forms a copolymer network with water-filled nanopores that exhibits reversible, pH-dependent swelling behavior (pH $\sim$1–8) [128]. In the acidic environment of the stomach (pH 1–2) the network collapses and traps insulin, whereas in the neutral environment of the lower intestine (pH 6–7) the network swells allowing insulin release. However, enzymatic degradations of insulin by GI proteases still occurs and this needs to be minimized. The steric hindrance of enzymes caused by the presence of PEG decreases the proteolysis of labile peptide residues.

Potential amino acid targets available for insulin PEGylation most commonly investigated are at the *N*-terminals of the A and B chains, glycine and phenylalanine, respectively, and the lysine at the 29th residue of the B chain as seen in Figure 13.6. Derivatization of the GlyA1 residue significantly reduced

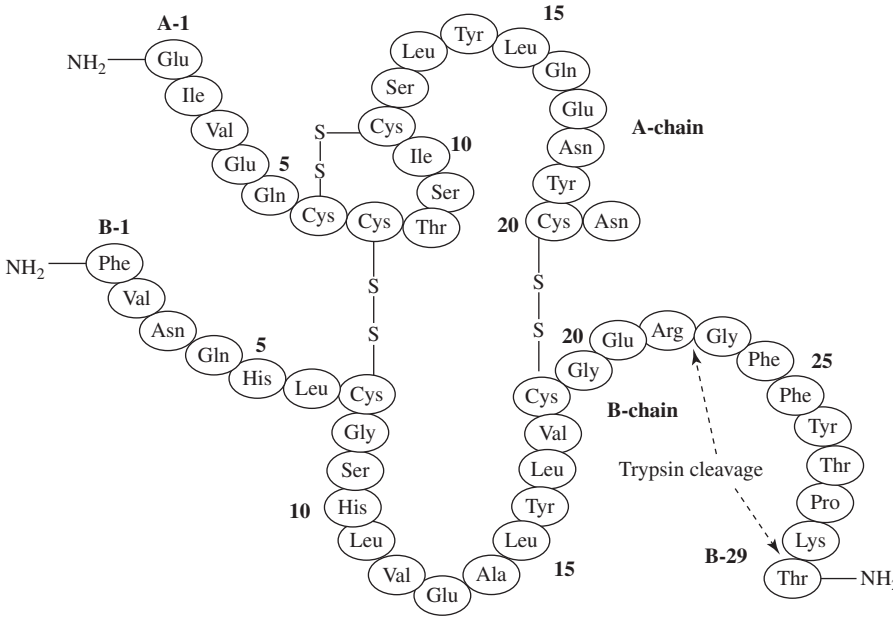

FIGURE 13.6 Amino acid sequences of human insulin. (Redrawn from [52].)

bioactivity of the insulin; therefore, the PheB1 and the LysB29 sites are now generally targeted for modification. Poly(methacrylic acid-*g*-ethylene glycol) [P(MAA-*g*-EG)]–insulin hydrogel conjugate, successfully induced hypoglycemia that exceeded 100% after both intravenous (IV) and subcuteanous (SC) administration [129]. While it may seem that modification of insulin would reduce its ability to lower circulating glucose levels, this effect can be explained not by an increased potency of PEGylated insulin but in its increased residence time in the bloodstream. The reduced rate of clearance causes it to have an extended action and therefore a prolonged hypoglycemic effect. This may also be influenced by a reduced affinity of PEGylated insulin for the insulin receptor such that it is released and binds to subsequent receptors.

13.6 CONCLUSIONS

PEGylation technology has taken several years to serve as a tool in production of PEGylated drugs. There are improvements in technology in order to eliminate toxicity/undesirable effect and improve PEGylated drug efficiency. Numbers of new chemical classes have been introduced to the PEGylation world. It is expected that PEGylation will play an important role in pharmaceuticals.

REFERENCES

1. Dubin, C. H. Protiens & peptides: Dependent on advances in drug delivery? *Drug Deliv. Tech.* 2009;**9**(3):36–41.
2. Roberts, M. J., M. D. Bentley, and J. M. Harris. Chemistry for peptide and protein PEGylation. *Adv. Drug Deliv. Rev.* 2002;**54**(4):459–476.
3. Mateo, C., J. Lombardero, E. Moreno, A. Morales, G. Bombino, J. Coloma, L. Wims, S. L. Morrison, and R. Pérez. Removal of amphipathic epitopes from genetically engineered antibodies: Production of modified immunoglobulins with reduced immunogenicity. *Hybridoma* 2000;**16**(9):463–471.
4. Lyczak, J. B. and S. L. Morrison. Biological and pharmacokinetic properties of a novel immunoglobulin-CD4 fusion protein. *Arch. Virol.* 1994;**139**(1):189–196.
5. Syed, S., P. D. Schuyler, M. Kulczycky, and W. P. Sheffield. Potent antithrombin activity and delayed clearance from the circulation characterize recombinant hirudin genetically fused to albumin. *Blood* 1997;**89**(9):3243–3252.
6. Szebeni, M. D. P. D. J. The interaction of liposomes with the complement system. *Crit. Rev. Therap. Drug Carrier Syst.* 1998;**15**(1):32.
7. Bailon, P. and W. Berthold. Polyethylene glycol-conjugated pharmaceutical proteins. *Pharm. Sci. Tech. Today* 1998;**1**(8):352–356.
8. Zalipsky, S. Chemistry of polyethylene glycol conjugates with biologically active molecules. *Adv. Drug Deliv. Rev.* 1995;**16**:157–182.
9. Gabizon, A. and F. Martin. Ger: Polyethylenglykol-umhulltes (pegyliertes) liposomales Doxorubicin: Grundlagen fur die Anwendung bei soliden Tumoren. *Drugs* 1997;**54**:15–21.

10. Hamidi, M., A. Azadi, and P. Rafiei. Pharmacokinetic consequences of pegylation. *Drug Deliv*. 2006;**13**(6):399–409.
11. Veronese, F. M. and G. Pasut. PEGylation, successful approach to drug delivery. *Drug Discov. Today* 2005;**10**(21):1451–1458.
12. Mahmood, I. and M. D. Green. Pharmacokinetic and pharmacodynamic considerations in the development of therapeutic proteins. *Clin. Pharmacokin*. 2005;**44**:331–347.
13. Harris, J. M., N. E. Martin, and M. Modi. Pegylation: A novel process for modifying pharmacokinetics. *Clin. Pharmacokin*. 2001;**40**:539–551.
14. Greenwald, R. B., Y. H. Choe, J. McGuire, and C. D. Conover. Effective drug delivery by PEGylated drug conjugates. *Adv. Drug Deliv. Rev*. 2003;**55**(2):217–250.
15. Harris, J. M. and R. B. Chess. Effect of pegylation on pharmaceuticals. *Nat. Rev. Drug Discov*. 2003;**2**(3):214–221.
16. Chapman, A. P. PEGylated antibodies and antibody fragments for improved therapy: A review. *Adv. Drug Deliv. Rev*. 2002;**54**(4):531–545.
17. Veronese, F. M. Peptide and protein PEGylation: A review of problems and solutions. *Biomaterials* 2001;**22**:405–417.
18. Delgado, C., G. E. Francis, and D. Fisher. The uses and properties of PEG-linked proteins. *Crit. Rev. Therap. Drug Carrier Syst*. 1992;**9**(3,4):249–304.
19. Pasut, G. and F. M. Veronese. Polymer-drug conjugation, recent achievements and general strategies. *Prog. Polym. Sci*. 2007;**32**:933–961.
20. Harris, J. M., L. Gou, and Z. H. Fang. PEG-protein tethering for pharmaceutical applications. Seventh International Symposium on Recent Advances in Drug Delivery. Salt Lake City, 1995.
21. Harris, J. M. and A. Kozlowski. Polyethylene glycol and related polymers monosubstituted with propionic or butaonic acids and functional derivatives thereof for biotechnical applications. U.S. Patent US672662, 1997.
22. Goodson, R. J. and N. V. Katre. Site-directed pegylation of recombinant interleukin-2 at its glycosylation site. *Nat. Biotech*. 1990;**8**(4):343–346.
23. El Tayar, N., M. J. Roberts, J. M. Harris, and W. Sawlivich. Polyol-IFN-β conjugates. Patent WO1999/55377, 1999.
24. Zalipsky, S., M. Qazen, J. A. Walker, N. Mullah, Y. P. Quinn, and S. K. Huang. New detachable poly(ethylene glycol) conjugates: Cysteine-cleavable lipopolymers regenerating natural phospholipid, diacyl phosphatidylethanolamine. *Bioconj. Chem*. 1999;**10**(5):703–707.
25. Bentley, M. D., J. M. Harris, and A. Kozlowski. Heterobifunctional polyethylene glycol derivatives and methods for their preparation. U.S. Patent US7642323, 2001.
26. Yokoyama, M., T. Okano, Y. Sakurai, A. Kikuchi, N. Ohsako, Y. Nagasaki, and K. Kataoka. Synthesis of poly(ethylene oxide) with heterobifunctional reactive groups at its terminals by an anionic initiator. *Bioconj. Chem*. 1992;**3**(4):275–276.
27. Yamasaki, N., A. Matsuo, and H. Isobe. Novel polyethylene glycol derivatives for modification of proteins. *Agric. Biol. Chem*. 1988;**52**(8):2125–2127.
28. Veronese, F.M., P. Caliceti, and O. Schiavon. Branched and linear poly(ethylene glycol): Influence of the polymer structure on enzymological, pharmacokinetic, and immunological properties of protein conjugates. *J. Bioact. Compat. Polym*. 1997;**12**(3):196–207.

29. Caliceti, P. and F. Veronese. Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. *Adv. Drug Deliv. Rev.* 2003;**55** (10):1261–1277.
30. Kang, J. S., P. P. DeLuca, and K. C. Lee. Emerging PEGylated drugs. *Expert Opin. Emerg. Drugs* 2009;**14**(2):363–380.
31. Davis, F. F. The origin of pegnology. *Adv. Drug Deliv. Rev.* 2002;**54**(4):457–458.
32. Guiotto, A., M. Pozzobon, C. Sanavio, O. Schiavon, P. Orsolini, and F. M. Veronese. An improved procedure for the synthesis of branched polyethylene glycols (PEGs) with the reporter dipeptide met-[beta]ala for protein conjugation. *Bioorg. Med. Chem. Lett.* 2002;**12**(2):177–180.
33. Chapman, A. P., P. Antoniw, M. Spitali, S. West, S. Stephens, and D. J. King. Therapeutic antibody fragments with prolonged in vivo half-lives. *Nat. Biotech.* 1999;**17**(8):780–783.
34. Sato, H. Enzymatic procedure for site-specific pegylation of proteins. *Adv. Drug Deliv. Rev.* 2002;**54**(4):487–504.
35. Schiavon, O., G. Pasut, S. Moro, P. Orsolini, A. Guiotto, and F. M. Veronese. PEG-Ara-C conjugates for controlled release. *Eur. J. Med. Chem.* 2004;**39** (2):123–133.
36. Gaberc-Porekar, V., I. Zore, B. Podobnik, and V. Menart. Obstacles and pitfalls in the PEGylation of therapeutic proteins. *Curr. Opin. Drug Disc.* 2008;**11**(2): 242–250.
37. DeFrees, S., Z-G. Wang, R. Xing, A. E. Scott, J. Wang, D. Zopf, D. L. Gouty, E. R. Sjoberg, K. Panneerselvam, and E. C. M. B-Vd. Linden, R. J. Bayer, M. A. Tarp, and H. Clausen. GlycoPEGylation of recombinant therapeutic proteins produced in Escherichia coli. *Glycobiology* 2006;**16**(9):833–843.
38. Veronese, F. Peptide and protein PEGylation: A review of problems and solutions. *Biomaterials* 2001;**22**(5):405–417.
39. Woghiren, C., B. Sharma, and S. Stein. Protected thiol-polyethylene glycol: A new activated polymer for reversible protein modification. *Bioconj. Chem.* 1993;**4** (5):314–8.
40. Amunix. Amunix home information. Retrieved Feb. 10, 2010, from http://www .amunix.com/.
41. Schellenberger, V., W. P. Stemmer, C-W. Wang, M. D. Scholle, M. Popkov, N. C. Gordon, and A. Crameri. Unstructured recombinant polymers and uses thereof. U.S. Patent US20080039341, 2008.
42. Lee, S., R. B. Greenwald, J. McGuire, K. Yang, and C. Shi. Drug delivery systems employing 1,6-elimination: Releasable poly(ethylene glycol) conjugates of proteins. *Bioconj. Chem.* 2001;**12**(2):163–169.
43. Filpula, D. and H. Zhao. Releasable PEGylation of proteins with customized linkers. *Adv. Drug Deliv. Rev.* 2008;**60**(1):29–49.
44. Sinhababu, A. K. and D. R. Thakker. Prodrugs of anticancer agents. *Adv. Drug Deliv. Rev.* 1996;**19**: 241–273.
45. Maeda, H., L. Seymour, and Y.Miyamoto. Conjugates of anticancer agents and polymers: Advantages of macromolecular therapeutics in vivo. *Bioconj. Chem.* 1992;**3**(5):351–362.
46. Maeda, H. SMANCS and polymer-conjugated macromolecular drugs: Advantages in cancer chemotherapy. *Adv. Drug Deliv. Rev.* 2001;**46**:169–185.

47. Kelland, L. Discontinued drugs in 2005: Oncology drugs. *Expert Opin. Invest. Drugs* 2006;**15**(11):1309–1318.
48. Xuan, Z. and J. M. Harris. Novel degradable poly(ethylene glycol) hydrogels for controlled release of protein. *J. Pharm. Sci.* 1998;**87**(11):1450–1458.
49. Gillinov, A. M. and W. L. Bruce. A novel synthetic sealant to treat air leaks at cardiac reoperation. *J. Card. Surg.* 2001;**16**(3):255–257.
50. Wain, J. C., L. R. Kaiser, D. W. Johnstone, S. C. Yang, C. D. Wright, J. S. Friedberg, R. H. Feins, R. F. Heitmiller, D. J. Mathisen, and M. R. Selwyn. Trial of a novel synthetic sealant in preventing air leaks after lung resection. *Ann. Thor. Surg.* 2001;**71**(5):1623–1629.
51. Ferland, R., D. Mulani, and P. K. Campbell. Evaluation of a sprayable polyethylene glycol adhesion barrier in a porcine efficacy model. *Human Reprod.* 2001;**16**(12):2718–2723.
52. Hinds, K. D. and S. W. Kim. Effects of PEG conjugation on insulin properties. *Adv. Drug Deliv. Rev.* 2002;**54**(4):505–530.
53. Weir, A., A. Nesbitt, A. Chapman, A. Popplewell, P. Antoniw, and A. Lawson. Formatting antibody fragments to mediate specific therapeutic functions. *Biochem. Soc. Trans.* 2002;**30**(4):512–516.
54. Rocca, M., G. Giavaresi, P. Caliceti, F. Veronese, and R. Giardino. Pathophysiological and histomorphological evaluation of polyacryloylmorpholine vs polyethylene glycol modified superoxide dismutase in a rat model of ischaemia/reperfusion injury. *Int. J. Artif. Organs* 1996;**19**(12):730–734.
55. Zalipsky, S. and J. M. Harris. Chemistry and biological applications of polyethylene glycol (American Chemical Society Symposium Series 680) San Francisco, 1997, pp. 1–15.
56. Calvo, P., B. Gouritin, H. Chacun, D. Desmaële, J. D'Angelo, J-P. Noel, D. Georgin, E. Fattal, J. P. Andreux, and P. Couvreur. Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. *Pharm. Res.* 2001;**18**(8):1157–1166.
57. Shi, N., R. J. Boado, and W. M. Pardridge. Receptor-mediated gene targeting to tissues in vivo following intravenous administration of pegylated immunoliposomes. *Pharm. Res.* 2001;**18**(8):1091–1095.
58. Avramis, V. I., S. Sencer, A. P. Periclou, H. Sather, B. C. Bostrom, L. J. Cohen, A. G. Ettinger, L. J. Ettinger, J. Franklin, P. S. Gaynon, J. M. Hilden, B. Lange, F. Majlessipour, P. Mathew, M. Needle, J. Neglia, G. Reaman, J. S. Holcenberg, and L. Stork. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: A Children's Cancer Group study. *Blood* 2002;**99**(6):1986–1994.
59. Schwarz, K. B., P. Mohan, M. Narkewicz, J. P. Molleston, H. S. Te, S. Hu, S. Sheridan, M. Lamb, S. C. Pappas, and G. Harb. The safety, efficacy and pharmacokinetics of peginterferon Alfa-2a (40KD) in children with chronic hepatitis C. *Gastroenterology* 2003;**124**(4, Suppl 1):A700.
60. Tetsuji Y., T. Yasuhiko, and I. Yoshito. Distribution and tissue uptake of poly (ethylene glycol) with different molecular weights after intravenous administration to mice. *J. Pharm. Sci.* 1994;**83**(4):601–606.
61. Brannon-Peppas, L. Poly(ethylene glycol): Chemistry and biological applications. *J. Control. Release* 2000;**66**(2–3):321.

62. Richter, A. W. and E. Kerblom. Antibodies against polyethylene glycol produced in animals by immunization with monomethoxy polyethylene glycol modified proteins. *Int. Arch. Allergy Immunol.* 1983;**70**(2):124–131.

63. Cheng, T-L., P-Y. Wu, M-F. Wu, J-W. Chern, and S. R. Roffler. Accelerated clearance of polyethylene glycol-modified proteins by anti-polyethylene glycol IgM. *Bioconj. Chem.* 1999;**10**(3):520–528.

64. Monfardini, C., O. Schiavon, P. Caliceti, M. Morpurgo, J. M. Harris, and F. M. Veronese. A branched monomethoxypoly(ethylene glycol) for protein modification. *Bioconj. Chem.* 1995;**6**(1):62–69.

65. Kozlowski, A., S. A. Charles, and J. M. Harris. Development of pegylated interferons for the treatment of chronic hepatitis C. *BioDrugs* 2001;**15**:419–429.

66. Hershfield, M. S. PEG-ADA replacement therapy for adenosine deaminase deficiency: An update after 8.5 years. *Clin. Immunol. Immunopath.* 1995;**76**(3, Part 2): S228–S232.

67. Burnham, N. L. Polymers for delivering peptides and proteins. *Am. J. Health-Syst. Pharm.* 1994;**51**(2):210–218.

68. Hillman, B. C. and R. U. Sorensen. Management options: SCIDS with adenosine deaminase deficiency. *Ann. Allergy* 1994;**72**(5):395–403.

69. Booth, C., M. Hershfield, L. Notarangelo, R. Buckley, M. Hoenig, N. Mahlaoui, M. Cavazzana-Calvo, A. Aiuti, and H. B. Gaspar. Management options for adenosine deaminase deficiency; proceedings of the EBMT satellite workshop (Hamburg, March 2006). *Clin. Immunol.* 2007;**123**(2):139–147.

70. Chan, B., D. Wara, J. Bastian, M. S. Hershfield, J. Bohnsack, C. G. Azen, R. Parkman, K. Weinberg, and D. B. Kohn. Long-term efficacy of enzyme replacement therapy for adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID). *Clin. Immunol.* 2005;**117**(2):133–143.

71. Keating, M. J., R. Holmes, S. Lerner, and D. H. Ho. L-Asparaginase and PEG asparaginase past, present, and future. *Leuk. Lymph.* 1993;**10**(s1):153–157.

72. Holle, L. M. Pegaspargase: An alternative? *Ann. Pharmacother.* 1997;**31**(5): 616–624.

73. Liang, T. J., B. Rehermann, L. B. Seeff, and J. H. Hoofnagle. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann. Internal Med.* 2000;**132**(4): 296–305.

74. Reddy, K. R., M. W. Modi, and S. Pedder. Use of peginterferon alfa-2a (40 KD) (Pegasys(R)) for the treatment of hepatitis C. *Adv. Drug Deliv. Rev.* 2002;**54** (4):571–586.

75. Kozlowski, A. and J. M. Harris. Improvements in protein PEGylation: Pegylated interferons for treatment of hepatitis C. *J. Control. Release* 2001;**72**(1–3):217–224.

76. Wang, Y-S., S. Youngster, M. Grace, J. Bausch, R. Bordens, and D. F. Wyss. Structural and biological characterization of pegylated recombinant interferon alpha-2b and its therapeutic implications. *Adv. Drug Deliv. Rev.* 2002;**54**(4): 547–570.

77. Bailon, P., A. Palleroni, C. A. Schaffer, C. L. Spence, W-J. Fung, J. E. Porter, G. K. Ehrlich, W. Pan, Z-X. Xu, M. W. Modi, A. Farid, W. Berthold, and M. Graves. Rational design of a potent, long-lasting form of interferon: A 40 kDa branched polyethylene glycol-conjugated interferon alfa-2a for the treatment of hepatitis C. *Bioconj. Chem.* 2001;**12**(2):195–202.

78. Grace, M. J., S. Lee, S. Bradshaw, J. Chapman, J. Spond, S. Cox, M. DeLorenzo, D. Brassard, D. Wylie, S. Cannon-Carlson, C. Cullen, S. Indelicato, M. Voloch, and R. Bordens. Site of pegylation and polyethylene glycol molecule size attenuate interferon-alfa antiviral and antiproliferative activities through the JAK/STAT signaling pathway. *J. Biol. Chem.* 2005;**280**(8):6327–6336.

79. Glue, P., J. W. S. Fang, R. Rouzier-Panis, C. Raffanel, R. Sabo, S. K. Gupta, M. Salfi, and S. Jacobs. PEG-interferon-α2b: Pharmacokinetics, pharmacodynamics, safety and preliminary efficacy data. *Clin. Pharm. Therap.* 2000;**68**:556–567.

80. Lindsay, K. L., C. Trepo, T. Heintges, M. L. Shiffman, S. C. Gordon, J. C. Hoefs, E. R. Schiff, Z. D. Goodman, M. Laughlin, R. Yao, and J. K. Albrecht. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001;**34**(2):395–403.

81. Sulkowski, M., E. Lawitz, M. L. Shiffman, A. J. Muir, G. Galler, J. McCone, L. Nyberg, W. M. Lee, R. Ghalib, E. Schiff, J. Galati, B. Bacon, M. Davis, P. Mukhopadhyay, S. Noviello, L. Pedicone, J. Albrecht, and J. McHutchison. Final Results of the IDEAL (Individualized Dosing Efficacy Versus Flat Dosing to Assess Optimal PEGylated Interferon Therapy) phase IIIB study. *J. Hepatol.* 2008;**48**(Suppl 2):S370–S371.

82. Datamonitor. Pipeline Insight: Hepatitis C. Retrieved Feb. 10, 2010, from http://www.datamonitor.com/kc/pharma/Download.asp?R = DMHC2188&type = Report &itemHref = 001DMHC2188.pdf.

83. Hadziyannis, S. J., H. Sette, T. R. Morgan, V. Balan, M. Diago, P. Marcellin, G. Ramadori, H. Bodenheimer, D. Bernstein, M. Rizzetto S. Zeuzem, P. J. Pockros, A. Lin, and A. M. Ackrill. Peginterferon-alfa2a and ribavirin combination therapy in chronic hepatitis C. *Ann. Internal Med.* 2004;**140**(5):346–355.

84. Manns, M. P., J. G. McHutchison, S. C. Gordon, V. K. Rustgi, M. Shiffman, R. Reindollar, Z. D. Goodman, K. Koury, M-H. Ling, and J. K. Albrecht. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. *Lancet* 2001;**358** (9286):958–965.

85. Michael, W. F. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;**36**(S1):S237–S244.

86. O'Brien, C. A double-blind, multi-center randomized, parallel dose-comparison study of six regimens of 5kD linear peginterferon α-2a compared with Roferon-A in patients with chronic hepatitis C. *Antiviral Therapy* 1999;**4**:15.

87. Bailon, P. Pharmacological properties of five polyethylene glycol conjugates of interferon α-2a. *Antiviral Therapy* 1999;**4**:27.

88. Veronese, F. M. and A. Mero. The impact of PEGylation on biological therapies. *BioDrugs* 2008;**22**:315–329.

89. Schering-Plough. PegIntron™ product information. Retrieved Feb. 10, 2010, from http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/103949s5172lbl.pdf.

90. Roche. Pegasys™ product information. Retrieved Feb. 10, 2010, from http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/103964s5147,%20103964s5163lbl.pdf.

91. Bruno, R., P. Sacchi, C. Scagnolari, F. Torriani, L. Maiocchi, S. Patruno, F. Bellomi, G. Filice, and G. Antonelli. Pharmacodynamics of peginterferon alfa-2a and

peginterferon alfa-2b in interferon-naive patients with chronic hepatitis C: A randomized, controlled study. *Alimentary Pharmacol. Therap.* 2007;**26**(3):369–376.

92. Algranati, N. E., S. Sy, and M. Modi. A branched methoxy 40 kDa polyethylene glycol moiety optimizes the pharmacokinetics of PEG–IFN. *Hepatology* 1999;**30**: (190A).
93. Zeuzem, S., S. V. Feinman, J. Rasenack, E. J. Heathcote, M-Y. Lai, E. Gane, J. O'Grady, J. Reichen, M. Diago, A. Lin, J. Hoffman, and M. J. Brunda. Peginterferon alfa-2a in patients with chronic hepatitis C. *N. Engl. J. Med.* 2000;**343**(23):1666–1672.
94. Heathcote, E. J., M. L. Shiffman, W. G. E. Cooksley, G. M. Dusheiko, S. S. Lee, L. Balart, R. Reindollar, R. K. Reddy, T. L. Wright, A. Lin, J. Hoffman, and J. D. Pamphilis. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. *N. Engl. J. Med.* 2000;**343**(23):1673–1680.
95. McHutchison, J. G., S. C. Gordon, E. R. Schiff, M. L. Shiffman, W. M. Lee, V. K. Rustgi, Z. D. Goodman, M-H. Ling, S. Cort, and J. K. Albrecht. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N. Engl. J. Med.* 1998;**339**(21):1485–1492.
96. Poynard, T., P. Marcellin, S. S. Lee, C. Niederau, G. S. Minuk, G. Ideo, V. Bain, J. Heathcote, S. Zeuzem, C. Trepo, and J. Albrecht. Randomised trial of interferon [alpha]2b plus ribavirin for 48 weeks or for 24 weeks versus interferon [alpha]2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998;**352**(9138):1426–1432.
97. Fried, M. W., M. L. Shiffman, K. R. Reddy, C. Smith, G. Marinos, F. L. Goncales, D. Jr., Haussinger, M. Diago, G. Carosi, D. Dhumeaux and others. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* 2002;**347**(13):975–982.
98. Parkinson, C., J. A. Scarlett, and P. J. Trainer. Pegvisomant in the treatment of acromegaly. *Adv. Drug Deliv. Rev.* 2003;**55**(10):1303–1314.
99. Drake, W. M., C. Parkinson, S. A. Akker, J. P. Monson, G. M. Besser, and P. J. Trainer. Successful treatment of resistant acromegaly with a growth hormone receptor antagonist. *Eur. J. Endocrinol.* 2001;**145**(4):451–456.
100. van der Auwera, P., E. Platzer, Z. X. Xu, R. Schulz, O. Feugeas, R. Capdeville, and D. J. Edwards. Pharmacodynamics and pharmacokinetics of single doses of subcutaneous pegylated human G-CSF mutant (Ro 25-8315) in healthy volunteers: Comparison with single and multiple daily doses of Filgrastim. *Am. J. Hematol.* 2001;**66**(4):245–251.
101. Edwards, C. PEGylated recombinant human soluble tumour necrosis factor receptor type I (r-Hu-sTNF-RI): Novel high affinity TNF receptor designed for chronic inflammatory diseases. *Ann. Rheum. Dis.* 1999;**58**:173–181.
102. Moreland, L., D. C. McCabe, J. R., M. Sack, M. Weisman, G. Henry, J. Seely, S. Martin, C. Yee, A. Bendele, J. Frazier, T. Kohno, M. E. Cosenza, S. A. Lyons, J. M. Dayer, A. M. Cohen, and C. K. Edwards 3rd. Phase I/II trial of recombinant methionyl human tumor necrosis factor binding protein PEGylated dimer in patients with active refractory rheumatoid arthritis. *J. Rheum.* 2000;**27** (3):601–609.
103. Choy, E. H. S., B. Hazleman, M. Smith, K. Moss, L. Lisi, D. G. I. Scott, J. Patel, M. Sopwith, and D. A. Isenberg. Efficacy of a novel PEGylated humanized

anti-TNF fragment (CDP870) in patients with rheumatoid arthritis: A phase II double-blinded, randomized, dose-escalating trial. *Rheumatology* 2002;**41** (10):1133–1137.

104. Upjohn, P. P. (2008). Somavert product information. Retrieved Feb. 10, 2010, from http://www.pfzer.com/fles/products/uspi_somavert.pdf.
105. Kinstler, O., N. Gabriel, C. Farrar, and R. DePrince. Amgen Inc., is the assignee. N-terminally chemically modifed protein compositions and methods. U.S. Patent US5824784, 1998.
106. Amgen. Neulasta brand profile Annotation: Product information on Neulasta. Retrieved Feb. 10, 2010, from http://www.amgen.com/medpro/products_neulasta .html.
107. Amgen. Amgen signs multi-year agreement with international oncology network for Aranesp, Neulasta and Neupogen. Retrieved Feb. 10, 2010, from http://www .amgen.com/media/media_pr_detail.jsp?year = 2002;&releaseID = 268988.
108. Crawford, J. Clinical uses of pegylated pharmaceuticals in oncology. *Cancer Treat. Rev*. 2002;**28**(Suppl. 1):7–11.
109. Bence, A. K. and V. R. Adams. Pegfilgrastim: A new therapy to prevent neutropenic fever. *J. Am. Pharm. Assoc*. 2002;**42**(5):806–808.
110. Morgensztern, D., M. Q. Baggstrom, G. Pillot, B. Tan, P. Fracasso, R. Suresh, J. Wildi, and R. Govindan. A phase I study of PEGylated liposomal doxorubicin and irinotecan in patients with solid tumors. *Chemotherapy* 2009;**55**(6): 441–445.
111. Trudeau, M. E., M. J. Clemons, L. Provencher, L. Panasci, L. Yelle, D. Rayson, J. Latreille, T. Vandenberg, R. Goel, L. Zibdawi, Y. Rahim, and J. F. Pouliot. Phase II multicenter trial of anthracycline rechallenge with pegylated liposomal doxorubicin plus cyclophosphamide for first-line therapy of metastatic breast cancer previously treated with adjuvant anthracyclines. *J. Clin. Oncol*. 2009;**27** (35):5906–5910.
112. Kourlas, H. and D. S. Schiller. Pegaptanib sodium for the treatment of neovascular age-related macular degeneration. *Rev. Clin. Ther*. 2006;**28**(1):36–44.
113. Topf, J. CERA: Third-generation erythropoiesis-stimulating agent. *Expert Opin. Pharm*. 2008;**9**(5):839–849.
114. Mircera® Scientifc. Mircera® Scientifc Discussion. Retrieved Feb. 10, 2010, from http://www.emea.europa.eu/humandocs/PDFs/EPAR/mircera/H-739-en1.pdf.
115. Athwal, D., B. Singh, D. Thomas, A. Weir, C. Neil, A. Pepplewell, C. A. George, P. King, and D. John. Antibody molecules having specificity for human tumor necrosis factor alpha, and use thereof. Patent WO094585, 2001.
116. Voulgari, P. V. Emerging drugs for rheumatoid arthritis. *Expert Opin. Emerg. Dr*. 2008;**13**(1):175–196.
117. Di Minno, G., A. M. Cerbone, A. Coppola, E. Cimino, M. Di Capua, F. Pamparana, A. Tufano, and M. N. D. Di Minno. Longer-acting factor VIII to overcome limitations in haemophilia management: the PEGylated liposomes formulation issue. *Haemophilia* 2010;**16**:2–6.
118. Savient. Puricase (PEGloticase) meets pre-specified primary efficacy endpoint in two replicate phase 3 studies. Retrieved Feb. 10, 2010, from http://investor.savient .com/releasedetail.cfm?ReleaseID = 281657.

119. Levay, P. F. and M. Viljoen. Lactoferrin: A general review. *Haematologica* 1995;**80** (3):252–267.

120. Nojima, Y., Y. Suzuki, K. Iguchi, T. Shiga, A. Iwata, T. Fujimoto, K. Yoshida, H. Shimizu, T. Takeuchi, and A. Sato. Development of poly(ethylene glycol) conjugated lactoferrin for oral administration. *Bioconj. Chem.* 2008;**19** (11):2253–2259.

121. Ueki, K., H. Onishi, M. Sasatsu, and Y. Machida. Preparation of carboxy-PEG-PLA nanoparticles loaded with camptothecin and their body distribution in solid tumor-bearing mice. *Drug Develop. Res.* 2009;**70**(7):512–519.

122. Yu, D., P. Peng, S. S. Dharap, Y. Wang, M. Mehlig, P. Chandna, H. Zhao, D. Filpula, K. Yang, V. Borowski, G. Borchard, Z. Zhang, and T. Minko. Antitumor activity of poly(ethylene glycol)-camptothecin conjugate: The inhibition of tumor growth in vivo. *J. Control. Release* 2005;**110**(1):90–102.

123. Penson, R. T., M. V. Seiden, A. Goodman, A. F. Fuller, R. S. Berkowitz, U. A. Matulonis, C. Krasner, H. Lee, T. Atkinson, and S. M. Campos. Phase I trial of escalating doses of topotecan in combination with a fixed dose of pegylated liposomal doxorubicin in women with mullerian malignancies. *Gynecol. Oncol.* 2004;**93**(3):702–707.

124. Scott, L., J. Yao, A. Benson, A. Thomas, S. Falk, R. Mena, J. Picus, J. Wright, M. Mulcahy, J. Ajani, and T. R. Evans. A phase II study of pegylated-camptothecin (pegamotecan) in the treatment of locally advanced and metastatic gastric and gastro-oesophageal junction adenocarcinoma. *Cancer Chemother. Pharm.* 2009;**63**(2):363–370.

125. Sugahara, S., M. Kajiki, H. Kuriyama, and T. R. Kobayashi. Paclitaxel delivery systems: The use of amino acid linkers in the conjugation of paclitaxel with carboxymethyldextran to create prodrugs. *Biol. Pharm. Bull.* 2002;**25**(5):632–641.

126. Wolff, R., S. Routt, J. Riggs-Sauthier, W. Zhang, H. Persson, and R. K. Johnson. NKTR-105, a novel PEGylated-docetaxel, demonstrates superior anti-tumor activity compared to docetaxel in human non-small cell lung and colon cancer xenografts. *Eur. J. Cancer Suppl.* 2008;**6**(12):141.

127. Von Hoff, D. D., G. Jameson, M. J. Borad, L. S. Rosen, J. Utz, S. Dhar, L. Acosta, T. Barker, J. Walling, and J. T. Hamm. First phase I trial of NKTR-102 (PEG-irinotecan) reveals early evidence of broad anti-tumor activity in three schedules. *Eur. J. Cancer Suppl.* 2008;**6**(12):186.

128. Kim, J. J. and K. Park. Modulated insulin delivery from glucose-sensitive hydrogel dosage forms. *J. Control. Release* 2001;**77**(1–2):39–47.

129. Tuesca, A. D., C. Reiff, J. I. Joseph, and A. M. Lowman. Synthesis, Characterization and in vivo efficacy of PEGylated insulin for oral delivery with complexation hydrogels. *Pharm. Res.* 2009;**26**(3):727–739.

PART VI

INORGANIC POLYMERS

CHAPTER 14

CALCIUM-PHOSPHATE-BASED CERAMICS FOR BIOMEDICAL APPLICATIONS

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14.1 INTRODUCTION

Calcium phosphate (CaP) is the principal inorganic component of hard tissues (bone and teeth) in vertebrates [1]. To mimic natural hard tissue structures, researchers have investigated calcium phosphate materials in particular, hydroxyapatite (HA) for various applications. This has lead to the development of a variety of procedures to synthesize various CaP materials with different properties. Based on composition, calcium phosphate ceramics can be categorized into different types, as summarized in Table 14.1. These calcium phosphate compounds have different formula and Ca/P ratios, yielding materials with significantly different properties.

Calcium phosphates have been extensively investigated as implant or coating materials for bone replacement, repair, augmentation, or regeneration. Various forms and combinations of calcium phosphates are widely utilized for a variety of applications. For example, the combination of HA and β-TCP are known as biphasic phosphate (BCP). The commonly used forms of CaP materials include coatings and cements with flexible compositions.

In this chapter, we will introduce the synthesis and characterization of CaP materials and HA in particular. The properties of CaP materials discussed in this chapter include crystallinity, chemical composition, degradability, and mechanical properties. Moreover, the biocompatibility and toxicity of the material in vitro and in vivo are reviewed. Finally, a brief review on some of the clinical results and the commercial CaP products are presented.

14.2 CHEMISTRY

14.2.1 Synthesis of Calcium Phosphate

Hydroxyapatite (HA) is the most widely studied CaP. It is a naturally occurring inorganic calcium phosphate mineral component of bone and teeth in mammals, with the formula $Ca_5(PO_4)_3(OH)$, but it is often written as $Ca_{10}(OH)_2(PO_4)_6$ to denote the two entities present in the crystal unit cell [1]. The calcium phosphate ratio in bone is 1.67. Other nonapatitic calcium phosphates are distinguished from one another by their chemical formulae and Ca/P ratio. Approximately 70% of human bone is made up of HA,

TABLE 14.1 Calcium Phosphate Compound Classification. Modified from [2]

Ca/P Ratio	Compound	Formula	Abbreviation
2	Tetracalcium phosphate	$Ca_4(PO_4)_2O$	TTCP
1.67	Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	HA
1.5	Tricalcium phosphate	$Ca_3(PO_4)_2$	TCP
1.33	Octacalcium phosphate	$Ca_8(HPO_4)_2(PO_4)_4$	OCP
1	Dicalcium phosphate	$CaHPO_4$	DCP
0.5	Monocalcium phosphate	$Ca(H_2PO_4)_2$	MCP

therefore, extensive research efforts have been undertaken to synthesize HA in vitro as a bone substitute and/or replacement for clinical purposes [3, 4].

Several research groups have successfully developed different methods for synthesizing calcium phosphates. These methods include chemical precipitation [5], hydrothermal methods [6], and the sol–gel growth method [7]. Newer methods, such as ultrasonic spray freeze–drying, have also been developed to produce HA [8].

Synthesis of HA from Chemical Precipitation. Of all the techniques mentioned, chemical precipitation method has been most often used to produce calcium phosphate because of the simple processing route and relatively inexpensive cost of operation. Moreover, this method can be easily scaled up to achieve higher production yields. The morphology and size of calcium phosphate particles can be controlled by different experimental conditions e.g. the aging process and growth kinetics of the particles. The raw materials, pH, temperature, and reaction time are the main parameters that can be varied to produce monophase calcium phosphates.

Calcium Hydroxide and Orthophosphoric Acid. One of the typical methods for producing HA is the precipitation of calcium hydroxide and orthophosphoric acid [5]. The chemical equation that describes this reaction is

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

The morphologies of HA particles obtained by this method are very sensitive to the reactant addition rate and reaction temperature. The phosphoric acid is added at a controlled rate, with stirring being maintained throughout the process [5]. Reaction temperatures between 25 and 90°C are common, with the reaction temperature determining whether HA particles will form monocrystalline or polycrystalline structure. A transition temperature ($T = 60°C$) exists where synthesized HA particles are monocrystalline ($T < 60°C$) or polycrystalline ($T > 60°C$) [9, 10].

Calcium Nitrate and Diammonium Hydrogen Phosphate. HA has also been synthesized by a homogeneous precipitation using aqueous solutions of calcium nitrate [$Ca(NO_3)_2$] and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$)][11–13] according to the following reaction:

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12NH_4NO_3 + 8HNO_3$$

A diammonium hydrogen phosphate solution is slowly added to a calcium nitrate solution. To increase the rate of reaction, the pH of the solution (pH 10) is maintained by adding ammonium hydroxide. After the chemical reaction, the HA

precursor solution is aged, decanted, and rinsed with deionized water followed by addition of 95% ethanol to remove nitrates and ammonium hydroxide. The precipitate is then dried at 250°C for 3h and calcined at 850°C. Since calcium is slowly incorporated into the HA structure, continued stirring and aging can help the material approach desired stoichiometric Ca/P of 1.67. Moreover, the size of the HA particles can be controlled by changing the time and the temperature of precipitation. For instance, if the reaction is stirred overnight at room temperature, the particle size of HA will usually be less than 100 nm.

Synthesis of HA from Hydrothermal Reaction. Hydrothermal synthesis is the second most popular synthetic method for producing HA [6]. Hydrothermal synthesis of HA has several advantages: (1) single-step process [14], (2) nanocrystalline structured materials [15], and (3) chemically homogeneous. This synthesis method is characterized by the reaction of aqueous reactants such as calcium nitrate tetrahydrate [$Ca(NO_3)_24H_2O$] and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$] in a closed reactor (e.g., Teflon-lined hydrothermal reactor) under controlled temperature and pressure. The temperature of the reaction can be raised above the boiling point of the water, reaching the pressure of vapor saturation (usually 275°C and 12,000 psi). The reaction proceeds between 24 and 72 h. After which the HA precipitates are filtered and washed by distilled water and the HA is collected by centrifugation. Finally, the HA particles are dried for several hours.

It has been reported that HA particles can be synthesized from natural resources using hydrothermal reaction [16–18]. HA derived from natural materials has an advantage that they inherit some properties of the raw materials such as the pore structure and interconnectivity that mimics the human bone. Coral, marine algae, and eggshell are the natural resources containing a large content of calcium. Eggshell waste is available in large quantity from food industries, thus the utilization of eggshell waste as a starting material for HA synthesis provides an environmentally beneficial and cost-effective strategy [19]. Sopyan and co-workers described a process to synthesis HA from eggshell using the hydrothermal wet chemistry method [19]. It involves thermal decomposition of eggshell to calcium oxide (CaO) followed by hydrothermal reaction with $(NH_4)_2HPO_4$ and water. The HA powders are oven dried overnight. The chemical reactions involved in the system are

$$CaCo_3 \rightarrow CaO + CO_2$$

$$5CaO + 3(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_5(PO_4)_3(OH) + 6NH_4OH$$

It is important to point out that pure HA is not always obtained. Many of the HA synthesis routes lead to significant production of side products. For instance, Tricalcium phosphate (TCP) is one of the common side products as a result of decomposition during sintering [20, 21].

Synthesis of Tricalcium Phosphates. Tricalcium phosphate has been synthesized as scaffold material for supporting tissue regeneration because

of its excellent bioresorbability [2]. There are two forms of TCPs (α and β). Beta-TCP can be synthesized by heating a mixture of dicalcium hydrogenphosphate anhydride and calcium carbonate above 1100°C for 48 h [22]. Synthesis of α-TCP is similar to the method of synthesizing β-TCP except that sintering temperature of 1400°C is used.

Synthesis of CaP from Calcium Phosphate Cement. As a prevalently used form of CaP, calcium phosphate cement (CPC) has been developed for biomedical applications due to its biocompatibility and osteoconductivity [23]. Several different CPC compositions have been developed and approved by FDA for clinical applications [23–25]. The first CPC was synthesized in 1986 and it consisted of tetracalcium phosphate and dicalcium phosphate anhydrous [23]. The CPC powder could be mixed with an aqueous solution, i.e. water to form a paste that could adapt to irregularly shaped bones. The CPC paste could harden in situ to form HA as the end product [23].

14.2.2 Characterization

The chemical composition, purity, structure, and morphology of calcium phosphates, in particular HA, can be characterized by the following techniques:

Chemical Analysis. The Ca/P ratios of the calcium phosphate samples can be determined by conventional chemical analysis. The calcium phosphate samples are first dissolved in a dilute solution of hydrochloride acid. The concentration of phosphorus is determined by a colorimetric method [26], and the concentration of Ca is determined by atomic absorption spectrophotometry, and, subsequently, the Ca/P ratio can be calculated. For instance, pure HA and TCP have ratios of 1.67 and 1.5, respectively [27]. The drawbacks of this method include labor-intensive sample preparation and the inability to determine Ca and P simultaneously.

Chemical Composition and Purity Analysis. Fourier transform infrared spectrometry (FTIR) is one of the most powerful techniques for identifying functional groups in a molecule. FTIR spectra of pure molecules are usually so unique that they are like a "human fingerprint." Thus for most common materials such as HA, the spectrum produced can be easily identified by comparison to a reference standard of known compounds.

Figure 14.1 shows a typical FTIR spectrum of HA. According to the data from Poinern et al., [29] the characteristic frequencies derived from PO_4^{-3} functional groups can be seen at around 1092, 1032, 964, 601, and 564 cm^{-1}. The two weak peaks at 725 and 832 cm^{-1} are associated with carbonate, which reveals the presence of carbonates in the sample. Bands appearing at wavenumber values of around 1384 and 1644 cm^{-1} are indicative of carbonate ion substitution, which might have come from a reactant source (e.g., eggshell) or from the reaction of HA alkaline samples with atmospheric carbon dioxide

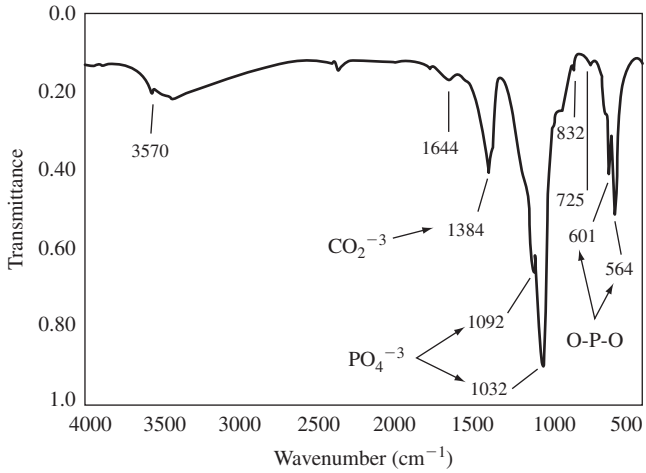

FIGURE 14.1 Typical FTIR spectrum of HA. (Modified from [29] with permission.)

[28, 29]. The weak peak at 3570 cm^{-1} corresponds to the vibrations of the OH^- ions in the HA lattice.

Crystallinity Analysis. X-ray diffraction (XRD) is used to study the atomic and molecular structure of crystalline substances. The HA sample is exposed to X rays at different angles. The diffraction patterns produced can be easily identified by comparing with reference standards. XRD is also used to verify composition and purity of HA samples. Figure 14.2 shows the XRD pattern of HA samples sintered at different temperatures. HA sintered at higher temperature shows sharp and well-defined peaks (upper panel) compared to HA sintered at a lower temperature (lower panel) [30].

Morphological Analysis. Scanning electron microscopy (SEM) is used to investigate the morphology and particle size of synthesized HA. HA can appear in different morphologies such as aggregate, rough, powdery, and its particles showed different shapes as shown in Figure 14.3 [31].

14.2.3 Biodegradability

Degradability is one of the most important criteria for biomaterial design. The driving forces for calcium phosphate biomaterials biodegradation are both solution-driven and mediated by cell processes. Furthermore, the Chemical composition (Ca/P ratio), purity, physical properties, and crystal structures plays an important role in the degradation behavior of calcium phosphates such as implantation sites and animal models, also contribute to the biodegradation [34, 35]. Typically, in vitro degradation of calcium phosphate

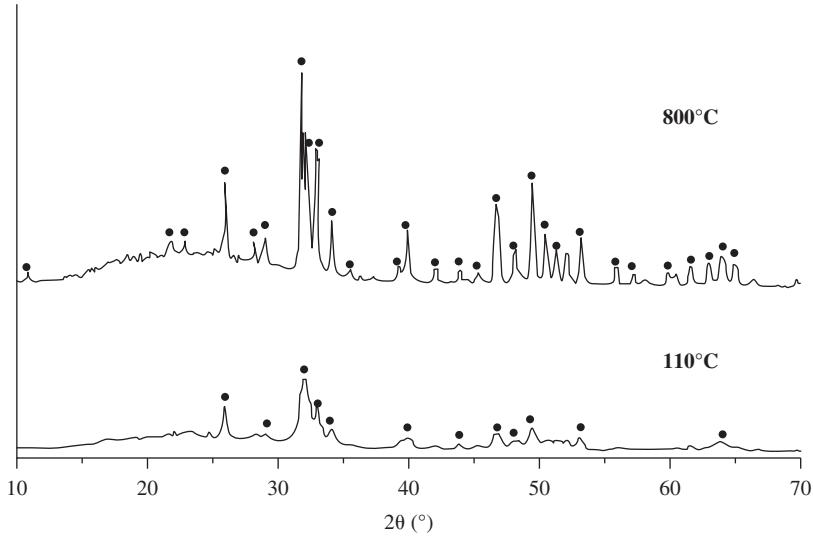

FIGURE 14.2 XRD of HA sample obtained by the precipitation process. Peaks marked with black dots indicating HA. (Modified from [30].)

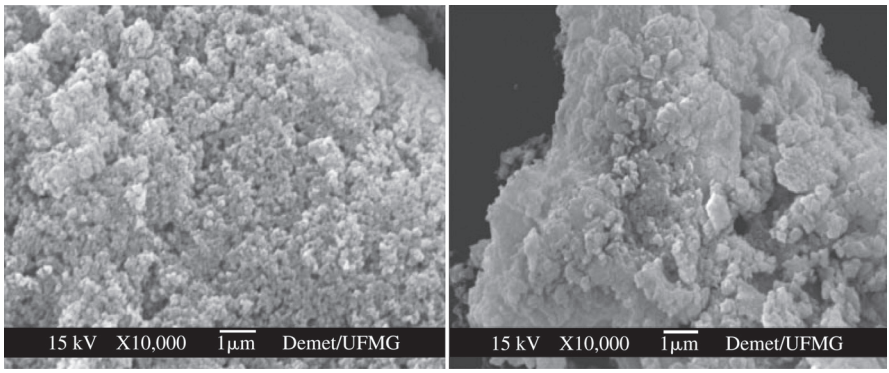

FIGURE 14.3 (a) SEM image of synthesized HA before thermal treatment and (b) SEM image of synthesized HA after thermal treatment. (Adapted from [31] with permission.)

has been evaluated by soaking in physiological saline [36], and in vivo degradation and osteoinductivity have been evaluated by implanting constructs in animal models [36]. In general, crystalline HA has much slower in vivo degradation time compared to α, β-tricalcium phosphate and amorphous HA [37]. While this can impact sustained mechanical integrity, it is also the

TABLE 14.2 Comparison of Mechanical Properties of Human Bone and Calcium Phosphate Data were Obtained from [43–47].

Ceramics	Compressive Strength (MPa)	Elastic Modules (GPa)	Tensile Strength (MPa)	Fracture Toughness (MPa $\sqrt{m}$)
Dense HA	>400	~ 100	~ 40	~ 1.0
Porous HA	0.21–0.41	$0.83-1.6\times10^{-3}$	—	—
Calcium phosphates	30–900	30–103	30–200	<1.0
Human cortical bone	130–180	12–18	50–151	6–8

disadvantageous due to low resorbability and the slow rate of integration with bone.

The study of in vivo degradation of calcium phosphate is important because it can enhance bioactivity of these ceramic biomaterials. The underlying mechanism of the bioactivity of calcium phosphate involves the dissolution of calcium and phosphate ions from the HA [38]. This process mainly occurs through the surrounding environment and may also be mediated by cells [39]. For instance, studies show that dissolution of HA increases calcium and phosphate ion concentrations in the space between the implant and the existing bone [40]. Precipitation of calcium and phosphate ions into this space ensures incorporation of the implant into the existing bone. [41].

14.2.4 Mechanical Properties

The mechanical properties of calcium phosphates vary significantly with their crystallinity, grain size, porosity, and composition [42]. The mechanical properties of dense and porous HA, other types of calcium phosphates (e.g., α-TCP, β-TCP, and TTCP), and bone are summarized in Table 14.2. It is generally believed that the mechanical strength of CaP decreases with increasing amount of amorphous phase, microporosity, and larger grain size [42]. On the other hand, high crystallinity, low porosity, and small grain size tends to yield greater values of stiffness, compressive and tensile strength, and toughness [42]. For instance, dense HA has significant compressive strength and elastic moduli, while porous HA has a small compressive strength and elastic moduli. In comparison, cortical bone has good fracture toughness and moderate compressive and tensile strength, largely due to the presence of dense HA crystals in bone [42].

14.3 TOXICITY AND SAFETY

Calcium phosphate ceramics were introduced commercially in the 1980s. Calcium phosphate was introduced for medical applications in granular form

for alveolar ridge augmentation and periodontal lesion filling. In the following decade, calcium phosphate continued to be applied in restorative dental and orthopedic implants in the forms of blocks and coatings [48]. Various tests have been developed to evaluate the toxicity of calcium phosphate in vitro and in vivo.

14.3.1 Cytotoxicity (in Vitro)

Every new material should be carefully evaluated and characterized in terms of its chemical and biological behavior for any biomedical application. In the literature, in vivo studies are used prevailingly to assess the biological properties of new materials. There are relatively few reports on the in vitro biocompatibility of calcium phosphate materials.

In general, calcium phosphate is considered biocompatible with cells and tissues. However, depending on various compositions, different cellular responses be elicited in vitro. In vitro cytotoxicity can be tested in two ways by culturing cells either with direct contact with the material or with extracts of the material.

Calcium phosphate cement (CPC) is a common form of calcium phosphate material that has been applied in the grafting and replacement of damaged bone. Initially introduced by Gruninger et al., CPC is a paste that can be prepared by mixing a calcium phosphate salt with an aqueous solution and reacting at room or body temperature. The basic and acid components in the cement react when mixed with water, producing products with an intermediary acidity [49]. The advantage of this form of material lies in the biocompatibility and in situ hardening properties, which allows easy manipulation and adaptation to the shape and dimensions of defects [50]. Therefore, cements with different compositions were actively developed by various groups for biomedical applications.

In one study conducted by Rossa et al. two formulations (α and β) of calcium phosphate cements were evaluated in vitro with fibroblasts. Alpha and β formulation of cements are composed mainly of α-TCP and β-TCP. The effect of the tested materials on both cell proliferation and viability was assessed via direct and indirect contact with the cements. The results showed reduced cell proliferation and viability with β formulations whereas α formulations showed similar results as positive controls [51]. The difference might be related to the composition of the liquid component of the CPCs. Beta formulation of CPC contained a liquid composed of phosphoric acid, which might lead to a greater change in pH of the culture medium, resulting in more cellular damage. Another possible reason is the production of H_3PO_4 as hydrolyzation product of the TCP, which can result in pH change as well [51].

Another study by dos Santos et al. showed that α-TCP-based cement was cytotoxic to CHO cells [50]. α-TCP had a high pH value of 9.3 in aqueous solution initially. However, because of the transformation reaction $\alpha\text{-}Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(HPO_4)(PO_4)_5OH$, the pH value slowly returns to

neutral [52]. This gradual drop in pH is related to the hydration process of α-TCP [$10Ca_3(PO_4)_2 + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2H_3PO_4$]. This reaction could cause the accumulation of H_3PO_4 in solution resulting in long term changes in pH values.

An injectable calcium phosphate composite containing biphasic calcium phosphate (BCP) was developed by Grimandi et al. [53] BCP is composed of 60% HA and 40% β-TCP. In vitro biocompatibility was tested by direct-contact cytotoxicity and cell proliferation assays. Fibroblast cells were viable after 24-h culture with direct contact with these materials. In long-term cell proliferation study with extracts of the tested materials, cells kept proliferating until day 4. From day 4 to day 21, BCP showed an inhibitory effect on cells. Such in vitro inhibition of cell proliferation could be attributed to the changes in the ionic homeostasis of the media [53]. It is reported that incubation with HA for only 3 h resulted in a decrease in calcium and phosphate concentration in the media [54]. Since HA is a major component of BCP, the long-term culture with BCP may result in a marked change in the ionic concentration of the media. In addition, other forms of calcium phosphate can be transformed into HA after long-term incubation [55]. Therefore, β-TCP in BCP could be transformed into HA as well, resulting in change of pH and ionic concentrations.

In summary, the in vitro biocompatibility of calcium phosphate materials can be tested by direct contact with the materials or with extracts from the materials. The reported adverse cellular response can be attributed to either change in pH values or in ionic concentrations.

14.3.2 In Vivo Biocompatibility

In vitro studies often cannot fully predict in vivo results; therefore, in vivo studies are important and provide ultimate means to test the biocompatibility of a new material. There are numerous in vivo studies that have evaluated calcium phosphate materials and so far have yielded positive results. Favorable biologic data have been reported in the literature with calcium phosphate implant materials of various compositions and structures. The profile is probably generic to all calcium phosphate materials. The in vivo responses commonly tested include local or systemic toxicity, inflammatory response, fibrous tissue capsule thickness, osteoconductivity and osteointegrativity, and the like Cement and coating are two most widely investigated forms of calcium phosphate materials in medical applications.

Calcium Phosphate Cement. CPC was invented two decades ago and was believed to be a breakthrough in biomaterials for bone reconstruction. Different compositions of CPC are still under active research today and continue to show improvement in performance in vivo, which has been tested widely in various types of animals, including rabbits [56], dogs [57, 58], goats [59], and sheep [60]. Calcium-deficient hydroxyapatite porous scaffolds fabricated from CPC were implanted in the bone cavities of rabbit mandibles for up

to 8 weeks. During the healing, no signs of implant rejection, necrosis, or infection were found. The newly formed bone was indistinguishable from the original bone, and the defect area was completely filled with newly formed bone [56]. A new kind of CPC composed of α-TCP, dibasic calcium phosphate dehydrate, and hydroxyapatite was developed, and its in vivo properties were investigated by implantation into femoral bone and dorsal muscles of dogs for 3 and 6 months. In both bony site and muscle, no foreign body reaction, no inflammation, and no necrosis were found. No connective tissue layer between the cement and bone were detected. A creeping substitution of bone was found as osteoblast-like cells resorbed the cement as if the cement is part of the bone, and new bone was formed directly on the resorption line of the cement. These results suggested that this new CPC is biocompatible, resorbable, osteoconductive, and osteoinductive [57]. A monocalcium phosphate bone cement, namely Norian craniofacial repair system, was tested with either titanium or resorbable polylactic acid mesh in a canine model to repair dual calvarial defects. Eighteen adult male mongrel dogs were used in this experiment and all of them survived their respective time line (3, 6, and 12 months). It was revealed that good integration of the newly formed bone with the host bone was observed after 12 months in both titanium and polylacitc acid mesh groups. No long-term adverse effects were observed with usage of the Norian craniofacial repair system, indicating its excellent biocompatibility [58]. Bodde et al. [59] investigated the osteoinductivity of macroporous CPC, consisting of α-TCP, CaHPO4, CaCO3, and HA in goats. Porous CPC was embedded subcutaneously in four goats, each receiving six implants. The histological sections revealed that fibrous capsules of medium thickness with a few inflammatory cells surrounding the implants after 3 months. The thickness of this capsule decreases significantly after 6 months. A massive inflammatory response in the interstice was seen after 3 months, but disappeared by 6 months [59]. A resorbable calcium phosphate paste composed of resorbable calcium phosphate particles, combined with HA particles were implanted in 3-mm segmental tibial defects in 28 sheep, and results were compared with groups receiving autologous bone grafts. On histological examination, no adverse effects were observed in the calcium phosphate groups. In the groups that received HA particles, bone formation was limited and fibrous encapsulation of the particles was observed. However, no explicit inflammatory response was detected, suggesting the nontoxic, nonallergenic, and noninflammatory properties of HA. More importantly, the new bone was formed in direct contact with HA particles. In the calcium phosphate particle group, bone formation was less evident. In the group with calcium phosphate paste, significantly more amounts of bone were found as compared with other groups, and direct contact between newly formed bone and implant was present in all animals [60]. Therefore, the in vivo responses of calcium phosphate materials depend on both composition and form.

Calcium Phosphate Coating. The brittleness of calcium phosphate materials has been the primary limitation for their application in weight-bearing

implants. In comparison, metallic implants have the advantage of strong mechanical strength. To combine the advantages of metallic strength and calcium phosphate biocompatibility, calcium phosphate coatings appear to attain the fatigue properties of metal [61]. Besides, there were early studies that reported high aseptic loosening rates of orthopedic implants, such as the hip femoral component [62, 63]. It is generally agreed that implant stabilization can be achieved by bone growth into a porous surface or by bone apposition to a macrotextured surface if there is no movement at the implant site and the porous structure has some minimum pore size [64]. Therefore, a layer of biocompatible coating with appropriate pore size could enhance the implant stabilization to a much greater extent than the implant alone.

The most commonly used calcium phosphate coating is HA. There are different ways of depositing HA coatings onto metal implants, such as dip coating, electrophoretic deposition, immersion coating, hot isostatic pressing, solution deposition, sputter coating, and thermal spraying [65]. The interface mechanical characteristics between HA-coated implant and native bone were determined using a transcortical push-out model in dogs after 3, 5, 6, 10, and 32 weeks. It was found that the HA-coated titanium alloy implants exhibited five to seven times the mean interfacial strength of the uncoated, bead-blasted pure titanium implants. Histologic evaluations revealed the mineralization of interfacial bone directly onto the HA-coated implant surface and the absence of fibrous tissue layer interposed between the bone and HA confirmed by light microscropy [66]. HA-coated Ti-6Al-4V implants with different degrees of crystallinity were tested in terms of their osseointegration and gap-healing ability in a goat model with uncoated implants as control. Implants were inserted in the femoral condyles of both femora of eight goats. After 24 weeks, histology showed that significantly more bone contact with implants was observed in coated groups in comparison with the uncoated control group. In the group with 25–30% crystalline HA, coating degraded after 24 weeks, however, bone contact was present without fibrous tissue interposition. HA coating with higher crystallinity degraded to a less extent and did not show significant differences in terms of bone contact or ingrowth in comparsion to the control [67]. Similar conclusions were drawn from other studies with an identical model in a 6-week study [68]. However, other groups have obtained different results. Biphasic calcium phosphate composed of 40% TCP and 60% HA was employed as coating and was compared with pure HA coating. It was found by Delecrin et al. that accelerated bone apposition on BCP coating was observed as compared with HA coating with no difference in anchorage strength after 3 and 6 weeks [69]. It has been suggested that a certain amount of dissolution of the coating may accelerate the dissolution and reprecipitation reaction during new bone formation [70]. Lind et al. compared TCP- and HA-coated cylindrical grit-blasted titanium implants in proximal humerus of 20 skeletally mature dogs. After 6 weeks, the animals were sacrificed and the implants were tested by histomorphometry and mechanical push-out test. The results demonstrated that bone anchorage to HA-coated implants were

10-fold stronger than TCP-coated implants, and bone ongrowth on HA-coated implants was significantly higher as well. This result may be related to the almost complete resorption of TCP after 6 weeks, which was in distinct comparison from the HA-coated implants that showed almost no resorption [71]. Hence, in coating applications, degradability of calcium phosphate ceramics could influence bone ongrowth and fixation of implants.

14.3.3 Mechanism of Biocompatibility of Calcium Phosphate Materials

Numerous studies have shown the lack of local or systemic toxicity and excellent biocompatibility of calcium phosphate materials in different animal models. The underlying mechanism could be due to the chemical nature, composed of only calcium and phosphate ions [48]. Calcium phosphate materials are also attractive because of their lack of inflammatory response stimulation in vivo, of which has been attributed to their close resemblance to bone mineral. The property that separates calcium phosphate from other biomaterials is its ability to bond to bone directly.

It is commonly believed that a "dissolution–reprecipitation" theory for apatite nucleation may be used to explain the mechanism of bone bonding to HA [72]. It is proposed that dissolution of calcium and phosphate ions from the outermost surface of the HA implants leads to a local increase in the ion concentration, which causes apatite precipitation heterogeneously on proteins in the vicinity of the coating or directly on the coating surface itself [38, 73].

The mechanism of the formation and strengthening of bone bonding of crystalline HA has been investigated by Chen et al. [74] using high-resolution transmission electron microscope and energy-dispersive X-ray analysis. Bioactive bone cement composed of strontium-containing HA, and a resin blend was implanted in 21 New Zealand white rabbits in cavities of 3 mm in diameter and 12 mm in depth. The results of this study suggested bone to HA implant bonding following dissolution–precipitation process contained the following steps: (i) crystalline HA transforms into amorphous HA; (ii) the amorphous HA dissolves into the surrounding solution, resulting in oversaturation; and (iii) the nanocrystallites are precipitated from the oversaturated solution in the presence of collagen fibers [74]. The direct bonding between HA and mature bone was established after 6 months as observed in this study.

14.4 CLINICAL APPLICATIONS

In the past 20 years, calcium phosphate material, in particular hydroxyapatite, have been widely investigated as implant and coating material in various clinical applications, such as for filling bone defects [75], bone augmentation in

spinal arthrodesis [76, 77], periodontal treatment [78, 79], and as coatings on metal implants [80].

It is commonly agreed that the cancellous bone defect zone should be filled with a bone substitute when treating tibia compression fractures. Artificial bone substitutes have been used as void fillers in these cases. Huber et al. conducted a prospective functional and radiological evaluation of the application of a resorbable nanocrystalline hydroxyapatite paste (Ostim) in combination with a solid HA ceramic core (Cerabone) for treatment of large metaphyseal defects following reduction of tibia compression fractures in 24 patients (9 women and 15 men). No local or systemic adverse reactions were observed in this study. Moreover, new formation of well-structured bone tissue with lack of inflammation or osteonecrosis was observed from the defect zone refilled with HA. The complication rate was within a reasonable margin (4 in 24). It is concluded that the use of angularly stable osteosynthesis together with the HA paste in combination with the central HA ceramic core for the treatment of tibia head compression fractures presents a reliable surgical procedure [75].

Hydroxyapatite-based ceramics have been investigated in various applications in spinal surgery. In the clinic, HA ceramics have shown efficacy as a bone graft extender in posterior spinal fusion surgery for childhood scoliosis and as a structure bone graft substitute in anterior cervical spine fusions [81]. Calcium phosphate cements hold promise for use as bone substitutes for vertebroplasty because of their injectability, ease of handling, high radiopacity, adapted viscosity, low curing temperature, lasting mechanical properties, biocompatibility, and bioactivity [82].

Calcium-phosphate-based biomaterials have a long history in periodontal treatment, tracing back to 1975 [83]. More recently, injectable CaP materials have been applied in dental applications. Weiss et al. report a clinical evaluation of an injectable bone substitute for the first time. This injectable bone substitute, consisting of BCP particles suspended in an aqueous polymer solution and was used to fill bone defects after tooth extraction in 11 patients. Radiographic density of the surgical sites gradually increased to those of the surrounding teeth in 3 years. A gradual substitution of the filler by bone tissue was observed and the BCP granules were in direct contact with mineralized bone tissue.

The effectiveness of HA coatings in enhancement of the attachment of implants to native bone has been evaluated by investigators in clinical settings and showed significant improvement over implant alone. Long-term results of the first clinical trial of HA-coated total hip arthroplasty were reported by Miyakawa et al. Stable fixation with bone ongrowth was achieved in 46% of the acetabular cups and 89% of the femoral stems, indicating that porous coating would be beneficial [80].

There is a spectrum of commercially available bone substitutes made of CaP materials that are currently in clinical use and are manufactured by Merck, Stryker Howmedica, Zimmer, Bioland biomateriaux/DePuy, Biomet, and so forth. Some of these products are listed in Table 14.3.

TABLE 14.3 Commercial CaP-Based Bone Substitutes Modified from [82]

Name	Component	Manufacturer
Tricalcium Phosphate		
Calciresorb	β-TCP	Ceraver Osteal
Chronos	β-TCP	Synthes
Vitoss	β-TCP	Orthovita
Biosorb	β-TCP	SBM France
Bio Base	α-TCP	Bio Vision
Vitoss	β-TCP	Orthovita
Biologic HA		
Interpore Pro Osteon 500/ 200 HA/CC	Coral Calcium Carbonate → HA	Interpore International
Endobon	Bovine HA	Merck KGaA
Pyrost	Bovine HA	Osteo/Stryker Howmedica
Synthetic HA		
ApaPore	HA	Apatech
Synatite	HA	SBM France
Cerapatite	HA	Ceraver Osteal
PermaOS	HA	Mathys
Pro Osteon	HA, calcium carbonate	Biomet
Biphasic ceramics		
Triosite	60%HA, 40% TCP	Zimmer
BCP	60%HA, 40% TCP	Bioland biomateriaux/ Sofamor Danek
Ceraform	65%HA, 35% TCP	Teknimed
Biocer−Biogel 2 (Phocalcite, Biogreffe)	75%HA, 25% TCP	Bioland biomateriaux/ Depuy
Calciresorb 35-Cerapatite 65	65%HA, 35% β-TCP	Ceraver Osteal
Bi Ostetic	HA, TCP	Berkeley Advanced Biomaterials
Calcium Phosphate Cements		
Bone Source	TTCP, DCP	Orthofix/Howmedica
Norian SRS	α-TCP, $CaCO_3$, MCPM	Norian
A BSM=Biobon=embarc	ACP, DCPD	Etex/Merck/Biomet/ Lorenz Surgical
Mimix	TCP, TTCP, citric acid	Lorenz Surgical
Biocement D	α-TCP, DCP, $CaCO_3$, PHA	Merck/Biomet
Cementek	α-TCP, TTCP, $Ca(OH)_2$, H_3PO_4	Teknimed
Fracture Grout	TTCP, $CaCO_3$, H_3PO_4	Norian
Biopex	α-TCP, TTCP, DCPD	Mitsubishi

14.5 SUMMARY

It has been more than two decade since HA was introduced commercially for medical applications. Tremendous efforts have been put into research focusing on calcium phosphate materials. A broad understanding of the physical, chemical, physiochemical, and biological properties of these materials has been gained. As a biomaterial, CaP has shown excellent biocompatibility evidenced by the lack of local or systemic toxicity, allergy, and inflammatory response in vivo. In addition, clinical success of CaP material has also been demonstrated. In the future, improvements of CaP materials and their continuous success in medical applications are anticipated.

REFERENCES

1. Fellah, B. H. and P. Layrolle. Sol-gel synthesis and characterization of macroporous calcium phosphate bioceramics containing microporosity. *Acta Biomaterialia* 2009;**5**(2):735–742.
2. Kamitakahara, M., C. Ohtsuki, and T. Miyazaki. Review paper: Behavior of ceramic biomaterials derived from tricalcium phosphate in physiological condition. *J. Biomater. Appl.* 2008;**23**(3):197–212.
3. Habraken, W. J., J. G. Wolke, and J. A. Jansen. Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. *Adv. Drug. Deliv. Rev.* 2007;**59** (4–5):234–248.
4. Hutmacher, D. W., J. T. Schantz, C. X. F. Lam, K. C. Tan, and T. C. Lim. State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J. Tissue Eng. Regen. Med.* 2007;**1**(4):245–260.
5. Akao, M., H. Aoki, and K. Kato. Mechanical properties of sintered hydroxyapatite for prosthetic applications. *J. Mater. Sci.* 1981;**28**:809.
6. Zhang, H. and S. Li. Morphology and formation mechanism of hydroxyapatite coating by hydrothermal method on CaO-SiO_2-$B2O_3$-Na_2O glass. *Biomed. Mater. Eng.* 2000;**10**(3–4):205–212.
7. Kamiya, K., T. Yoko, K. Tanaka, and Y. Fujiyama. Growth of fibrous hydroxyapatite in the gel system. *J. Mat. Sci.* 1988;**24**(3):827–832.
8. Itatani, K., K. Iwafune, F. S. Howell, and M. Aizawa. Preparation of various calcium-phosphate powders by ultrasonic spray freeze-drying technique. *Mater. Res. Bull.* 2000;**35**(4):575–585.
9. Bouyer, E., F. Gitzhofer, and M. Boulos. Morphological study of hydroxyapatite nanocrystal suspension. *J. Mater. Sci.* 2000;**11**:523–531.
10. Ferraz, M. P., F. J. Montbro, and C. M. Manuel. Hydroxyapatite nanoparticles: A review of preparation methodologies. *J. Appl. Biomater. Biomech.* 2004;**2**: 74–80.
11. Taş, A. C., F. Korkusuz, M. Timucin, and N. Akkas. An investigation of the chemical synthesis and high-temperature sintering behaviour of calcium hydroxyapatite (HA) and tricalcium phosphate (TCP) bioceramics. *J. Mater. Sci.: Mater. Med.* 1997;**8**:91–96.

12. Jarcho, M., J. F. Kay, K. I. Gumaer, R. H. Doremus, and H. P. Drobeck. Tissue cellular and subcellular events at a bone—ceramic hydroxyapatite interface. *J. Bioeng*. 1976;**1**:79–92.
13. Zhang, H., S. Li, and Y. Yan. Dissolution behavior of hydroxyapatite powder in hydrothermal solution. *Ceram. Int*. 2001;**27**(4):451–454.
14. Chaudhry, A. A., S. Haquea, S. Kellicia, P. Boldrina, I. Rehmanb, F. A. Khalidc, and J. A. Darr. Instant nano-hydroxyapatite: A continuous and rapid hydrothermal synthesis. *Chem. Commun*. 2006:2286–2288.
15. Prakash Parthiban, S., K. Elayaraja, E. K. Girija, Y. Yokogawa, R. Kesavamoorthy, M. Palanichamy, K. Asokan, and S. Narayana Kalkura. Preparation of thermally stable nanocrystalline hydroxyapatite by hydrothermal method. *J. Mater. Sci. Mater. Med*. 2009;(20):S77–S83.
16. Siva Rama Krishna, D., A. Siddharthan, S. K. Seshadri, and T. S. Sampath Kumar. A novel route for synthesis of nanocrystalline hydroxyapatite from eggshell waste. *J. Mater. Sci. Mater. Med*. 2007;**18**(9):1735–1743.
17. Lee, S. J., Y. S. Yoon, M. H. Lee, and N. S. Oh. Nanosized hydroxyapatite powder synthesized from eggshell and phosphoric acid. *J. Nanosci. Nanotechnol*. 2007;**7** (11):4061–4064.
18. Felicio-Fernandes, G. and C. M. Laranjeira. Calcium phosphate biomaterials from marine algae. Hydrothermal synthesis and characterisation. *Quím. Nova* 2000;**23** (4):441–446.
19. Sopyan, L., M. F. Raihana, M. Hamdi, and S. Ramesh. Novel chemical conversion of eggshell to hydroxyapatite powder. *Biomed 2008, Proc*. 2008;**21**:333–336.
20. Cihlar, J., A. Buchal, and M. Trunec. Kinetics of thermal decomposition of hydroxyapatite bioceramics. *J. Mater. Sci*. 1999;**34**:6121–6131.
21. Muralithran, G. and S. Ramesh. The effects of sintering temperature on the properties of hydroxyapatite. *Ceram. Int*. 2000;**26**:221–230.
22. Ermrich, M. and F. Peters. X-ray powder diffraction data of synthetic β-tricalcium phosphate. *Zeitschrift Kristallographie Suppl*. 2006:523–528.
23. Brown, W. E. and L. C. Chow. A new calcium phosphate water setting cement. In *Cements Research Progress*. American Ceramic Society: Westerville, OH, 1986, pp. 352–379.
24. Julien, M., I. Khairouna, R. Z. LeGerosc, S. Delplacea, P. Pileta, P. Weissa, G. Daculsia, J. M. Boulera, and J. Guicheuxa. Physico-chemical-mechanical and in vitro biological properties of calcium phosphate cements with doped amorphous calcium phosphates. *Biomaterials* 2007;**28**(6):956–965.
25. del Real, R. P., E. Ooms, J. G. C. Wolke, M. Vallet-Regí, and J. A. Jansen. In vivo bone response to porous calcium phosphate cement. *J. Biomed. Mater. Res. A* 2003;**65**(1):30–36.
26. Fogg, D. N. and N. T. Wilkinson, *The colorimetric determination of phosphorus*. *Analyst*. 1958;**83**:406–414.
27. Rameshbabu, N. and K. P. Rao. Microwave synthesis, characterization and in-vitro evaluation of nanostructured biphasic calcium phosphates. *Curr. Appl. Phys*. 2009;**9** (1, Suppl. 1):S29.
28. Panda, R. N., M. F. Hsieha, R. J. Chunga, and T. S. Chin. FTIR, XRD, SEM and solid state NMR investigations of carbonate-containing hydroxyapatite

nano-particles synthesized by hydroxide-gel technique. *J. Phy. Chem. Solids* 2003;**64**:193–199.

29. Poinern, G. E., R. K. Brundavanama, N. Mondinosa, and Z.-T. Jianga. Synthesis and characterisation of nanohydroxyapatite using an ultrasound assisted method. *Ultrason. Sonochem.* 2009;**16**(4):469–474.
30. Vazquez, C. G., C. P. Barba, and N. Munguia. Stoichiometric hydroxyapatite obtained by precipitation and sol gel processes. *Rev. Mexi. Fisica* 2005;**51** (3):284–293.
31. Santos, M. H., M. Oliveira, L. P. F. Souza, H. S. Mansur, and W. L. Vasconcelos. Synthesis control and characterization of hydroxyapatite prepared by wet precipitation process. *Mater. Res.* 2004;**7**:625–630.
32. Radin, S. R. and P. Ducheyne. Effect of bioactive ceramic composition and structure of in vitro behavior. III. Porous versus dense ceramics. *J. Biomed. Mater. Res.* 1994;**28**:1303–1309.
33. Li, R., A. E. Clark, and L. L. Hench. An investigation of bioactive glass powders by sol-gel processing. *J. Appl. Biomater. Biomech.* 1991;**2**:231–239.
34. Lu, J. X., A. Gallur, B. Flautre, K. Anselme, M. Descamps, B. Thierry, and P. Hardouin. Comparative study of tissue reactions to calcium phosphate ceramics among cancellous, cortical, and medullar bone sites in rabbits. *J. Biomed. Mater. Res.* 1988;**42**:357–367.
35. Daculsi, G., R. Z. LeGeros, M. Heughebaert, and I. Barbieux. Formation of carbonate-apatite crystals after implantation of calcium phosphate ceramics. *Calcif. Tissue Int.* 1990;**46**:20–27.
36. So, K., S. Fujibayashia, M. Neoa, Y. Ananb, T. Ogawab, T. Kokuboc, and T. Nakamuraa. Accelerated degradation and improved bone-bonding ability of hydroxyapatite ceramics by the addition of glass. *Biomaterials* 2006;**27**(27): 4738–4744.
37. Nagano, M., T. Nakamura, T. Kokubo, M. Tanahashi, and M. Ogawa. Differences of bone bonding ability and degradation behaviour in vivo between amorphous calcium phosphate and highly crystalline hydroxyapatite coating. *Biomaterials* 1996;**17**(18):1771–1777.
38. Weng, J., et al. Formation and characteristics of the apatite layer on plasma-sprayed hydroxyapatite coatings in simulated body fluid. *Biomaterials* 1997;**18** (15):1027–1035.
39. Doi, Y., Q. Liu, J. G. C. Wolke, X. Zhang, and K. de Groot. Sintered carbonate apatites as bioresorbable bone substitutes. *J. Biomed. Mater. Res.* 1998;**39**(4): 603–610.
40. Amrah-Bouali, S., C. Rey, A. Lebugle, and D. Bernache. Surface modifications of hydroxyapatite ceramics in aqueous media. *Biomaterials* 1994;**15**(4):269–272.
41. Porter, A. E., C. M. Botelho, M. A. Lopes, J. D. Santos, S. M. Best, and W. Bonfield. Ultrastructural comparison of dissolution and apatite precipitation on hydroxyapatite and silicon-substituted hydroxyapatite in vitro and in vivo. *J. Biomed. Mater. Res. A* 2004;**69**(4):670–679.
42. Rezwan, K., Q. Z. Chena, J. J. Blakera, and A. R. Boccaccinia. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* 2006;**27**(18):3413–3431.

43. Chen, Q. Z., O. Bretcanu, and A. R. Boccaccini. Inorganic and composite bioactive scaffolds for bone tissue engineering. In *Biomaterials Fabrication and Processing Handbook*. World Scientific Publishing Co. Pte Ltd: Singapore, 2008, pp. 3–44.
44. LeGeros, R. Z. and J. P. LeGeros. *Dense Hydroxyapatite. An Introduction to Bioceramics*, 2nd ed. Word Scientific: London, 1999; pp. 139–180.
45. Kim, H. W., J. C. Knowles, and H. E. Kim. Hydroxyapatite porous scaffold engineered with biological polymer hybrid coating for antibiotic Vancomycin release. *J. Mater. Sci. Mater. Med.* 2005;**16**(3):189–195.
46. Zioupos, P. and J. D. Currey, Changes in the stiffness, strength, and toughness of human cortical bone with age. *Bone* 1998;**22**(1):57–66.
47. Keaveny, T. M. and W. C. Hayes. Mechanical properties of cortical and trabecular bone. In *Bone growth*. CRC Press: Boca Raton, FL, 1993, pp. 285–344.
48. Manley, M. T. Calcium phosphate biomaterials: A review of the literature. In *Hydroxyapatite Coatings in Orthopaedic Surgery*. M. T. M. Rudolph and G. T. Geesink (Ed.). Raven Press: New York, 1993, pp. 1–18.
49. Gruninger, S. Evaluation of the biocompatibility of a new calcium-phosphate setting cement. *J. Dent. Res.* 1984;**63**:h200.
50. dos Santos, L. A., R. G. Carrodéguas, S. O. Rogero, O. Z. Higa, A. O. Boschi, and A. C. de Arruda. AlphaTricalcium phosphate cement: "in vitro" cytotoxicity. *Biomaterials* 2002;**23**(9):2035–2042.
51. Carlos Rossa, Jr., E. Marcantonio, Jr., L. A. Santos, A. O. Boschi, and M. S. G. Raddi, Cytotoxicity of two novel formulations of calcium phosphate cements: A comparative in vitro study. *Artif. Organs* 2005;**29**(2):114–121.
52. Fernandez, E., F. J. Gil, M. P. Ginebra, F. C. M. Driessens, J. A. Planell, and S. M. Best. Production and characterization of new calcium phosphate bone cements in the CaHPOv4-alpha-$Ca_3(PO_4)_2$ system: pH, workability and setting times. *J. Mater. Sci.: Mater. Med.* 1999;**10**(4):223–230.
53. Grimandi, G., P. Weiss, F. Millot, and G. Daculsi. In vitro evaluation of a new injectable calcium phosphate material. *J. Biomed. Mater. Res.* 1998;**39**(4):660–666.
54. Pizzoferrato A., S. Stea, E. Cenni, C. R, Arciola, D. Granchi, and L. Savarino. Cell culture methods for testing biocompatibility. *Clin. Mater.*, 1994;**15**(3):173–190.
55. Takagi, S., L. C. Chow, and K. Ishikawa. Formation of hydroxyapatite in new calcium phosphate cements. *Biomaterials* 1998;**19**(17):1593–1599.
56. Guo, H., J. Sub, J. Weia, H. Kongc, and C. Liua. Biocompatibility and osteogenicity of degradable Ca-deficient hydroxyapatite scaffolds from calcium phosphate cement for bone tissue engineering. *Acta Biomater* 2009;**5**(1):268–278.
57. Yuan, H., Y. Li, J. D. de Bruijn, K. de Groot, and Xingdong Zhang. Tissue responses of calcium phosphate cement: A study in dogs. *Biomaterials* 2000;**21**(12):1283–1290.
58. Genecov, D. G., M. Kremer, R. Agarwal, K. E. Salyer, C. R. Barcelo, H. M. Aberman, and L. A. Opperman. Norian craniofacial repair system: Compatibility with resorbable and nonresorbable plating materials. *Plast. Reconstr. Surg.* 2007;**120**(6):1487–1495.
59. Esther W. H. Bodde, C. T. R. Cammaert, J. G. C. Wolke, P. H. M. Spauwen, and J. A. Jansen. Investigation as to the osteoinductivity of macroporous calcium phosphate cement in goats. *J. Biomed. Mater. Res. Part B: Appl. Biomater* 2007;**83B**(1):161–168.

60. Bloemers, F. W., T. J. Blokhuis, P. Patka, F. C. Bakker, B. W. Wippermann, and H. J. Th. M. Haarman. Autologous bone versus calcium-phosphate ceramics in treatment of experimental bone defects. *J. Biomed. Mater. Res. Part B: Appl. Biomat* 2003;**66B**(2):526–531.

61. Geesink, R., K. de Groot, and C. Klein. Bonding of bone to apatite-coated implants. *J. Bone Joint Surg. Br*. 1988;**70-B**(1):17–22.

62. Thomas, K. A. and S. D. Cook. An evaluation of variables influencing implant fixation by direct bone apposition. *J. Biomed. Mater. Res*. 1985;**19**(8):875–901.

63. Sutherland, C. J., A. H. Wilde, L. S. Borden, and K. E. Marks. A ten-year follow-up of one hundred consecutive Muller curved-stem total hip-replacement arthroplasties. *J. Bone Joint Surg. Am*. 1982;**64**(7):970–982.

64. Bobyn, J. D., R. M. Pilliar, H. U. Cameron, and G. C. Weatherly. The optimum pore size for the fixation of porous-surfaced metal implants by the ingrowth of bone. *Clin. Orthop. Relat. Res*. 1980;**150**:263–270.

65. Sun, L., C. C. Berndt, K. A. Gross, and A. Kucuk. Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings: A review. *J. Biomed. Mater. Res*. 2001;**58**(5):570–592.

66. Cook, S. D, K. A. Thomas, J. F. Kay, and M. Jarcho. Hydroxyapatite-coated titanium for orthopedic implant applications. *Clin. Orthop. Relat. Res*. 1988;**232**: 225–243.

67. Clemens, J. A. M., C. P. A. T. Klein, R. C. Vriesde, P. M. Rozing, and K. de Groot. Healing of large (2 mm) gaps around calcium phosphate-coated bone implants: A study in goats with a follow-up of 6 months. *J. Biomed. Mater. Res*. 1998;**40**(3): 341–349.

68. Clemens, J. A. M., C. P. A. T. Klein, R. J. B. Sakkers, W. J. A. Dhert, K. de Groot, and P. M. Rozing, Healing of gaps around calcium phosphate-coated implants in trabecular bone of the goat. *J. Biomed. Mater. Res*. 1997;**36**(1):55–64.

69. Delecrin, J., G. Daculsi, N. Passuti, and B. Duquet. Specific resorbable calcium phosphate coating to enhance osteoconduction. *Cells Mater*. 1994;**4**:51–62.

70. Daculsi, G., R. Z. LeGeros, E. Nery, K. Lynch, and B. Kerebel, Transformation of biphasic calcium phosphate ceramics I in vivo: Ultrastructural and physicochemical characterization. *J. Biomed. Mater. Res*. 1989;**23**(8):883–894.

71. Lind, M., S. Overgaard, C. Bünger, and K. Søballe. Improved bone anchorage of hydroxyapatite coated implants compared with tricalcium-phosphate coated implants in trabecular bone in dogs. *Biomaterials* 1999;**20**(9):803–808.

72. Porter, A. E. Nanoscale characterization of the interface between bone and hydroxyapatite implants and the effect of silicon on bone apposition. *Micron* 2006;**37**(8):681–688.

73. Radin, S. R. and P. Ducheyne. The effect of calcium phosphate ceramic composition and structure on in vitro behavior. II. Precipitation. *J. Biomed. Mater. Res*. 1993;**27**(1):35–45.

74. Chen, Q. Z., C. T. Wong, W. W. Lu, K. M. C. Cheung, J. C. Y. Leong, and K. D. K. Luk. Strengthening mechanisms of bone bonding to crystalline hydroxyapatite in vivo. *Biomaterials* 2004;**25**(18):4243–4254.

75. Huber, F.-X., N. McArthur, J. Hillmeier, H. J. Kock, M. Baier, M. Diwo, I. Berger, and P. J. Meeder. Void filling of tibia compression fracture zones using a novel

resorbable nanocrystalline hydroxyapatite paste in combination with a hydroxyapatite ceramic core: First clinical results. *Arch. Ortho. Trauma Surg.* 2006;**126** (8):533–540.

76. Cavagna, R., G. Daculsi, and J. M. Bouler. Macroporous calcium phosphate ceramic: A prospective study of 106 cases in lumbar spinal fusion. *J. Long Term Eff. Med. Implants* 1999;**9**(4):403–412.
77. Daculsi G., N. Passuti, S. Martin, C. Deudon, R. Z. Legeros, and S. Raher. Macroporous calcium phosphate ceramic for long bone surgery in humans and dogs. Clinical and histological study. *J. Biomed. Mater. Res.* 1990;**24**(3):379–396.
78. Kasaj, A., A. Kasaj, M. Teich, and S. Jepsen. Clinical evaluation of nanocrystalline hydroxyapatite paste in the treatment of human periodontal bony defects? A randomized controlled clinical trial: 6-month results. *J. Periodontol.* 2008;**79** (3):394–400.
79. Weiss, P., P. Layrolle, L. P. Clergeau, B. Enckel, P. Pilet, Y. Amouriq, G. Daculsi, and B. Giumelli. The safety and efficacy of an injectable bone substitute in dental sockets demonstrated in a human clinical trial. *Biomaterials* 2007;**28**(22): 3295–3305.
80. Miyakawa, S., H. Kawamura, H. Mishima, and J. Yasumoto. Grit-blasted and hydroxyapatite-coated total hip arthroplasty: An 11-to 14-year follow-up study. *J. Orthop. Sci.* 2004;**9**(5):462–467.
81. Spivak, J. and A. Hasharoni. Use of hydroxyapatite in spine surgery. *Eur. Spine J.* 2001;**10**:S197–S204.
82. Heini, and U. Berlemann. Bone substitutes in vertebroplasty. *Eur. Spine J.* 2001;**10**: S205–S213.
83. Nery, E. B., K. L. Lynch, W. M. Hirthe, and K. H. Mueller. Bioceramic implants in surgically produced infrabony defects. *J. Periodontol.* 1975;**46**(6):328–347.

PART VII

BIODEGRADABLE POLYMERS FOR EMERGING CLINICAL USES

CHAPTER 15

BIOCOMPATIBLE POLYMERS FOR NUCLEIC ACID DELIVERY

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CONTENTS

Biodegradable Polymers in Clinical Use and Clinical Development, First Edition. Edited by Abraham J. Domb, Neeraj Kumar, and Aviva Ezra
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15.1 INTRODUCTION

The completion of the human genome project and discovery of inhibitory ribonucleic acid (RNA) has produced new hope in treatment modalities that involve gene manipulation, anticipating benefits to humanity in ways that were unimaginable before. Despite these promising new developments, barriers in nucleic acid delivery remain the major hurdle in the pursuit of nucleic acid therapeutics. The promise of viral vectors for deoxyribonucleic acid (DNA) delivery has been hampered by serious safety concerns in the clinic despite good preclinical data. Nonviral delivery systems such as cationic liposomes were considered a safe alternative to viral vectors, but lack of convincing clinical data due to poor transfection efficiency, acute toxicity, and structural rigidity to chemical functionalization has slowed their progress in the clinic. Delivery systems that are structurally amenable to chemical modifications are preferred since multiple delivery attributes may be incorporated to overcome the delivery challenges. Synthetic polymers fit this profile due to the high degree of structural variation inherent in this class of delivery systems. Several types of polymeric carriers for nucleic acid delivery have been described in the field, including (a) cationic polymers that condense nucleic acid into nanoparticles via electrostatic interaction, (b) nonionic polymers that protect nucleic acid from nuclease action and facilitate nucleic acid entry to the target cell, and (c) biodegradable polymers that serve as nucleic acid depots and release their payload as they erode over time and under specific physiological stress. Most of the early-generation polymeric carriers failed to reach the clinic due to suboptimal activity, which has lead to their chemical functionalization with molecular entities designed to overcome the delivery limitations. For example, poly-*L*-lysine (PLL), despite its extensive use for gene delivery, failed to advance to clinical development due to high polydispersity, poor in vivo stability, high cytotoxicity, and dependence on endosomal disruptive agents for activity. The discovery of polyethyleneimine (PEI) offered an alternative to PLL due to its high endosomal disruptive activity, which is attributed to its high amine density. Despite better transfection activity than PLL, PEI has not advanced to significant clinical development due to toxicity concerns, which has led to the design of new PEI approaches focusing on backbone modifications to improve biocompatibility and transfection activity. The lessons learned from the early-generation carriers have proven useful in designing new and improved systems.

This chapter focuses on delivery polymers that have been described in the last five years. Special emphasis is given to systems with promising in vivo activity. Greater emphasis is given to the DNA delivery systems. However, a brief overview of RNA interference (RNAi) literature is also provided. The structural design, physicochemical properties, and preclinical evaluation of different polymeric carriers is described in Sections 15.2–15.4 and progress in clinical development is described in Section 15.5.

15.2 CATIONIC POLYMERS

A common structural characteristic of all cationic polymers is the presence of protonable amines, which at physiological pH provide electrostatic interaction with negatively charged DNA molecule and form polymer–DNA complexes (polyplexes). This electrostatic interaction forces the DNA structure to collapse into nanoparticles and assume a positive surface charge. The magnitude of size reduction and increase in charge density varies with polymer structure, number and location of protonable amines, pK_a of the amines, DNA–polymer molar ratio, and the presence of functional groups but is largely independent of DNA size, nucleotide sequence, and physical form [1–5]. It is generally believed that the size of polyplexes should be <150 nm for optimal cell uptake through endocytosis [6–8]. However, this may not be the only mechanism for particle uptake since much larger complexes also transfect cells in culture [9, 10]. The density of positive charge on polyplexes depends on the concentration of the polycation. Generally, high polymer:DNA molar ratio is required to achieve good DNA compaction, physical stability, nuclease protection, cell surface interaction, and transfection [10–13]. Nevertheless, DNA cationization is also one of the major reasons for poor in vivo performance. The positively charged polyplexes are subjected to interaction with anionic macromolecules such as sulfated glycosaminoglycans, hyaluronan, or other anions in the biological milieu promoting DNA release from polyplexes and ultimately the degradation by nucleases [14–17]. Considerable attention has been given to circumvent this problem by modifying existing cationic polymers or designing new systems to withstand the in vivo barriers. A detailed description of the conventional predecessor systems and their new and improved derivative polymeric carriers is provided in the following sections. The discussion is circumscribed to delivery polymers that have advanced into clinical development or those that have exhibited promising results in preclinical studies.

15.2.1 Polyethyleneimine

Since the demonstration of its DNA delivery properties by Boussif in 1995 [18], PEI has become the most widely used polymeric delivery system for nucleic acids. A distinct advantage of PEI over early-generation cationic polymers is its ability to escape from the endosomes due to its relatively dense amine-containing structure (Fig. 15.1). The overall protonation level of PEI doubles from pH 7 to pH 5 [19–21], resulting in PEI becoming heavily protonated within the endosome. This so-called proton sponge effect results in the influx of chloride ions to neutralize the compartmental charge, which is then followed by a corresponding destructive influx of water to dilute the high salt concentration. The resulting endosomal lysis occurs without damage to the nucleic acid cargo.

PEIs vary in molecular weight and backbone configuration. Linear polyethylenimine (LPEI) contains only secondary amines, while branched polyethylenimine (BPEI) contains primary, secondary, and tertiary amines in the ratio of

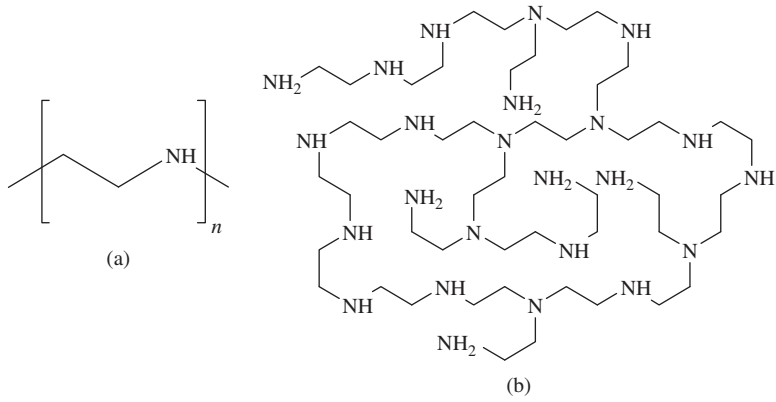

FIGURE 15.1 Structure of (a) LPEI and (b) BPEI.

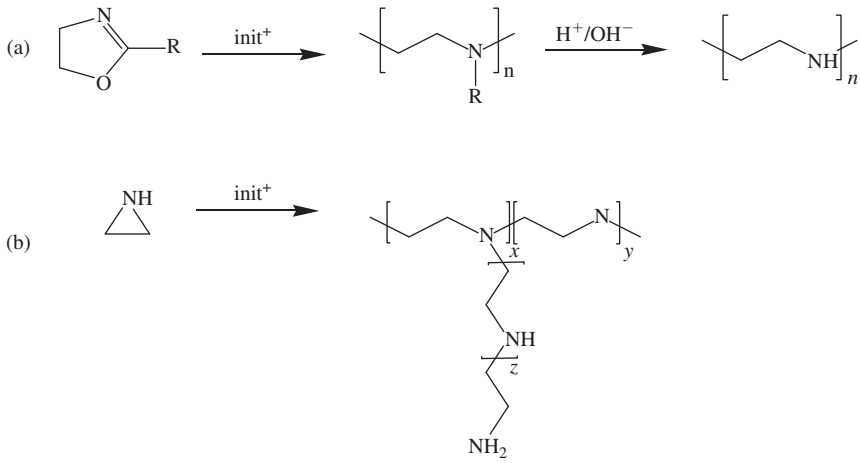

FIGURE 15.2 Synthetic routes for (a) LPEI and (b) BPEI.

1 : 2 : 1, respectively. These structural differences influence the synthesis schemes and transfection properties of PEIs. LPEI may be synthesized by the cationic ring-opening polymerization of oxazolines in the presence of an initiator. The resulting polymer contains side chains that are hydrolyzed by sulfuric acid and then precipitated as the polysulfate salt. BPEI may be synthesized by the cationic polymerization of ethyleneimine (Fig. 15.2), and the pure product obtained by precipitation. Although under certain conditions PEI may be analyzed using high-performance liquid chromatography (HPLC), the most widely used method of molecular weight and polydispersity characterization is

viscometry. An overview of the various different types of PEIs is described in the subsequent sections.

The effect of PEI molecular weight and structural configuration on transfection activity and cytotoxicity has been studied in vitro and in vivo. Both enhancement and reduction in PEI activity has been observed with increasing PEI molecular weight. Goodbey et al. [20] found higher transfection efficiencies with increasing molecular weight of PEI (70 kDa >10 kDa > > > 1.8 kDa) in vitro. Kunath et al. [22] reported high transfection efficiency and reduced toxicity of low-molecular-weight PEI (5 kDa) in several different cell lines. Abdallah et al. [23] found reduced transfection efficiencies with increasing molecular weight of PEI (25 kDa >50 kDa >800 kDa) in vivo. The influence of backbone configuration (linear or branched) on PEI transfection activity and toxicity has also been examined. Initially, BPEI was reported to yield higher transfection activity than LPEI following intravenous delivery to the kidney [24]. However, in later reports, LPEI was found to be more active and less toxic than BPEI [25–27]. The higher transfection efficiency of LPEI is thought to be due to its inherent kinetic instability under salt conditions.

Both LPEI and BPEI have been examined extensively in preclinical studies. Their clinical development has been hampered by poor in vivo stability and acute systemic toxicity. Dissociation of PEI–DNA complexes by negatively charged proteins in blood and extracellular matrix is believed to be the principal mechanism of their low in vivo activity [28]. Acute inflammatory response, characterized by activation of lung endothelium, platelet aggregation, and liver damage, has also been observed following systemic delivery of PEI–DNA complexes [26, 29]. While systemic delivery of nucleic acid with PEIs faces significant delivery and safety challenges, local delivery has generally been found to be more amenable. Gene delivery of diphtheria toxin with LPEI (22 kDa) in human and murine bladder carcinoma was well-tolerated, efficacious, and localized at the injected site [30]. Despite some success in the clinic, the future of PEI as a versatile nucleic acid delivery system remains uncertain unless a substantial improvement is made in its in vivo stability and biocompatibility. Several PEI functionalization approaches have been examined in this regard. These include attachment of tissue-specific or biocompatible components to the PEI backbone, intermolecular crosslinking of low-molecular-weight backbones via degradable linkages, and encapsulation of PEI complexes into biodegradable microspheres. Each of these different approaches to functionalize PEIs is described in the subsequent sections.

15.2.2 Functionalized PEIs

For PEI to be clinically viable it must be made more efficient and tolerable in vivo. Several modifications of PEI have been reported to accomplish this. In one of the approaches, PEI was conjugated with biocompatible polymers such as poly(ethylene glycol) (PEG), polycaprolactone (PCL), chitosan, or cyclodextrins. In another approach, low-molecular-weight PEI was crosslinked to

give a larger total molecular weight polymer to improve in vivo stability. Since the crosslinking was achieved with degradable bonds, the size increase was not permanent and the resulting polymer was potentially more biocompatible than the conventional high-molecular-weight PEIs. PEI was also conjugated with targeting ligands to improve tissue specificity. Each of these approaches is described in the following sections.

Copolymers of PEI. Biocompatible polymers such as PEG, dextran, chitosan, cyclodextrin, PCL or poly(*N*-(2-hydroxypropyl) methylacrylamide have been conjugated to PEI to improve its hydrophilicity, stability, and safety for in vivo application. PEG is a widely used polymer for drug delivery [31]. Strong hydration and high conformation flexibility of PEG provide for steric stabilization of the PEG conjugates in the biological milieu. The principal advantages of PEG conjugation are good serum stability, decreased renal clearance, and overall better pharmacokinetics and bioavailability [32–35]. PEG conjugation to cationic lipids, PLL, poly(dimethyoaminoethyl methacrylate)-(N-vinyl-2-pyrrolidone) (poly(DMAEMA-NVP)), peptides, and other DNA delivery systems have been shown to reduce positive charge density, improve salt stability, and reduce cytotoxicity of the delivery systems [32, 36, 37]. Merdan et al. [38] have characterized the effect of PEG conjugation on the physical stability, in vivo distribution, and transfection activity of BPEI (25 kDa)–DNA complexes. BPEI conjugation with PEG (2000 Da) did not affect particle size but reduced the positive zeta potential (+26 mV) to neutral values. Intravenous administration of PEGylated polyplexes resulted in reduced toxicity, improved organ deposition, and lowered transfectability as compared to the non-PEGylated BPEI. Hong et al. [39] examined the effect of PEG conjugation density relative to BPEI. PEGylation with 10% NHS-activated PEG (2000 Da) produced significantly higher improvement in PEI tolerability and transfection activity than with 6% PEG. Lipiodolized emulsion of BPEI–PEG (10%) successfully delivered plasmid DNA into skeletal muscle following intravenous administration into surgically occluded rat femoral artery. The magnitude of gene expression was three-fold higher than that of the BPEI control. These results demonstrate that gene transfer into skeletal muscle by intravenous route is feasible with emulsified PEG–BPEI administered into locally occluded blood vessels. Neu et al. [40, 41] examined the effect of larger PEGs than those described in the above two studies. PEG (20 kDa) or PEG (30 kDa) was grafted on to BPEI (25 kDa) in di-block (PEG–BPEI) or tri-block (PEG–BPEI–PEG) configuration. Due to the higher chain termini dilution inherent in higher molecular weight polymers, the more reactive linking chemistry involving diisocyanates used here may be more advantageous, rather than the NHS-activated esters used by Hong et al. [39] for lower molecular weight PEGs. All four PEG–BPEIs [PEG (20 kDa)–BPEI, PEG (20 kDa)–BPEI–PEG, PEG (30 kDa)–BPEI, and PEG (30 kDa)–BPEI–PEG] exhibited significantly lower zeta potential, better salt stability, and higher blood concentrations when formulated with DNA. The

best results were obtained with PEG (30 kDa) in both configurations, which suggests that PEG size and incorporation density are important factors. Despite good surface modification, PEGylation did not improve transfection activity as its high occluded volume hinders the electrostatic attraction between positively charge polyplexes and the anionic cell surface. However, Fewell et al. [42] reported a PEGylated BPEI that had significantly higher transfection activity than a control BPEI. A low-molecular-weight BPEI (1800 Da) was functionalized with cholesterol chloroformate and a low-molecular-weight NHS-activated PEG (550 Da) at different PEG : BPEI ratios, and the authors found a PEG–dependent improvement in gene transfer to solid tissues. A 10-fold enhancement over BPEI–cholesterol was achieved at a PEG : BPEI molar ratio of 2 : 1. Increasing the PEG concentration higher than 2 : 1 : 1 (PEG : BPEI : cholesterol) ratio led to a significant decrease in transfection efficiency. PEGylation also lowered the particle zeta potential, which was consistent with the charge neutralization effect exerted by the PEG molecule. The enhancement of BPEI in vivo activity in this study could be due to the use of low-molecular-weight BPEI and low-molecular-weight PEG. Previous studies used much higher molecular weight PEG and did not find significant improvement in transfection activity. Fewell et al. [42] demonstrated that these modifications significantly increased the in vivo activity of BPEI. Together, the cholesterol and PEG modification of BPEI produced approximately 20-fold enhancement in the activity of BPEI (1800 Da), a poorly active transfection agent alone. The PEG–BPEI–cholesterol (PPC) polymer has been successfully used for interleukin-12 (IL-12) gene delivery and anticancer efficacy in several subcutaneously implanted and peritoneally disseminated mouse cancer models. The safety and tolerability of escalating doses of IL-12 plasmid (10, 50, and 250 μg DNA) and PPC complexes at N : P ratio of 11 has been demonstrated by intraperitoneal and subcutaneous administration in normal mice [43]. Biodistribution studies following intraperitoneal or subcutaneous administration showed DNA delivery by PPC was primarily localized at the injection site and only a small amount escaped into the systemic circulation, demonstrating PPC suitability for local gene delivery [44]. In addition, intracranial delivery of IL-12 plasmid–PPC in mice with B16 glioma produced significant IL-12 expression in tumor tissue and prolonged animal survival without causing significant toxicity [45]. The clinical development of PPC-mediated IL-12 gene delivery for the treatment of recurrent ovarian cancer is described in Section 15.5.1. of this chapter.

Another approach to functionalization of low-molecular-weight PEI is to crosslink the polymer chains with a biocompatible polysaccharide. Tang et al. [46] described the use of β-cyclodextrin for this purpose. Cyclodextrins are attractive targets for PEI functionalization due to their low immunogenicity and low toxicity. Activated β-cyclodextrin was reacted with BPEI (600 Da) in the presence of base and then purified into dry powder. The final molar ratio of BPEI : β-cyclodextrin was calculated to be 1 : 1 based on relative integration of characteristic peaks in the proton nuclear magnetic resonance (^{1}H-NMR)

spectrum. The molecular weight of the copolymer was determined to be 61 kDa by gel permeation chromatography (GPC), corresponding to an average degree of polymerization of 35 units for each BPEI–β-cyclodextrin molecule. Although the degradation of the sugar residues was not investigated, albumin was shown to degrade the newly formed carbamate bonds between each PEI–sugar unit significantly within 2 days. The BPEI–β-cyclodextrin was able to condense the DNA into nanoparticles of < 200 nm with a corresponding zeta potential of +40 mV at N : P ratio of 20. In neuronal cells, the efficiency of luciferase gene transfer was 100-to 1000-fold higher than that of BPEI (600 Da) and comparable to that of BPEI (25 kDa). Intrathecal administration of DNA–BPEI–β-cyclodextrin complexes produced two- to four fold higher gene transfer than that of DNA complexes with BPEI (600 Da). The exact mechanism by which cyclodextrins enhance transfection efficiency is not yet understood. Disruption of biological membranes by complexation with phospholipids and cholesterol is believed to be one of the mechanisms by which cyclodextrins mediate their action.

Chitosan is another polysaccharide that has been used for functionalization of PEI. Rather than graft chitosan onto PEI, Wong et al. [47] used the primary amines of chitosan to initiate aziridine polymerization, resulting in a copolymer containing a chitosan backbone and BPEI side chains. Polymerization was efficient, as PEI grafts were observed at 100% of chitosan amines. BPEI-*g*-chitosan fully condensed plasmid DNA into 200- to 400-nm particles of positive zeta potential at high N : P ratios. In comparison, unmodified chitosan (3400 Da) failed to condense plasmid DNA. BPEI-*g*-chitosan had lower toxicity and two- to fivefold higher transfection activity than BPEI (25 kDa) in vitro. Direct infusion of BPEI-*g*-chitosan–luciferase plasmid complexes into the bile duct of rat liver at 0.8 mg/kg plasmid dose produced significant gene transfer in liver, the efficiency of which was 3, 58, and 141 times higher than that of chitosan, BPEI (25 kDa), and naked DNA, respectively. The expression was highly localized in the bile duct and negligible in the lungs, spleen, kidney, and heart. Higher transfection activity was achieved in vivo when particles were prepared at low N : P ratio and displayed large particle diameters. Conversely, higher transfection activity was achieved in vitro when particles were prepared at high N : P ratios and displayed small particle diameters. This discrepancy was believed to be due to difference in DNA concentration used in the in vitro and in vivo formulations. Efficient DNA release from BPEI-*g*-chitosan at low N : P ratios is thought to be one of the reasons for higher transfection efficiency in vivo.

Crosslinked PEIs. One of the approaches to improve PEI transfection activity without augmenting toxicity is to crosslink low-molecular-weight PEI through degradable linkages to create larger, degradable structures. For example, Gosselin et al. [48] synthesized several degradable polymers by crosslinking BPEI (800 Da) via dithiobissuccinimidylpropionate or dimethyl-dithiobispropionimidate linkers. These crosslinked polymers gave significantly higher transfection activity than that of the BPEI monomer but also exerted

significant cytotoxicity. The in vivo properties of these crosslinked polymers were not determined. Thomas et al. [49] synthesized a degradable crosslinked polymer from a mixture of 400 and 2000 Da BPEI, which gave higher in vivo transfection activity than BPEI (25 kDa). Since no in vivo safety data is available, it is difficult to ascertain true potential of this polymer in the clinic. Matar et al. [50] reported a disulfide-crosslinked polymer of LPEI (3600 Da), which had a total average molecular weight of approximately 8 kDa and in vitro and in vivo transfection activity of significantly higher magnitude than that of the LPEI monomer. The crosslinked polymer expressed 20-fold higher transfection activity than that of the monomer LPEI and produced significantly lower toxicity than BPEI (25 kDa) in vitro. Following intravenous administration, plasmid/crosslinked polymer complexes elicited significant gene transfer in lungs, while complexes prepared with LPEI monomer did not yield measurable activity. The transfection activity of the crosslinked LPEI was 2.5 times and 70 times higher than that of LPEI and BPEI (25 kDa), respectively [50]. This crosslinked polymer also exhibited better tolerability than both LPEI and BPEI following in vivo administration, demonstrating that the crosslinking of low-molecular-weight LPEI via degradable linkage is a viable approach to improving PEI transfection activity without augmenting toxicity. This degradable crosslinked polymer of LPEI is distinct from a crosslinked polymer of BPEI [51] with respect to water solubility and transfection activity. The crosslinked polymer of LPEI [50] was highly soluble in aqueous solution and showed transfection activity that was superior to its monomer, whereas the crosslinked polymer of BPEI [51] was poorly soluble in aqueous solution (presumably due to excessive intramolecular crosslinking) and only marginally better than the corresponding monomer in transfection activity. Matar et al. [50] used the *t*-butoxycarbonyl (BOC) anhydride protection step to minimize excessive crosslinking between the monomer amines. The difference between the water solubility and transfection activity of LPEI- and BPEI-based crosslinked polymers shows that the PEI geometry and synthesis scheme are important factors to consider in designing the crosslinking strategy. Neu et al. [52] have described a different approach to PEI crosslinking in which BPEI (25 kDa)/plasmid DNA complexes were stabilized with a crosslinking agent, dithiobis (succinimidylpropionate) (DSP). The size of DNA particles prepared from this crosslinked BPEI increased at higher crosslinking density. This is presumably due to the relatively high local concentration of ester-reactive primary and secondary amines resulting in high intramolecular looping of the linkers. Consequently, this reactivity promoted particle aggregation due to muting of the surface amines and lowering of charge repulsion. Upon intravenous administration the crosslinked polyplexes gave significantly higher tissue plasmid concentrations than control polyplexes of unmodified BPEI. Park et al. [53] synthesized a degradable crosslinked PEI with small molecular weight linear ethyleneimine oligomers (423 Da) using comparatively long-chain bis-acrylate-terminated PEG linkers. Such amine-acrylate reactions are a convenient way to generate labile ester linkages between PEIs in that the

reactive amines are still available for protonation after linking. The resultant poly(ester amine) (PEA) displayed lower cytotoxicity and higher transfection activity in certain cell lines when compared to BPEI (25 kDa). Unlike the previous example in which reactive amines are consumed leading to aggregation [52], the reactive amines in this case retained their cationic nature, which generated repulsion between the particles and hence prevented aggregation in aqueous solution. In addition, due to the internal ester linkages, these polymers degraded rapidly at 37°C in phosphate-buffered saline (PBS) with a half-life of 72 h. DNA complexes prepared with the crosslinked polymer exhibited 100- to 250-nm particle size and 20−30 mV zeta potential, depending upon the N : P ratio. The in vitro gene transfer activity of the crosslinked polymer was significantly higher, and cytotoxicity was lower compared to BPEI (25 kDa). Aerosolization or intravenous administration of luciferase plasmid with the crosslinked polymer yielded high levels of gene expression in the lungs, liver, spleen, heart, and kidney, and expression levels were higher than those from DNA complexes with BPEI (25 kDa).

Ligand-Modified PEI for Tissue Targeting. As described earlier, most PEI polyplexes are rapidly intercepted by serum proteins when administered into systemic circulation. Only a fraction of the injected formulation escapes the surveillance system and enters nonspecifically into the first-pass organs where barriers of the extracellular matrix further attenuate the passage to the target cells. To achieve gene targeting to a specific tissue by systemic route, the carrier must exhibit stability in serum and extracellular matrix, evade immune recognition, and express tissue-specific homing devices to minimize nontarget uptake. A number of strategies have been described to target gene delivery to a specific tissue using tissue-specific ligands. These strategies are described below.

Many of the targeting ligands used for PEI functionalization are based on peptides and polypeptides. Arg-Gly-Glu (RGD)-containing RGD peptides are one of the most prominent ligands for tumor targeting due to the fact that their receptor (fibronectin-binding peptide) is overexpressed on tumor cell surfaces [54]. Sakae et al. [55] conjugated an RGD peptide to PEG with an anionic terminus. The anionic RGD−PEG conjugate was adsorbed onto the positively charged BPEI (25 kDa)−DNA polyplexes via electrostatic interaction. The concept behind this approach was to reduce the cationic surface charge of the polyplexes in order to minimize particle opsonization by serum proteins and then to incorporate RGD peptide to promote specific binding to RGD receptor overexpressed on tumor surface. The addition of RGD−PEG lowered the zeta potential of the DNA complexes from +37.8 to −25.4 mV. The particle size (150 nm) of the ternary complexes was stable over several hours without aggregation. The ternary complexes were also resistant to albumin-induced aggregation while binary complexes of BPEI−DNA were not. High levels of green fluorescent protein (GFP) gene transfer were observed in subcutaneously implanted tumors, liver, and lungs after tail vein injection of the RGD-coated complexes. Much lower levels were obtained with uncoated complexes.

Although this study attempted to address a fundamental problem in the systemic use of cationic polyplexes, it lacks sufficient information on the in vivo stability and target specificity of the delivery system. In addition to its use for surface modification, the RGD ligand has also been directly attached to the PEI polymer [56, 57]. In this approach, RGD was attached to the BPEI (25 kDa) backbone through a heterobifunctional PEG, N-hydroxysuccinimide-PEG-vinyl sulfone (NHS–PEG–VS). The PEG was first conjugated to the peptide amine terminus and the resultant RGD–PEG–VS was then conjugated to BPEI at various molar ratios (1, 5, 10, 20). The RGD–PEG–BPEI/DNA polyplexes displayed a uniform particle distribution with an average particle size of 180 nm. In tumor-bearing mice, tail vein injection of RGD–PEG–BPEI/DNA complexes yielded tumor-specific uptake of the plasmid DNA with tumor specificity linearly related to the degree of RGD incorporation [58]. The RGD-conjugated polyplexes were efficacious as their intravenous delivery carrying IL-12 and Flk-1 plasmid in tumor-bearing mice reduced tumor metastases and improved animal survival.

Human immunodeficiency virus (HIV) derived TAT, a potent membrane penetrating peptide, has been used to improve gene delivery in vivo. Kleeman et al. [59] utilized TAT peptide to enhance the transfection efficiency of BPEI (25 kDa) for local gene delivery to the lungs. The conjugation of BPEI to TAT peptide containing a terminal cysteine residue was achieved via a heterobifunctional NHS–PEG–VS (3400 Da). BPEI was attached through the NHS group and TAT through the VS group. BPEI–PEG–TAT was purified by removal of the unreacted material by ultrafiltration. The degree of PEG substitution was 2.0% for PEG and 0.8% for TAT as a fraction of all amines present in the BPEI backbone. In 5% glucose media, TAT–PEG–BPEI/DNA polyplexes displayed a relatively constant particle size of 100 nm and resisted aggregation in high-salt media (150 mM NaCl) over 24 h. The complexes also protected DNA from degradation upon incubation in bronchial alveolar fluid and DNase solution. Intratracheal instillation of TAT–PEG–BPEI/DNA complexes in mice was well-tolerated and produced significantly higher gene expression than the BPEI control.

Nerve growth factor peptide has been used for gene targeting into neuronal tissue [60]. A low-molecular-weight BPEI (600 Da) was functionalized with nerve growth factor loop 4 hairpin. A ternary complex of plasmid DNA, BPEI, and a 39-amino-acid peptide containing NGF loop 4 motif and DNA-interacting lysine 10mer stretch was prepared. The resulting polyplexes were 180 nm in diameter and displayed a +22-mV zeta potential. Transfection of cortical neurons with targeted complexes yielded 1000-fold higher gene transfer than with nontargeted complexes. In vivo gene transfer following intrathecal administration in rats produced gene transfer in NGF receptor-expressing dorsal root ganglia. The level of gene transfer with the TAT–ligand complexes was 59-fold higher than that with the nontargeted complexes. In a cell viability assay, targeted complexes did not display significant toxicity compared to BPEI (25 kDa).

Rudolph et al. [61] have described the use of triiodo-L-thyronine (T3) for targeting gene delivery to the liver. The T3–PEG–BPEI was synthesized by

reacting T3 with PEG-bis acid in the presence of initiators and then with BPEI (25 kDa). T3−PEG−BPEI/DNA complexes of particle size 11−27 nm were prepared for gene transfer studies in vitro and in vivo. T3-specific gene transfer was achieved in HepG2 cells as verified by reduction in transfection efficiency by excess T3. Intravenous injection of T3−PEG−BPEI complexes in mice resulted in sevenfold higher gene expression than PEG−BPEI control in the liver. High levels of gene expression were also obtained in lungs, spleen, thyroid gland, heart, and kidneys from both ligand and nonligand complexes without significant difference.

Elfinger et al. [62] used lactoferrin (Lf) to target lactoferrin receptors expressed on the surface of bronchoepithelial cells. Periodate-oxidized Lf was mixed with BPEI (25 kDa) at different ratios. Lf-modified BPEI successfully condensed plasmid DNA into <100 nm particles of 20−30 mV zeta potential. The transfection efficiency of Lf−BPEI polyplexes in bronchial epithelial cells was fivefold higher than that of Lf-free BPEI polyplexes. The cytotoxicity of Lf−BPEI polyplexes was lower than that of BPEI−DNA complexes. In addition, Lf conjugation was verified by the reduction of transfection activity of Lf−BPEI polyplexes by an excess of free Lf. In A549 cells, which do not express high levels of Lf receptors, the transfection efficiency of Lf−BPEI was comparable to that of BPEI alone. Increasing the Lf incorporation reduced the transfection efficiency in both alveolar and bronchial epithelial cells.

In another approach, a monoclonal antibody was used to target solid tumors with PEI−DNA complexes. Moffatt et al. [63] developed a unique targeting strategy where the targeting ligand was attached to preformed PEI−DNA complexes to ensure ligand exposure to particle surface. A monoclonal antibody (mAb) against prostate-specific membrane antigen J591 was used to target plasmid DNA to prostate tumors. A linking chemistry utilizing the high affinity interaction between phenyl(di)boronic acid (PDBA) and salicylhydroxamic acid, conjugated the antibody through a PEG linker to BPEI (25 kDa). Initially, a heterobifunctional PEG (H_2N−PEG−COOH) was capped with PDBA to form the PDBA−PEG−COOH conjugate, which was then NHS-activated to form PDBA−PEG−NHS. The DTT-reduced J591 antibody was reacted with PDBA−PEG−NHS and the unreacted PDBA−PEG−NHS was removed by dialysis. Intravenous administration of antibody-targeted complexes into mice with subcutaneously implanted prostate tumors resulted in tumor-specific gene transfer, which was 10- to 30-fold higher than that of nontargeted complexes. High tumor specificity of this targeted system is surprising since the targeted complexes had highly positive zeta potential (28−42 mV) and large particle size (600−800 nm). The same group later used this postcomplexation approach to attach Cys-Asp-Gly-Arg-Cys (CNGRC) peptide to BPEI (25 kDa). The CNGRC peptide binds to tumor cells that express aminopeptidase N or CD13 [64]. Physical characterization of the complexes was not performed, and it was assumed that the CNGRC targeted particles have a similar profile as that of the antibody-targeted complexes [63]. The tumor specificity of the CNGRC-modified complexes was demonstrated in CD31

positive cells in vitro. Intravenous administration of the CNGRC-targeted complexes in tumor-bearing mice produced up to 12-fold higher gene expression in the tumor than in the lungs. The transfection efficiency of targeted complexes was significantly higher than that of nontargeted complexes.

Natural saccharides including lactose, galactose, and mannose have been examined for gene targeting. Lactose and galactose have been used for liver targeting due to their specific affinity for asialoglycoprotein receptors on liver hepatocytes while mannose has been used for targeting antigen presenting cells via surface mannose receptors. Kim et al. [65] conjugated galactose to PEG–BPEI (25 kDa) for liver targeting. Unlike most reports of ligand attachment to distal ends of PEG linkers, Kim et al. [65] attached lactobionic acid (galactose-gluconic acid) (LBA) and mPEG-COOH (2000 Da) independently to BPEI (25 kDa) backbone. This one-pot synthesis does not require the isolation and purification of the activated acid form of LBA. The NHS-activated form of LBA is generated *in situ*, and is followed by the subsequent reaction with a primary amine group of BPEI. The attachment of mPEG-COOH was performed in an identical procedure. The LBA–BPEI–PEG conjugates were purified by extensive dialysis and lyophilized to give the final product. Two forms of the conjugate were synthesized, one with 10% PEG and the other with 50% PEG. The LBA content in both polymers was 10 mol%. PEGylation increased the particle size of LBA–BPEI–PEG to >200 nm and decreased the zeta potential from 19.1 to 5.75 mV. The liver specificity of the targeted systems was confirmed in vitro using asialoorosomucoid (ASOR)-high and -low expressing cells. Intravenous administration of LBA–BPEI–PEG/DNA complexes in mice with liver tumors was well tolerated and produced significant gene uptake and expression in rapidly dividing liver tumor cells but not in nondividing normal liver cells.

In another approach, poly(vinyl pyrrolidone) (PVP) was coupled to BPEI-galactose to improve delivery safety [66]. BPEI (25 kDa) was first conjugated with galactose residues via reductive amination, then with monocarboxylic acid-terminated poly(vinyl pyrrolidone) (PVP) in varying ratios via NHS/EDC coupling. Galactose content was determined to be 4.4 mol% and the PVP content estimated to be 4.0, 6.0, and 7.5 mol% compared to BPEI. The galactosylated-BPEI-*g*-PVP (GPP)/DNA complexes showed good DNA binding ability and high DNA protection from nuclease attack. The particle size of DNA complexes decreased with increased charge ratio with a minimum value of 60 nm at the charge ratio of 40 for the GPP–DNA complex (PVP content: 4.1 mol%). The GPP showed low cytotoxicity and the transfection efficiency of GPP–DNA complexes at charge ratio of 40 in HepG2 cells was higher than that of BPEI–DNA alone.

Sugar ligands have also been examined for gene targeting to lung epithelium [67, 68]. LPEI was covalently conjugated to lactose, glucose, or mannose and evaluated for gene delivery to lung epithelium by nebulization. All three sugar conjugates of LPEI were as efficient as the unsubstituted LPEI but were considerably less cytotoxic. The polyplexes were taken up primarily by alveolar

epithelial cells. Interestingly, despite their high positive zeta potential, the particles were not taken up by alveolar macrophages. Fluorescence microscopy studies showed prenuclear decomplexation of glycosylated complexes, raising concern about the efficiency of nuclear delivery of this sugar-targeted system. Lisziewicz et al. [69] synthesized mannose-LPEI (22 kDa) to target an HIV antigen DNA to mannose receptors on antigen presenting cells in the skin. Topical application of mannosylated LPEI particles (100 nm) containing HIV DNA in naïve rhesus macaques induced virus-specific cluster of differentiation 4 (CD4) helper and cluster of differentiation 8 (CD8) memory T cells. The treatment also produced virological, immunological, and clinical benefits in SIV-infected macaques during chronic infection and acquired immunodeficiency syndrome (AIDS) [70]. When administered in combination with antiviral drugs, mannosylated particles augmented SIV-specific T-cell responses and enhanced control of viral load rebound during treatment interruptions. In the intermittent antiviral therapy, viral load significantly rebounded during the treatment interruption cycles. In contrast, intermittent treatment with combination therapy induced a progressive containment of viral load rebound during treatment interruption cycles in the majority of animals. The HIV vaccine based on mannosylated particles is now in human clinical trials.

Encapsulation of PEI Polyplexes. The PEI approaches described above deal with exposing the tissue to large concentrations of DNA formulations, which could be an important factor contributing to activation of the immune system and rapid clearance of the material. In this section, we have described approaches where PEI-based transfection complexes were delivered from a reservoir under a controlled delivery rate to allow a slow release of the transfection material over a long period of time. A slow release of transfection complexes may be more effective and tolerable for certain gene therapy applications. In some instances, PEI is simply added to encapsulating microspheres to achieve a favorable DNA release kinetic. For example, Nguyen et al. [71] has described a formulation approach to improve encapsulation properties and transfection activity of microsphere systems. Blending poly(orthoesters) (POE) with BPEI (25 kDa) at 0.04 wt% doubled the total release time of plasmid DNA and enhanced gene transfection efficiency of the microspheres up to 50-fold without any significant cytotoxicity. Upon degradation, the microspheres released DNA as complexes with BPEI. Addition of BPEI into the POE microsphere induced 50–60% greater phenotypic maturation and activation of bone-marrow-derived dendritic cells in vitro. Addition of a cationic polymer into POE is a simple approach for modulating the DNA release kinetics and gene transfection efficiency of POE microspheres. Combined with the ability to induce maturation of antigen-presenting cells, POE–BPEI blended microspheres may be excellent carriers for DNA vaccines.

Turk et al. [72] reported a diblock copolymer for temperature-regulated gene transfer properties. This copolymer was composed of poly(*N*-isopropylacrylamide) (NIPA) and PEI with temperature-sensitive and DNA binding

properties, respectively. Carboxyl-terminated NIPA was coupled to LPEI (25 kDa) using standard EDC chemistry, and both the plasmid DNA encapsulation efficiency and release kinetics could be controlled by changing the temperature of the polyplex solutions. Particle sizes ranged from 400 to 1000 nm and remained relatively unchanged upon heating the samples from 25 to 37°C. In addition, zeta potentials of 8–13 mV at polymer : DNA weight ratios were nearly identical to those observed for LPEI (25 kDa) alone. GFP expression was increased from 30 to 50% in vitro as the temperature of the culture environment was lowered to 28 from 37°C. The increase in expression was 10–15% after both subcutaneous and intratibial injection of polyplexes.

Lei et al. [73] used matrix metalloproteinase (MMP)-degradable PEG hydrogels for encapsulation of DNA–BPEI complexes and transfection of mesenchymal stem cells. Mesenchymal cells were seeded inside hydrogels composed of four-arm PEGs terminated with cysteine-reactive vinyl sulfone groups. Crosslinking was accomplished via cysteine-terminated MMP-specific peptide segments in the presence of plasmid DNA–BPEI (25 kDa) polyplexes, which resulted in in situ loading of the hydrogels. Polyplex loading did not significantly affect the viability of mesenchymal cells in transfection. Significant DNA release was observed only after the complexes were treated with trypsin, with over 90% release after 48 h. Transgene expression was reported throughout the 21-day incubation period with maximal values observed on the seventh day.

15.2.3 Cationic Polyesters

The most widely studied polymers in nucleic acid delivery applications that degrade in pharmaceutically relevant time frames are cationic polyesters. The main chain ester bonds may result in in vivo half-lives ranging from minutes to several days. The amine component of the polymers may be primary, secondary, and/or tertiary. The sensitivity to hydrolytic and aminolytic degradation is strongly dependent upon the local environment and may be influenced by the concentrations of amine groups in the polymer, relative hydrophobicity of the polymer and its tendency toward aggregation, and the ratio of ester groups to total polymer molecular weight.

The most basic approach to the synthesis of polymers that contain both amines and esters, so called PEAs, is to form graft-copolymers composed of a polyamine and a polyester. Arote et al. [74] synthesized a low-molecular-weight PEI-based system that contained long stretches of ester-containing segments in the form of polycaprolactone (PCL) for the administration of plasmid DNA to the mouse lung. A range of BPEI–PCL copolymers were synthesized, each containing alternating stretches of BPEI and PCL in varying ratios. PCL was first converted from the diol to the bis-acrylate with acryloyl chloride to give PCL with a molecular weight of 530 Da. Several different molecular weight BPEIs (600, 1200, 1800 Da) were combined with bis-acrylate-PCL to give BPEI-*b*-PCL copolymers. The authors reported gradual variation in the molecular

weights of the copolymers through tuning of the stoichiometric ratio of BPEI to PCL from 1 to 4. Maximum molecular weights were obtained from polymerizations in which the BPEI : PCL ratio was 1 : 1, with molecular weights decreasing at 2 : 1 and 4 : 1. The rate of degradation for the copolymers was also measured at 37°C in PBS and the molecular weights found to decrease by 30% after one day, with extended degradation down to 50% over 10 days. Although the authors attribute the degradation solely to hydrolysis of the PCL ester linkages, it is also likely that the relatively high concentrations of primary and secondary amines from the BPEI segments caused additional chain cleavage via aminolysis of the PCL ester linkages. This additional degradation route was observed in other PEAs containing primary amines [36]. When formulated with DNA at N : P ratios of >10 : 1, those of the higher BPEI molecular weight (1200 and 1800 Da) gave particles sizes <200 nm at N : P ratios up to 30 : 1, while PCL–BPEI (600 Da)/DNA complexes had particle sizes >300 nm. However, the PCL–BPEI (600 Da)/DNA complexes exhibited lower toxicity and higher transfection efficiency than BPEI (25 kDa) in several different cell lines. Aerosolization of PCL–BPEI (600 Da)/GFP plasmid in mice yielded GFP expression. The magnitude of lung transfection with PCL–BPEI(600 Da)/DNA was significantly higher than with BPEI (25 kDa)/DNA complexes.

Similar PEAs may be synthesized with more precise control over the composition by the alternating addition of amine- and ester-containing monomers. Langer et al. reported the development of a technique to synthesize large libraries of PEAs based on the conjugate addition of small-molecule amines to bis-acrylates [75, 76]. Each individual polymer within the library of over 2000 polymers had within its backbone alternating units containing an ester and a tertiary amine. Some members of the group also had secondary or tertiary amines within the side chains, as well as many other structurally unique features including branched structures and aromatic rings. In the high-throughput screening method employed, roughly 2% of the polymers synthesized had equal or higher transfection efficiencies than BPEI (25 kDa) in COS-7 cells. PEAs of this type were shown to be >80% degraded after 24 h at pH 5–7 [76]. Although several polymers in this library possessed high transfection activity, the lack of primary amines in the basic structure, although preventing more rapid degradation, could result in less stable polyplexes with plasmid DNA. Green et al. [77] made further combinatorial changes to the most active members of the previously established library and compared the activity in cell culture, primary cells, and systemic treatment of ovarian cancer. The properties of each polymer in the library were tuned by adjusting the diacrylate : amine ratio in the reaction mixture. A group of the most active polymers in the library (C32, D60) were synthesized to contain acrylate-terminal groups. To further vary the structure in an attempt to enhance the properties of the polymers, a group of small bis-amine-terminated compounds, some containing short ethylene glycol repeat units, were added to the parent polymers to create an additional library of polymers. These advanced polymers were synthesized by dissolving the parent diacrylate-terminated polymer in

dimethyl sulfoxide (DMSO), and adding to them DMSO solutions of the amine end-capping reagents. In general, C32 polymers terminated with primary amine derivatives containing no ethylene glycol spacers were more active than those containing the PEG spacer. These active derivatives were found to bind DNA more tightly than the parent C32 polymer, and also formed smaller complexes with DNA at the same N : P ratios (~150 nm). Intraperitoneal administration of C32-117/pCAGluc DNA complexes in MISIIR/TAg female transgenic mice bearing bilateral ovarian tumors produced luciferase expression; however, C32/pCAGluc complexes were much less active. The efficiency of gene transfer with C32-117 complexes was >100-fold higher than with C32 complexes.

15.2.4 Dendrimers

The majority of polymers and polymeric systems utilized as delivery systems for nucleic acids are linear in nature. Others, such as BPEI, may be synthesized in a relatively uncontrolled manner that results in a branched, or dendritic, architecture. A true dendrimer, however, is built in steps referred to as "generations," which are unique, isolatable, and well-defined polymeric structures. The most widely studied dendrimer is poly(amido amine) (PAMAM), which consists of an alkyl-diamine core and tertiary amine branches containing amide linkages [78]. PAMAM dendrimers are commercially available in generations from 0 to 10 (G0 to G10) with different core types and functional surface groups, which has led to their use as slightly more degradable alternatives to BPEI. Little in vivo data has been reported on the degradation products of PAMAM dendrimers, however, Tang et al. [79] have demonstrated extensive and specific solvolysis of the amide linkages by refluxing in 1-butanol over 43 h. Mamede et al. [80] developed complexes employing PAMAM dendrimers (G4) and avidin-biotin systems (Av-bt) for liver targeting of antisense oligonucleotides. This fourth-generation dendrimer contained 64 active primary amine surface groups and had a molecular weight of 14 kDa. The dendrimers were reacted with NHS-activated biotin and mixed with avidin-bound, In-111-labeled oligonucleotides. PAMAM-bound particles showed high accumulation in the liver (50.95% at 15 min, 47.88% at 60 min) compared to naked particles, which showed low uptake in organs other than the kidneys (21.48% injected dose ID/g at 15 min, 18.48% ID/g at 60 min).

Although PAMAM dendrimers have been in use for over a decade, their toxicity in in vivo applications is a primary drawback [81]. Molecular functionalization has been employed to reduce dendrimer toxicity and improve specificity of delivery. Huang et al. [82, 83] functionalized fifth-generation (G5) PAMAM dendrimers with Lf and Tf to enhance plasmid DNA delivery to the brain. Dendrimers were synthesized with a PEG spacer [NHS–PEG–maleimide (NHS–PEG–MAL) (3400 Da)] between the surface amine groups and the peptide ligands in order to enhance the effectiveness of the ligands and to shield the surface-positive charges of the dendrimer. Lf and Tf, modified with surface thiols, were attached separately to the distal end of

the PAMAM–PEG–MAL, giving PAMAM–PEG–Lf and PAMAM–PEG–Tf, respectively. The extent of the conjugation reaction was examined by visualizing the mobility of Lf- and Tf-containing structures in a polyacrylamide gel electrophoresis (PAGE) experiment. Intravenous administration of PAMAM–PEG–Lf/GFP DNA complexed at a 10 : 1 N : P ratio produced significantly higher gene uptake and expression than PAMAM–PEG–Tf/DNA complexes in the brain. Interestingly, gene transfer in the liver, spleen, kidneys, and lungs was higher with PAMAM–PEG–Tf/DNA complexes than with PAMAM–PEG–Lf/DNA complexes, demonstrating high brain specificity of Lf compared to Tf.

A related dendrimer based on lysine was synthesized by Okuda et al. [84] and its ability to target tumors via intravenous injection examined. These bio-inspired dendritic polymers are theorized to derive their reduced toxicity to their strictly peptidic linking arrangement. Hexamethylenediamine was used as the dendrimer core, and an alternating series of amine protection, O-Benzotriazole-N,N,N′, N′-tetramethyl-uronium-hexafluoro-phosphate (HBTU)–hydroxy-benzotriazole (HOBT) coupling, and amine deprotection steps were used to build succeeding generations with lysine. Lysine dendrimers up to the sixth generation (G6) were synthesized in this manner. The G6 dendrimers were then PEGylated by reaction with mPEG–NHS (5000 Da). The degree of PEGylation was determined by the barium–iodine method and found to be directly proportional to the amount of mPEG–NHS added in the reaction mixture. The unmodified dendrimers were found to have particle diameters of 6 nm. At a degree of PEGylation of 10, the size increased slightly to 8 nm, and even more dramatically at a degree of PEGylation of 76 to 17 nm. The zeta potentials reported for the dendrimers were 20, 0, and −7 mV, respectively. Although no data was reported when these lysine-based dendrimers were complexed with nucleic acids, the ability of a delivery system to enhance uptake at tumor sites after systemic injection without producing significant immune response has potential to be an important improvement upon dendrimer technology. Biodistribution studies in normal and tumor-bearing mice showed rapid clearance of unmodified dendrimers from the bloodstream and nonspecific accumulation in the liver and kidney. In contrast, the PEGylated derivatives showed better retention in blood and low accumulation in organs dependent upon the degree of PEGylation. In addition, the 76-PEG dendrimer was accumulated effectively in tumor tissue, presumably via the enhanced permeability and retention (EPR) effect. Moreover, multiple administrations did not affect the biodistribution characteristics of a second dose of the same dendrimer.

Ribeiro et al. [85] encapsulated a bacterial DNA antigen with a slightly different lysine-based dendrimer and embedded them within PLGA particles using the standard double emulsion method. Two different dendrimers were synthesized, each containing low generation numbers totaling 7 lysine units and 18 total amine groups and differing by the presence or absence of three C_{18} units grafted to the core of the dendrimer. The intact delivery system was formed by mixing the dendrimer with DNA, then with poly(vinyl alcohol)

(PVA), and finally PLGA to form the dendriplexes. Particle sizes and zeta potentials were not influenced by the N : P ratio, and ranged from 400 to 450 nm and −15 to −19 mV for the C_{18}-containing variant and 500−600 nm and −13 to −18 mV for the C_{18}-lacking variant. Immunization of mice with the dendriplexes produced superior antibody response in comparison to animals immunized with the PLGA particles alone.

Repeated reaction/purification steps involved in a variety of dendrimer syntheses can influence the stability of such molecular arrangements containing more degradable ester linkages. In an effort to reduce dendrimer degradation during synthesis, pseudodendrimers have been reported by Russ et al. [86] utilizing amine/acrylate chemistry. The pseudodendrimers were synthesized in two steps: (1) Michael addition of low oligoethyleneimine (OEI; 800 Da) to dioldiacrylates and (2) modification of the surface acrylates with various oligoamines. In the first step, OEI was coupled with a 20-fold molar excess of either 1,2-ethyleneglycoldiacrylate (ED), 1,4-butanedioldiacrylate (BD), or 1,6-hexanedioldiacrylate (HD). In the second step, the acrylate-coated cores were modified at the surface with different oligoamines including ethanolamine (E), spermidine (Sp), spermine (S), and OEI (O). The group of polymers displayed a range of molecular weights from 1200 to 60,000 Da, and when formulated with plasmid DNA, displayed a range of particle sizes from 160 to 900 nm and a range of zeta potentials from −8.8 to 29.0 mV. A primary concern was the stability of the ester linkages to aminolysis during the extended reaction and purification times. The remaining percentage of ester content of the polymers was as low as 71% for the ED−O combination and as high as 98% for the HD−Sp combination (although several of the polymers retained >90% of esters). The stability of the HD−O variant was measured in aqueous media at pH 7.4 and 37°C and found to be comparable to other PEAs. Within the first 24 h, only 75% of the original ester bonds remained, after which the level decreased linearly over the next 7 days to <20%. The DNA-binding ability, cytotoxicity, and transfection efficiency were influenced by the pseudodendritic core characteristics and different surface modifications. In vitro reporter gene expression levels were similar to high-molecular-weight LPEI and BPEIs. Intravenous administration of HD−O/DNA polyplexes in mice yielded gene transfer in tumor tissue at levels comparable to that obtained with LPEI. However, HD−O was better tolerated than LPEI and transgene expression was more tumor specific and much lower in all other investigated organs, particularly in the lungs.

Schatzlein et al. [87] have developed lower generation poly(propylenimine) dendrimers (diaminobutane (DAB) 8, generation 2 and DAB 16, generation 3), and have demonstrated effective DNA delivery in vivo. DAB dendrimers are structurally analogous to low-molecular-weight BPEIs. Methyl quaternary ammonium derivatives of DAB 4 (generation 1, 4 surface primary amines), DAB 8, DAB 16, and DAB 32 were synthesized to give Q4 (generation 1, 4 surface methyl quaternary amines), Q8, Q16, and Q32, respectively. Quaternization of DAB 8 proved to be critical in improving DNA binding, based on data from ethidium bromide exclusion assays and dendrimer−DNA colloidal

stability tests. Particle sizes for the various complexes increased with increasing dendrimer molecular weight up to 300 nm, and all formulations were decidedly positively charged at N : P ratios of 3–5 : 1. The improved colloidal stability had a major effect on vector tolerability, as Q8/DNA formulations were well tolerated upon intravenous injection while a similar DAB 8/DNA dose was lethally toxic by the same route. Quaternization also improved the in vitro cell biocompatibility of DAB 16/DNA and DAB 32/DNA dendrimer complexes by about fourfold but not that of the lower generation DAB 4/DNA and DAB 8/DNA formulations. In contrast to previous reports with nonviral gene delivery systems, the intravenous administration of DAB 16/DNA and Q8/DNA formulations resulted in liver-targeted gene expression as opposed to the lung-targeted gene expression obtained with the control polymer [Exgen 500 (LPEI)].

15.2.5 Synthetic Polypeptides

Polypeptides were one of the first polymer types to be investigated for complexation and delivery of nucleic acids. These biopolymers are biodegradable, primarily through enzymatic processes and possess nearly identical backbones but with a wide range of functional side chains. The most widely used polypeptide for gene delivery is PLL, due to the side-chain terminal primary amine that has a relatively high pK_a in the range of 10–11, which results in a net positive charge at physiological pH. PLL with different molecular weights has been evaluated for gene transfer activity [88–90]. Although PLL binds very well to nucleic acids via electrostatic attraction, transfection levels achieved with PLL alone are relatively modest due to aggregation, lack of endosomal disruptive properties, and toxicity [91]. To improve both in vitro and in vivo gene transfer levels through increased complex stability and reduced toxicity, PLL has been modified by the grafting of PEG to the termini or to the side chains [92, 93]. In other cases, the intermittent addition of other cationic amino acids such as arginine and histidine within the polypeptide chain has led to improved performance. PLL segments have also been incorporated into polypeptides or shorter oligopeptides containing other amino acids to confer nucleic acid binding properties [94–96].

One of the most clinically advanced synthetic polypeptide-based systems was reported by Konstan et al. [97] for the treatment of cystic fibrosis [98]. The PEG–PLL block copolymer was formulated with plasmid DNA encoding the cystic fibrosis transmembrane regulator (CFTR) gene and delivered to the mucosal lining of the lungs. The PEG–PLL block copolymer consisted of a 30-unit PLL block terminated by a single cysteine residue (CK30) and a linear mPEG block containing a terminal maleimide group (PEG10K–MAL) (10 kDa). CK30–PEG was prepared by linking the lysine block to the mPEG block through the thiol-maleimide linkage. 4,4′-Dithiopyridine release assays were performed to confirm 100% substitution of CK30 with mPEG–MAL. CK30–PEG/DNA nanoparticles self-assembled in the form of rods having dimensions of 200 nm × 20 nm, and showed extended stability in saline. The nanoparticles possessed only slight positive charge and gave a zeta potential of

4 mV at an N : P ratio of 2 : 1 [99]. The authors also performed calculations based on the theoretical volumes occupied by both plasmid and the polymer in water and described the polyplexes as containing a single molecule of plasmid DNA. After intranasal or intratracheal administration of CK30–PEG/DNA nanoparticles to mouse airways, luciferase expression was observed to be 200-fold higher than plasmid-only doses after 48 h. In addition, only at elevated doses was the toxicity profile of CK30–PEG/DNA nanoparticles discernable from saline [100]. The safety, tolerability, and gene transfer activity of identical formulations carrying plasmid encoding the cystic fibrosis gene in cystic fibrosis patients is described in Section 15.5.4.

Itaka et al. [101] have developed a system composed of a PEG-poly(aspartic acid) block copolymer that has been modified to contain enhanced lysine-like cationic amines. These modified copolymers were used to deliver plasmid DNA encoding osteogenic differentiation-inducing factors to bone scaffolds. PEG-poly(β-benzyl-L-aspartate) (PEG–PBLA) was synthesized by the ring-opening polymerization of β-benzyl-L-aspartate *N*-carboxyanhydride from the terminal primary amino group of α-methoxy-ω-amino PEG (12 kDa). The *N*-terminal amino group of PEG–PLBA was acetylated to obtain PEG–PLBA-Ac. The polymer was modified by the addition of diethylenetriamine (DET) in order to add a triamine structure to the side chains of the polymer. The aspartic acid side chains were quantitatively converted to the DET derivative and contained a secondary amine and a terminal primary amine designed to both bind nucleic acids and to buffer the endosome. PEG-*b*-poly[Asp-(DET)] formed spontaneous micelles with DNA. The particles had average diameters of 80–90 nm and zeta potentials of 0–3 mV [102]. In vitro gene transfer of mouse calvarial cells with PEG-*b*-poly[Asp-(DET)]/DNA complexes expressing a constitutively active form of activin receptorlike kinase 6 (*caALK6*) and runt-related transcription factor 2 (*Runx2*) produced significant gene transfer with low cytotoxicity. The osteogenic differentiation induced by gene transfer with PEG-*b*-poly[Asp-(DET)]/DNA of calvarial cells was higher compared to BPEI (25 kDa) or FuGENE6. The polyplex nanomicelles were efficiently incorporated into and released from calcium phosphate cement scaffolds and transfected surrounding cells. In a skull bone defect mouse model, local delivery of *caALK6* and *Runx2* genes from nanomicelles incorporated into the calcium phosphate cement scaffold resulted in substantial bone formation covering the entire lower surface of the implant without any sign of inflammation.

15.2.6 Polysaccharides

Chitosan. Chitosan, a linear polysaccharide composed of β (1–4)-linked 2-amino-2-deoxy-*b*-d-glucose and the *N*-acetylated analog isolated from chitin within the exoskeleton of crustaceans, was first described as a delivery system for nucleic acids in 1995 by Mumper et al. [103]. Both the native and deacetylated versions were later examined for in vivo plasmid delivery [104]. However, commercially available chitosan is of high molecular weight

(100–400 kDa) and as a result exhibits low solubility at physiological pH. In addition, chitosan solutions possess high viscosity at concentrations required for effective delivery. In an effort to improve the in vivo properties of chitosan in nucleic acid delivery applications, Koping-Hoggard et al. [105] utilized high-molecular-weight fully deacetylated chitosan and employed nitrous acid to depolymerize the polymer into two distinct fractions containing populations of degree of polymerization 25 and 18. The 18-unit fraction was further fractionated using gel filtration into fractions based on degree of polymerization: 10–14, 15–21, 22–35, and 36–50. These low-molecular-weight fractions of chitosan displayed comparable behavior to low-molecular-weight PEIs in that there was no correlation between in vitro and in vivo activity among the fractions at the optimal N : P ratio of 60 : 1. In this study, the fraction containing 15–21 monomer units displayed the highest transfection levels after intratracheal administration into mouse lungs, which gave particle sizes in the range of 35–120 nm as the concentration ranged from 25 to 500 μg/mL; however, the particles showed very little activity in vitro. The authors argue that the moderate polyplex stability afforded by this fraction compared to those of higher molecular weight are optimal for uptake by the epithelial lining of the airways, but not for cells in culture. In addition, the lower viscosity of the 15–21 fraction resulted in a more favorable aerosol droplet size.

The galactosylated complexes of Jiang et al. were targeted to normal liver and showed good efficiency [106]. The synthesis approach employed was unique in that it used chitosan as the backbone polymer and conjugated LBA and BPEI to it using NHS/EDC and periodate chemistry. First, chitosan and LBA were combined to form a chitosan–LBA conjugate and then BPEI (1800 Da) was grafted onto chitosan in the presence of potassium periodate. The final polymer, LBA–chitosan–BPEI, displayed high molecular weight (25 kDa) and contained 16 mol% BPEI. Complexation of plasmid DNA with LBA–chitosan–BPEI polymer reduced the DNA size to <100 nm and protected DNA from nuclease degradation. The cytotoxicity of this functionalized polymer was considerably lower than that of BPEI (25 kDa). The hepatocyte specificity of the delivery system was verified by comparing transfection activity in asialoglycoprotein receptor-positive and -negative cells. Intraperitoneal administration of ^{99m}Tc-labeled LBA–chitosan–BPEI/DNA complexes in mice gave higher gene uptake and transfection in liver compared to BPEI (25 kDa)/DNA complexes.

Cyclodextrins. Cyclodextrins are water-soluble oligosaccharides that form inclusion complexes with a wide variety of substances including lipid-soluble drugs, dyes, fragrances, and food stuffs [107]. The ability of cyclodextrins to form inclusion complexes with small hydrophobic compounds such as adamantane may be utilized to modify the surface of DNA-containing particles without interfering with the polymer–DNA binding interactions and overall particle shape [108]. These systems have been combined with both BPEI and LPEI by Pun et al. [109] to transfect mouse liver. BPEI and LPEI (25 kDa) were

grafted with 6-monotosyl-β-cyclodextrin in varying ratios. In order to utilize the adamantane-binding properties of β-cyclodextrin, adamantane–PEG (5000 Da) (AD–PEG) was generated by reacting the NHS-activated derivative of mPEG with 1-adamantanemethylamine. To form polyplexes with plasmid DNA, an aqueous PEI-β-cyclodextrin (PEI–CD) solution was mixed with an equal volume of an aqueous PEG–adamantane (PEG–AD) solution. This mixture was then combined with a solution of plasmid DNA at the appropriate ratios to give the desired N : P ratios. At an N : P ratio of 10 : 1, BPEI–CD+PEG–AD gave particles of less than 150 nm in diameter, which did not aggregate in the presence of high salt concentrations. LPEI–CD+PEG–AD also resisted aggregation and resulted in even smaller particles (<100 nm). In each case, the PEG–AD component dramatically reduced aggregation compared to PEI–CD alone. The CD-grafted LPEI and BPEIs were investigated as in vitro and in vivo gene delivery agents. The in vitro toxicity and transfection efficiency were sensitive to the level of CD grafting. PEGylated LPEI-CD-based particles give in vitro gene expression equal to or greater than LPEI as measured by the percentage of EGFP-expressing cells. The authors reported decreased transfection ability with increasing CD grafting, which is attributed to the reduced endosomal release efficiencies caused by the consumption of available amines with grafted CD units and their subsequent effect on the pK_a profiles of neighboring secondary amines. Tail vein injections into mice of 120 μg of plasmid DNA formulated with CD–LPEI and PEG–AD did not reveal observable toxicities, and both nucleic acid accumulation and expression were observed in the liver.

15.3 SYNTHETIC NONCONDENSING POLYMERS

15.3.1 Poloxamers

Nucleic acid delivery to skeletal muscle is a potentially valuable therapeutic application. However, traditional condensing cationic polymer–DNA systems have shown poor activity, primarily due to poor diffusion and uptake at the site of administration [110]. As a result, alternative noncondensing delivery systems have been developed that offer a moderate amount of complexation and protection for DNA in vivo, and which also afford increased diffusivity of the formulation at the site of administration. The most prominent example is a group of amphiphilic copolymers termed poloxamers, which are composed of PEG and its more hydrophobic derivative, poly(propylene glycol) (PPG). A large number of poloxamers are commercially available under the trade name Pluronics, and which vary in the ratio of PEG : PPG blocks in the PEG–PPG–PEG triblock copolymer configuration. Poloxamers assume a weak micelle-type arrangement in aqueous solution and associate through hydrophobic/hydrophilic interactions with a variety of therapeutic molecules including plasmid DNA.

Poloxamer CRL1005, formulated with a small amount of the cationic surfactant benzalkonium (BAK) chloride, was developed to deliver a plasmid DNA vaccine for the treatment of cytomegalovirus (CMV)-associated disease and is

currently being tested in a phase II clinical trial [111]. To create the formulations, poloxamer and plasmid DNA were dissolved in separate PBS solutions and combined at appropriate ratios, after which BAK was added. The poloxamer suspension spontaneously assembled into particles, and the authors suggested the multiple small micelles likely fused to form micron-sized particles with variable diameters and slightly negative surface charges. This behavior is attributed to the high surface concentration of electronegative oxygen atoms within the PEG blocks of the poloxamer chains. Formulations containing the cationic surfactant (BAK) produced more uniform-sized particles with a mean hydrodynamic diameter of ~200 nm, presumably due to anchoring of BAK on poloxamer surface yielding a positive charge preventing particle fusion due to repulsion. Studies in rhesus macaques showed CRL1005–BAK/DNA formulated vaccines enhanced the antigen-specific cellular and humoral immune response, presumably by improving DNA delivery.

Roques et al. [112] demonstrated reduction in PEI–DNA complex toxicity after intrapericardial administration by formulating the PEI complexes in a poloxamer 407-based thermosensitive gel. A ternary solution was formed, first by the combination of plasmid DNA and BPEI (25 kDa), then by the addition of an aqueous suspension of poloxamer 407. Characterization of the size and zeta potential of the complexes suggested interactions between the polyplexes and the poloxamer gel increased the polyplex size and afforded shielding of the BPEI surface charges. However, in vivo evaluation revealed a moderate degree of toxicity toward the myocardium, likely due to the rapid unmasking of the positively charged BPEI–DNA particles as a result of their loose association with the poloxamer. Despite this observation, feasibility of intrapericardial injection of poloxamer-based formulations as well as their decreased toxicity was established.

Poloxamer 188 has also been used to deliver plasmid encoding the extracellular matrix protein Del-1 gene to promote neovascularization in ischemic muscle [113]. Intramuscular injection into mouse tibialis muscle of Del-1 or vascular endothelial growth factor (VEGF) plasmid formulated in 5% (w/v) poloxamer 188 resulted in significant transgene expression in the injected muscle measured 7 days after the treatment. In a mouse model of hind-limb ischemia, both formulations induced formation of new blood vessels and restored hind-limb function. The capillary/myofiber ratio in the treated muscle was approximately 1.7-fold greater than in control-treated muscles. Similar results were obtained in a rabbit model of hind-limb ischemia.

15.3.2 Encapsulating Systems

Poly(lactic acid-*co*-glycolic acid) (PLGA) is a degradable polyester that has found wide utility in small-molecule drug and protein delivery. Traditional PLGA particles have sizes in the micron range and are synthesized using standard emulsion techniques. PLGA delivery systems are often used in slow-release applications, which may be advantageous for nucleic acid delivery for several reasons, including (1) sustained and predictable release, (2) protection

from tissue degradation before release, (3) site-specific delivery by local implants, (4) low injection frequency, and (5) improved patient compliance [114]. Achieving high incorporation efficiencies and control over release kinetics are significant challenges in encapsulating hydrophilic molecules such as DNA within submicron particles fabricated from PLGA.

PLGA-based systems, like poloxamer-based systems, are noncondensing, and due to the ester linkages between monomer units are sensitive to hydrolytic degradation. Chang et al. [115] have developed the PLGA equivalent of poloxamer for the delivery of plasmid DNA to rat skeletal muscle. $PEG_{13}-PLGA_{10}-PEG_{13}$ (lactic : glycolic = 3 : 1) (total molecular weight 3750 Da) was synthesized by combining PEG (550 Da) and stannous 2-ethylhexanoate-copolymerized DL-lactide and DL-glycolide (3 : 1 molar ratio) [116]. Polymer/plasmid DNA working solutions were prepared by mixing various amounts of $PEG_{13}-PLGA_{10}-PEG_{13}$ stock solutions with plasmid DNA saline solution. At a lower weight ratio of 1 : 1 ($PEG_{13}-PLGA_{10}-PEG_{13}$: plasmid DNA), the supercoiled morphology of the DNA was retained and the overall zeta potential of the particles was -85 mV. The loose association indicative of nonionic amphiphilic polymers such as poloxamers and triblock copolymers of this type was apparent when the weight ratio was increased to 25 : 1. Based on atomic force microscopy (AFM) observations, the morphology of the DNA was compacted slightly and the zeta potential increased to -60 mV. However, the authors confirmed that this loose association was not sufficient to retard the mobility of plasmid DNA in gel electrophoresis experiments. Although no degradation studies were reported, reference was made to a previous report of similar triblock copolymers [117]. However, when cell viability was examined over time (9–24 h) in comparison to Pluronic P85, the fraction of live cells remained relatively constant (70 to 66%) while those in contact with the poloxamers decreased markedly (55 to 21%). The higher viability is attributed to the degradability afforded the PLGA-based polymer at the ester linkages, as such degradable linkages are absent in the poloxamer structure and can lead to increased toxicity. Intramuscular injection of VEGF or luciferase plasmid DNA formulated in a 0.25% $PEG_{13}-PLGA_{10}-PEG_{13}$ solution produced gene expression in muscle that was 2–3 times higher than that from naked plasmid DNA. In addition, injection of fluorescence-labeled plasmid DNA showed greater dispersion of $PEG_{13}-PLGA_{10}-PEG_{13}$/DNA compared to BPEI (25 kDa)/DNA.

Blum et al. [118] attempted to improve PLGA for nucleic acid applications by conjugating PLL to PLGA (PLGA–PLL) to create an electrostatic interaction between the carrier material and DNA. Conjugation proceeded through the carboxylic acid end groups to amine-terminated side chains of PLL via dicyclohexylcarbodiimide (DCC) coupling. PLGA–PLL/PLGA particles were prepared using standard double-emulsion techniques and then loaded with plasmid DNA. Particles fabricated with higher weight percentages of PLGA–PLL displayed remarkably increased loading ($>90\%$), as well as a reduction in "burst" release kinetics of DNA. The shift to a more gradual release pattern compared to PLGA-only particles could be the result of PLGA ester aminolysis by side-chain-terminal primary amine

groups of the PLL component. In addition, the authors used homogenization and sonication to improve encapsulation efficiency and release kinetics over PLGA nanoparticles. Particles prepared with homogenization expressed higher encapsulation efficiency and linear release profile. By comparison, sonication produced low encapsulation efficiency and a burst release profile.

In addition to grafting polymers to PLGA, applicability of physical blends of other noncondensing polymers with PLGA have been explored. Csaba et al. [119] reported blends of PLGA and poloxamer derivatives, which exhibited the capacity to associate and release plasmid DNA in a controlled manner for intranasal delivery. The poloxamer derivative used was poloxamine, a 4-arm branched poloxamer containing a bis-tertiary amine core that provides a small amount of cationic nature to the polymer. Nanoparticles were formed using a modified oil-in-water emulsion technique containing poloxamer or poloxamine. Particle size was similar for the poloxamer and poloxamine-containing formulations; however, the slightly cationic poloxamine-containing nanoparticles displayed an increase in DNA encapsulation efficiency. The ability of these nanoparticles to overcome cellular and mucosal barriers was studied in vitro and in vivo. Fluorescent labels showed nanoparticles entered the cells and transported the associated DNA molecule across the cell membrane. The poloxamer-containing nanoparticles elicited a fast and strong immune response, significantly more pronounced than that corresponding to both the poloxamine-containing nanoparticles and naked plasmid DNA for up to 6 weeks. In intranasal applications, the more hydrophobic poloxamer derivative confered increased uptake by the nasal-associated lymphoid tissues and, consequentially, delivery to the antigen presenting cells.

Bhavsar et al. [120] prepared a novel nanoparticle-in-microsphere oral system (NiMOS) for gene delivery to the gastrointestinal (GI) tract. Plasmid DNA, encoding for β-galactosidase or green fluorescent protein, was encapsulated in type B gelatin nanoparticles. NiMOS was prepared by further protecting the DNA-loaded nanoparticles in a PCL matrix to form microspheres < 5 μm in diameter. Lipase (to degrade PCL) and protease (to degrade gelatin) were found to enhance the release of DNA from the particles, with protease inducing full payload release after 5 h. Biodistribution studies following oral administration in rats showed that while gelatin nanoparticles traversed through the GI tract quickly with more than 85% of the administered dose per gram localizing in the large intestine within the first hour, NiMOS resided in the stomach and small intestine for a relatively longer duration. Transgene expression was observed in the small and large intestines of rats. NiMOS shows significant potential as a novel gene delivery vehicle for therapeutic and vaccination purposes.

15.4 siRNA DELIVERY POLYMERS

The dramatic increase in interest in small inhibitory RNA (siRNA)-based therapeutics has resulted in the extension of PEI as a carrier. Initial reports have suggested that paradigms developed for the in vivo delivery of plasmid

DNA do not necessarily apply for siRNA. The much smaller size (19–25 base pairs compared to the average 5000–10,000 base pairs for plasmid DNA) has proven to require different properties in delivery systems. The unique beneficial properties of PEI (polycationic structure, proton sponge ability, readily functionalizable) allow its continued development for delivery of siRNA therapeutics, however, its lack of degradability remains an issue. Other polymers traditionally used for plasmid delivery such as polysaccharides and polypeptides have been modified to fit siRNA applications. In addition, the small size of siRNAs allows the introduction of direct conjugation techniques that are not available with large plasmids.

Grzelinski et al. [121] and Urban-Klei et al. [122] have reported two studies in which a commercially available form of low molecular weight LPEI, jetPEI, was used for intraperitoneal and systemic delivery of siRNA formulations targeting pleiotrophin (PTN) and the c-erbB2/neu (HER-2) receptor, respectively. Physical characterization of the PEI/siRNA complexes was performed with AFM at an N : P ratio of 10 : 1 where the particles showed a mean complex diameter of 42 nm with no free siRNA visible in the field. In addition, RNase cleavage of the siRNA in the presence of fetal calf serum (FCS) was not observed. After intraperitoneal (IP) administration in murine models, subcutaneous xenograft tumor growth was reduced compared to naked siRNA and nonsilencing controls, which the authors attribute to siRNA-mediated down-regulation of the targets after tumor cell uptake. In addition, no interferon-alpha (IFN-α) or tumor necrosis factor-alpha (TNF-α) response was observed as a result of siRNA-induced off-target effects.

In an effort to utilize high-molecular-weight BPEI for siRNA delivery, Schiffelers et al. [123] reported the development of a BPEI–PEG–RGD system for the systemic delivery of siRNA to solid tumors. A heterobifunctional PEG (NHS–PEG–VS) (3400 Da) was used as a macrolinker between BPEI (25 kDa) and RGD. RGD was first attached to PEG via the NHS group, then the RGD–PEG–VS coupled to BPEI through the vinyl sulfone group. The degree of RGD–PEG conjugation to BPEI was found to be 7% of the primary amines, or an average of about 40 RGD–PEG molecules attached to each BPEI molecule. Over a range of N : P ratios, RGD–PEG–BPEI/siRNA complexes displayed particle sizes distributed around 100 nm and zeta potentials of 5 mV. Intravenous injection into tumor-bearing mice in luciferase plasmid DNA-cotransfection experiments resulted in ligand-dependent reduction in luciferase signal. RGD–PEG–BPEI was used to deliver siRNA targeting VEGF receptor 2, and although the authors were not able to report a reduction in VEGF receptor 2 messenger RNA (mRNA) or protein levels, tumor vascular was reduced compared to naked siRNA, which suggests that the observed efficacy was a result of low target inhibition or a nonspecific effect of the siRNA delivery.

Heidel et al. [124–126] employed β-cyclodextrin derivatives to target siRNA-containing nanoparticles via a Tf peptide ligand to tumors after intravenous injection in nonhuman primates. The β-cyclodextrin (CDP) derivative employed in this study contained Tf–PEG–adamantane units that

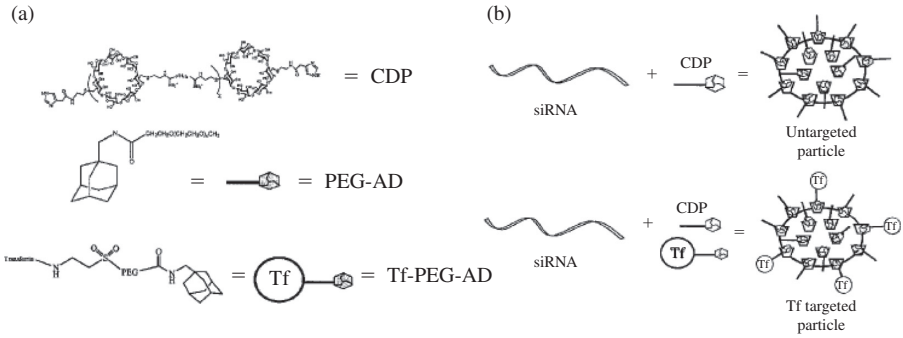

FIGURE 15.3 Configuration of CDP-AD-PEG-Tf delivery system [126].

form inclusion complexes within the β-cyclodextrin structure (CDP–AD–PEG–Tf). The cationic nature of the β-cyclodextrin units allowed the complexation of the nucleic acids (Figure 15.3). The CDP–AD–PEG–Tf/siRNA particle sizes were ~70 nm and were shown to be stable in physiologic fluid and demonstrated ability to protect the nucleic acid from nuclease degradation for up to 72 h. SiRNA targeting the M2 subunit of ribonucleotide reductase was formulated with CDP–AD–PEG–Tf and at a dose as high as 27 mg/kg produced only small diet-related toxicity responses. In addition, repeated doses over 18 days did result in an antibody response to the Tf ligand. In a separate study, CDP–AD–PEG–Tf was able to target Tf-receptor–expressing tumor cells after tail-vein administration in mice [108, 126].

Several groups have developed methods to eliminate the need for cationic polymers to bind anionic nucleic acids for delivery. An elegant approach was taken by Rozema et al. [127] to conjugate the siRNA molecule directly to the polymer via a degradable linkage in order to target apolipoprotein B (*apo*B) to hepatocytes. The structure of the polymeric system contained a latent endosomalytic segment that was designed to be triggered within the endosomes' acidic environment. The backbone polymer was synthesized by the polymerization of 2-vinyloxyethylphthalamide and either methyl vinyl ether, ethyl vinyl ether, propyl vinyl ether, or butyl vinyl ether. Among the vinyl polymers synthesized, the butyl derivative (PBAVE) produced the highest in vitro transfection levels and was therefore used for further modification [128]. The backbone polymer contained primary amine-terminated side chains that were first modified by the addition of 4-succinimidyloxycarbonyl-α-methyl-α-[2-pyridyldithio]toluene (SMPT) to give protected disulfide-terminated side chains. Amine-terminated siRNA was activated by the addition of N-Succinimidyl-S-Acetyl-Thioacetate (SATA) to give a protected thiol group. PBAVE–SMTP and siRNA–SATP were added to a glucose solution containing TAPS buffer, which served to cleave the thioacetyl group from SATP, thereby revealing free thiol-terminated siRNA for disulfide exchange with the SMTP group of the polymer. On average, 70–90% of the siRNA added to the mixture was successfully conjugated to

the polymer. Two additional components of the delivery system were then added that contained the triggered endosomalytic mechanism, carboxydimethyl maleic anhydride (CDM), *N*-acetyl galactosamine (NAG), and PEG (450 Da). The NAG–CDM and PEG–CDM were synthesized from the acid chloride generated by adding oxalyl chloride [129]. The resulting polymer–siRNA conjugate, termed dynamic polyconjugate (DPC), contained an average of one siRNA molecule, and as a result of the side-chain-terminal primary amines being "muted" by the addition of either NAG–CDM or PEG–CDM, the overall charge of the 10-nm particles was negative. The NAG ligand served to target the asialoglycoprotein receptor displayed on the surface of hepatocytes. The CDM linkage was reversible, cleaved at acidic pH, which then induced the proton sponge effect by revealing primary amines and also by dramatically increasing the overall molecule number within the endosome (1 molecular conjugate became 1 polymer, 1 siRNA, several PEG, and several NAG). SiRNA conjugated to DPC targeting *apo*B and delivered via tail-vain injection resulted in sequence-specific knockdown of *apo*B mRNA by 87% and in a reduction of total serum cholesterol levels by 42%. The reduced levels of *apo*B persisted for 10 days and returned to normal levels in 2 weeks. Using fluorescence staining, the activity of the DPCs was demonstrated to be confined to hepatocytes rather than Kupffur cells, thereby validating the effectiveness of liver targeting with the NAG ligand.

Kim et al. [130] have also developed a delivery system that involves the covalent modification of siRNA, with the added step of formulation with PEI. VEGF siRNA was synthesized bearing a 3′-terminal hexylamine group on the sense strand, which was capped with *N*-succinimidyl-3-(2-pyridylthio)propionate (SPDP), a protected disulfide terminated with an activated linker for conjugation to primary amines. The siRNA was coupled via disulfide exchange to mPEG-SH (5000 Da) to give siRNA-S-S-PEGm. The micelles were obtained by the addition of BPEI (25 kDa) to a concentrated solution of siRNA-S-S-PEGm. The siRNA conjugates were shown to be cleaved by 10 mM glutathione, however, the entire complex retained its integrity over 48 h in serum-conditioned medium [131]. The micelles displayed a spherical morphology with a size of <80 nm based on AFM measurements. Interestingly, the hydrodynamic diameters determined by light scattering of the same particles gave values of $\sim$20 nm larger. Intratumoral injection of the siRNA–PEG/BPEI micelles resulted in significant reduction in VEGF mRNA and protein levels within the treated tumors compared to nonsilencing and BPEI-only controls. In addition, IFN-α levels were largely unchanged by the administration, suggesting the siRNA was effectively shielded from immune system surveillance through PEG conjugation.

15.5 CLINICAL DEVELOPMENT OF POLYMERIC DELIVERY SYSTEMS

Clinical development of polymeric gene carriers has been sluggish. Only a small number of polymers have advanced into the clinic. An overview of the clinical

advancement of polymeric gene carriers by disease indications is provided in the following sections. The general synthetic methods, formulations, and gene transfection properties of these polymeric carriers have been described in earlier sections.

15.5.1 Oncology

Cancer represents a group of diseases that result from an uncontrolled proliferation of cells. Current cancer therapies are far from ideal as many of them have low response rates and serious side effects. There is an urgent need for safer and more effective therapies for cancer. Modulation of gene expression by gene therapy or RNAi therapy offers a new class of potential treatments that are distinct from conventional therapeutics. Cancer gene therapy approaches that utilize polymeric carriers are described in this chapter.

LPEI (22 kDa) was evaluated for local gene delivery of diphtheria toxin antigen H19 in patients with refractory superficial transitional cell carcinoma of the bladder [30]. Intravesicular administration of H19 gene plasmid formulated with LPEI into two bladder cancer patients was safe. Interestingly, there was no evidence of treatment-related local or systemic toxicity previously reported with the use of large molecular weight PEIs in animal studies. The antigen DNA was detected in urine samples but not in blood samples, which demonstrated that the plasmid DNA did not distribute into the systemic circulation. Analysis of bladder tissue showed significant uptake of the toxin plasmid by tumor tissue. Video-cystoscopy performed 6 weeks after treatment showed reduction in tumor size by 75% compared to pretreatment size. This study demonstrates that gene transfer with high-molecular-weight PEI is safe if administered locally.

A low-molecular-weight BPEI (1800 Da) functionalized by covalent attachment of cholesterol and PEG (550 Da) (PEG–PEI–cholesterol) (PPC) is in clinical development for local gene delivery of IL-12 into women with recurrent ovarian cancer. IL-12 is one of the most potent anticancer cytokines that works by activation of the natural and acquired immune systems against cancer and inhibition of tumor angiogenesis. A phase I trial to assess the safety and tolerability of human IL-12 plasmid (phIL-12) formulated with PPC polymer was conducted in women with chemotherapy-resistant recurrent ovarian cancer. A total of 13 patients were enrolled in four dose-escalating cohorts and treated with 0.6, 3, 12, or 24 mg/m^2 of the formulated plasmid once a week for 4 weeks. The phIL-12/PPC delivery was generally safe and well tolerated. Common side effects included low-grade fever and abdominal pain. High concentrations of IL-12 plasmid were detected in peritoneal fluid samples while approximately 1000-fold lower concentrations were detected in blood samples. The biological activity of IL-12 was also confined to peritoneal cavity as little activity was found in serum. These data demonstrate that IL-12 gene delivery with a functionalized low-molecular-weight BPEI is safe and offers therapeutic benefits to ovarian cancer patients. The safety and activity of PPC

formulated IL-12 plasmid is now being examined in conjunction with platinum/carboplatin/docetaxel in women with chemotherapy-sensitive ovarian cancer. The preliminary results have shown that the addition of IL-12/PPC to standard chemotherapy for treatment of ovarian cancer is safe. Based on promising phase I results the IL-12/PPC product is ready for advancement into phase II testing in ovarian cancer patients [42, 132, 133].

15.5.2 HIV–AIDS

A functionalized cationic polymer based on a mannose-conjugated PEI is being developed for the delivery of a DNA vaccine against HIV after a successful preclinical testing in macaques [70, 134]. The antigen DNA is formulated to mannose–LPEI nanoparticles to target antigen-presenting cells and to protect the DNA from intracellular degradation. In SIV-infected macaques, topically administered PEI-mannose-formulated DNA antigen successfully transfected dendritic cells and induced an immune response against the virus. This vaccine product is currently under clinical evaluation for safety and immunogenicity as a single agent or in combination with antiviral therapy in phase I and phase II human studies. To our knowledge, the clinical results from these trials have not been published.

15.5.3 Cardiovascular Disease

Peripheral vascular disease is a prevalent and disabling disease affecting over 10 million people in the United States. The disease is characterized by intermittent claudication resulting from arterial atherosclerosis of peripheral blood vessels, impairing blood flow to the lower limbs. Growth factors, such as vascular endothelial growth factor (VEGF-A), have been shown to promote angiogenesis and improve blood flow to the lower limbs in animal models. Gene therapy is an attractive approach to peripheral vascular disease since it can provide expression of a vascular growth factor for a long period of time in the affected limb. The use of naked DNA for this indication has limitations due to low transfection efficiency. Noncondensing polymers such as poloxamers have been shown to produce good efficacy results in preclinical models of peripheral vascular disease. In a phase II clinical trial a plasmid encoding an angiomatrix protein Del-1 was formulated with poloxamer 188 and administered by intramuscular injection into patients with moderate to severe peripheral arterial disease [135]. One-hundred five patients randomized to treatment and control groups received Del-1 plasmid formulation with poloxamer 188 or poloxamer 188 alone as 21 intramuscular injections to each lower extremity. The treatment was safe and produced a significant increase in the mean peak walking time, claudication onset time, and ankle brachial index compared to baseline values in both treatment and control groups. Both groups also demonstrated significantly improved quality of life at follow-up compared to baseline. None of the serious adverse events were determined to be treatment

related. Interestingly, both efficacy and adverse events were not significantly different between the treatment and control group, which suggests that the delivery polymer by itself has biological activity useful for treatment of peripheral arterial disease. In another human study, poloxamer 407 was used to deliver plasmid DNA encoding a zinc finger protein transcription factor engineered to enhance the VEGF expression in skeletal muscle of patients with intermittent claudication [136]. The results from this randomized, double-blind, dose-escalation, placebo-controlled study, designed to determine the safety, preliminary efficacy, and biological activity of the poloxamer-formulated plasmid, have not been published.

Wloch et al. [137] used a poloxamer-based formulation to deliver a viral vaccine in human subjects. VCL-CB01, a test cytomegalovirus (CMV) DNA vaccine that contains plasmids encoding CMV phosphoprotein 65 (pp65) and glycoprotein B (gB) was formulated with poloxamer CRL1005 and a cationic surfactant BAK to induce cellular and humoral immune responses in 44 healthy adult subjects. Thirty-two subjects received 1- or 5-mg doses of vaccine on a 0-, 2-, and 8-week schedule, and 12 subjects received 5-mg doses of vaccine on a 0-, 3-, 7-, and 28-day schedule. The vaccine was generally well tolerated, with no serious adverse events. Common adverse events included mild to moderate injection site pain and tenderness, induration, erythema, mild to moderate malaise, and myalgia. Evidence of immunogenicity including IFN-γ and T-cell responses were observed in several of the treated patients.

15.5.4 Cystic Fibrosis

Cystic fibrosis remains one of the very few genetic diseases that have been targeted by gene therapy. A genetic mutation results in abnormal fluid and electrolyte conductance across the bronchial airways leading to the clinical manifestation of this fatal disease. Previous gene therapy approaches have utilized viral vectors or cationic liposomes to achieve gene transfer of the cystic fibrosis gene with limited success. A polymeric gene carrier, PEG–PLL, has been evaluated for safe and efficient delivery of the cystic fibrosis gene in patients with cystic fibrosis after successful evaluation in animal models [97]. In a double-blind study, a single intranasal administration of escalating doses of cystic fibrosis gene plasmid (0.8, 2.67, and 8.0 mg DNA) formulated with PEG–PLL in 12 cystic fibrosis (CF) patients was safe and well tolerated. There was no evidence of a significant increase in inflammatory mediators in serum or nasal washing. Approximately 0.58 copies of the cystic fibrosis gene were quantified per cell in the nasal scrapings. Partial to complete biological response was achieved in eight subjects with some evidence of a dose trend. The corrections generally persisted for up to 6 days after gene transfer. This study shows gene transfer to nasal epithelium with PEG–PLL is safe and effective. It would be interesting to see if the duration of the observed effects can be extended by repeated delivery since the effect of single dosing lasted for about one week.

REFERENCES

1. Bloomfield, V. Condensation of DNA by multivalent cations: Considerations on mechanism. *Biopolymers* 1991;**31**:1471–1481.
2. Bloomfield, V. DNA condensation. *Curr. Opin. Struct. Biol.* 1996;**6**:334–341.
3. Wolfert, M. and L. Seymour. Atomic force microscopic analysis of the influence of the molecular weight of poly(L)lysine on the size of polyelectrolyte complexes formed with DNA. *Gene Ther.* 1996;**3**:269–273.
4. Adami, R., W. Collard, S. Gupta, K. Kwok, J. Bonadio, and K. Rice. Stability of peptide-condensed plasmid DNA formulations. *J. Pharm. Sci.* 1998;**87**:678–683.
5. Park, S., D. Harries, and W. Gelbart. Topological defects and the optimum size of DNA condensates. *Biophys. J.* 1998;**75**:714–720.
6. Lechardeur D. and G. Lukacs. Intracellular barriers to non-viral gene transfer. *Curr. Gene Ther.* 2002;**2**:183–194.
7. De Smedt, S., J. Demeester, and W. Hennink. Cationic polymer based gene delivery systems. *Pharm. Res.* 2000;**17**:113–126.
8. Wagner, E., M. Cotton, R. Foisner, and M. Birnstiel. Transferrin-polycation-DNA complexes: The effect of polycations on the structure of the complex and DNA delivery to cells. *Proc. Natl. Acad. Sci. U.S.A.* 1991;**88**:4255–4259.
9. Ogris, M., P. Steinlein, M. Kursa, K. Mechtler, R. Kircheis, and E. Wagner. The size of DNA/transferrin-PEI complexes is an important factor for gene expression in cultured cells. *Gene Ther.* 1998;**5**:1425–1433.
10. Pouton, C., P. Lucas, B. Thomas, A. Uduehi, D. Milroy, and S. Moss. Polycation-DNA complexes for gene delivery: A comparison of the biopharmaceutical properties of cationic polypeptides and cationic lipids. *J. Control. Release.* 1998;**53**:289–299.
11. Tang M. and F. Szoka. The influence of polymer structure on the interactions of cationic polymers with DNA and morphology of the resulting complexes. *Gene Ther.* 1997;**4**:823–832.
12. Bielinska, A., J. Kukowska-Latallo, and J. Baker. The interaction of plasmid DNA with polyamidoamine dendrimers: Mechanism of complex formation and analysis of alterations induced in nuclease sensitivity and transcriptional activity of the complexed DNA. *Biochim. Biophys. Acta* 1997;**1353**:180–190.
13. Haensler, J. and F. Szoka. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. *Bioconjug. Chem.* 1993;**4**:372–379.
14. Ruponen, M., S. Yla-Herttuala, and A. Urtti. Interactions of polymeric and liposomal gene delivery systems with extracellular glycosaminoglycans: Physico-chemical and transfection studies. *Biochim. Biophys. Acta* 1999;**1415**:331–341.
15. Kabanov, V. and A. Kabanov. Interpolyelectrolyte and block ionomer complexes for gene delivery: Physico-chemical aspects. *Adv. Drug Deliv. Rev.* 1998;**30**:49–60.
16. Smith, L., J. Duguid, M. Wadhwa, M. Logan, C. Tung, V. Edwards, and J. Sparrow. Synthetic peptide-based DNA complexes for nonviral gene delivery. *Adv. Drug Deliv. Rev.* 1998;**30**:115–131.
17. Izumrudov, V., M. Zhiryakova, S. Kargov, A. Zezin, and V. Kabanov. Competitive reactions in solutions of DNA-containing polyelectrolyte complexes. *Macromol. Symp.* 1996;**106**:179–192.

18. Boussif, O., F. Lezoualc'h, M. Zanta, M. Mergny, D. Scherman, B. Demeneix, and J. A. Behr. Versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo : Polyethyleneimine. *Proc. Natl. Acad. Sci. U.S.A.* 1995;**92**: 7297–7301.
19. Garnett, M. Gene delivery systems using cationic polymers. *Crit. Rev. Ther. Drug Carrier Syst.* 1999;**15**:147–207.
20. Godbey, W., K. Wu, and A. Mikos. Size matters: Molecular weight affects the efficacy of poly(ethylenimine) as a gene delivery vehicle. *J. Biomed. Mater. Res.* 1999;**45**:268–275.
21. Behr, J. Gene transfer with synthetic cationic amphiphiles: Prospects for gene therapy. *Bioconjug. Chem.* 1994;**5**:382–389.
22. Kunath K., A. von Harpe, D. Fisher, H. Peterson, U. Bickel, K. Voigt, and T. Kissel. Low-molecular-weight polyethylenimine as a non-viral vector for DNA delivery: comparison of physicochemical properties, transfection efficiency and in vivo distribution with high-molecular-weight polyethylenimine. *J. Control. Release* 2003;**89**:113–125.
23. Abdallah, B., A. Hassan, C. Benoist, D. Goula, J. P. Behr, and A. Mazabraud. A powerful nonviral vector for in vivo gene transfer into the adult mammalian brain: Polyethylenimine. *Hum. Gene Ther.* 1996;**7**:1947–1954.
24. Boletta, A., A. Benigni, J. Lutz, G. Remuzzi, M. Soria, and L. Monaco. Nonviral gene delivery to the rat kidney with polyethyleneimine. *Hum. Gene Ther.* 1997;**8**:1243–1251.
25. Furgeson, D. and S. Kim. Linear PEI-cholesterol conjugates for the LDL-R pathway. *Mol. Ther.* 2003;**7**:S372.
26. Gharwan, H., L. Wightman, R. Kercheis, R. Wagner, and K. Zatloukal. Nonviral gene transfer into fetal mouse livers (a comparison between the cationic polymer PEI and naked DNA). *Gene Ther.* 2003;**10**:810–817.
27. Wightman, L., R. Kircheis, V. Rossler, S. Carotta, R. Ruzicka, M. Kursa, and E. Wagner. Different behavior of branched and linear polyethylenimine for gene delivery in vitro and in vivo. *J. Gene Med.* 2001;**3**:362–372.
28. Burke, R. and S. Pun. Extracellular barriers to in vivo PEI and PEGylated PEI polyplex-mediated gene delivery to the liver. *Bioconj. Chem.* 2008;**19**:693–704.
29. Chollet, P., M. Favrot, A. Hurbin, and J. Coll. Side-effects of a systemic injection of linear polyethyleneimine-DNA complexes. *J. Gene Med.* 2002;**4**:84–91.
30. Ohana, P., O. Gofrit, S. Ayesh, W. Al-Sharef, A. Mizrahi, T. Birman, T. Schneider, I. Matouk, N. deGroot, E. Tavdy, A. Ami Sidi, and A. Hochberg. Regulatory sequences of the H19 gene in DNA based therapy of bladder cancer. *Gene Ther. Mol. Biol.* 2004;**8**:181–192.
31. Harris, J. M. *PEG: Chemistry and Biological Applications*. American Chemical Society: Washington, DC, 1997.
32. Kakizawa, Y. and K. Kataoka. Block copolymer micelles for delivery of gene and related compounds. *Adv. Drug Deliv. Rev.* 2002;**54**:203–222.
33. Oupicky, D., K. Howard, C. Konak, P. Dash, K. Ulbrich, and L. Seymour. Steric stabilization of poly-L-Lysine/DNA complexes by the covalent attachment of semitelechelic poly[N-(2-hydroxypropyl)methacrylamide]. *Bioconjug. Chem.* 2000; **11**:492–501.

34. Ogris, M., S. Brunner, S. Schuller, R. Kircheis, and E. Wagner. PEGylated DNA/transferrin-PEI complexes: Reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery. *Gene Ther.* 1999;**6**:595–605.

35. Katayose, S. and K. Kataoka. Remarkable increase in nuclease resistance of plasmid DNA through supramolecular assembly with poly(ethylene glycol)-poly (L-lysine) block copolymer. *J. Pharm. Sci.* 1998;**87**:160–163.

36. Lim, D., Y. Yeom, and T. Park. Poly(DMAEMA-NVP)-*b*-PEG-galactose as gene delivery vector for hepatocytes. *Bioconjug. Chem.* 2000;**11**:688–695.

37. Kwok, K., D. Mckenzie, D. Evers, and K. Rice. Formulation of highly soluble poly (ethylene glycol)-peptide DNA condensates. *J. Pharm. Sci.* 1999;**88**:996–1003.

38. Merdan, T., K. Kunath, H. Petersen, U. Bakowsky, K. H. Voigt, J. Kopecek, and T. Kissell. PEGylation of poly(ethylene imine) affects stability of complexes with plasmid DNA under in vivo conditions in a dose-dependent manner after intravenous injection into mice. *Bioconjugate Chem.* 2005;**16**:785–792.

39. Hong, J., J. Park, K. Huh, H. Chung, I. Kwon, and S. Jeong. PEGylated polyethyleneimine for in vivo local gene delivery based on lipiodolized emulsion system. *J. Control. Release* 2004;**99**:167–176.

40. Neu, M., J. Sitterberg, U. Bakowsky, and T. Kissel. Stabilized nanocarriers for plasmids based upon cross-linked poly(ethyleneimine). *Biomacromolecules* 2006;**7**:3428–3438.

41. Neu, M., O. Germershaus, M. Behe, and T. Kissel. Bioreversably crosslinked polyplexes of PEI and high molecular weight PEG show extended circulation times in vivo. *J. Control. Release* 2007;**124**:69–80.

42. Fewell, J., M. Matar, G. Slobodkin, S. Han, J. Rice, B. Hovanes, D. Lewis, and K. Anwer. Synthesis and characterization of a non-viral gene delivery system for immunogene therapy of cancer. *J. Control. Release* 2005;**109**:288–298.

43. Fewell, J., J. Rice, M. Matar, and K. Anwer. Safety and toxicity following intraperitoneal injection of murine interleukin-12 plasmid formulated with a novel polymeric delivery system. *Mol. Ther.* 2006;**13**:S109(#287).

44. Brunhoeber, E., M. Matar, K. Anwer, and J. Fewell. Biodistribution and clearance following intraperitoneal injection of murine interleukin-12 plasmid formulated with a novel polymeric delivery system. *Mol. Ther.* 2006;**13**:S109(#286).

45. Sonabend, A., S. Velicu, I. Ulasov, Y. Han, B. Tyler, H. Brem, M. Matar, J. Fewell, K. Anwer, and M. Lesniak. A safety and efficacy study of local delivery of IL-12 transgene by PPC polymer in a model of experimental glioma. *Anticancer Drug* 2008;**19**:133–142.

46. Tang, G., H. Guo, F. Alexis, X. Wang, S. Zeng, T. Lim, J. Ding, Y. Yang, and S. Wang. Low molecular weight polyethyeneimines linked by -cyclodextrin for gene transfer into the nervous system. *J. Gene Med.* 2006;**8**:736–744.

47. Wong, K., G. Sun, X. Zhang, H. Dai, Y. Liu, C. He, and K. Leong. PEI-*g*-chitosan, a novel gene delivery system with transfection efficiency comparable to polyethyleneimine in vitro and after liver administration in vivo. *Bioconj. Chem.* 2006;**17**:152–158.

48. Gosselin, M., W. Guo, and R. Lee. Efficient gene transfer using reversibly cross-linked low-molecular-weight polyethyleneimine. *Bioconj. Chem.* 2001;**12**:989–994.

49. Thomas, M., Q. Ge, J. Lu, J. Chen, and A. Klibanov. Cross-linked small polyethyleneimines: While still nontoxic, deliver DNA efficiently to mammalian cells in vitro and in vivo. *Pharm. Res*. 2005;**22**:373–380.

50. Matar, M., G. Slobodkin, A. Rea-Ramesy, E. Brunhoeber, J. Skoyen, J. Fewell, D. Lewis, and K. Anwer. Synthesis and characterization of low-molecular-weight linear polyethyleneimines for gene delivery. *J. Biomed. Nanotech*. 2006;**2**: 53–61.

51. Ahn, C. H., S. Y. Chae, Y. H. Bae, and S. W. Kim. Biodegradable poly(ethylenimine) for plasmid DNA delivery. *J. Control. Release* 2002;**23**:273–282.

52. Neu, M., O. Germershaus, S. Mao, K. Voigt, M. Behe, and T. Kissel. Crosslinked nanocarriers based upon poly(ethylene imine) for systemic plasmid delivery: In vitro characterization and in vivo studies in mice. *J. Control. Release* 2007;**118**,: 370–380.

53. Park., M., H. Kim, C. Hwang, K. Han, Y. Choi, S. Song, M. Cho, and C. Cho. Highly efficient gene transfer with degradable poly(ester amine) based on poly (ethylene glycol) diacrylate and polyethyleneimine in vitro and in vivo. *J. Gene Med*. 2008;**10**:198–207.

54. Hart, S., R. Harbottle, R. Cooper, A. Miller, R. Williamson, and C. Coutelle. Gene delivery and expression mediated by an integrin-binding peptide. *Gene Ther*. 1995;**2**:552–554.

55. Sakae, M., T. Ito, C. Yoshihara, N. Iida-Tanaka, H. Yanagie, M. Eriguchi, and Y. Koyama. Highly efficient in vivo gene transfection by plasmid/PEI complexes coated by anionic PEG derivatives bearing carboxyl groups and RGD peptide. *Biomed. Pharmacother*. 2008;**62**:448–453.

56. Yockman, J., W. Kim, C. Chang, and S. Kim. Non-viral delivery of interleukin-2 and soluble Flk-1 inhibits metastatic and primary tumor growth in renal cell carcinoma. *Gene Ther*. 2007;**14**:1399–1405.

57. Suh, W., S. Han, L. Yu, and S. Kim. An angiogenic, endothelial-cell-targeted polymeric gene carrier. *Mol. Ther*. 2002;**6**:664–672.

58. Kim, W., J. Yockman, J. Jeong, J. Christensen, M. Lee, Y. Kim, and S. Kim. Anti-angiogenic inhibition of tumor growth by systemic delivery of PEI-*g*-PEG-RGD/pCMV-sFlt-1 complexes in tumor-bearing mice. *J. Control. Release* 2006;**114**: 381–388.

59. Kleeman, E., M. Neu, N. Jekel, L. Fink, T. Schmehl, T. Gessler, W. Seeger, and T. Kissel. Nanocarriers for DNA delivery to the lung base upon a TAT-derived peptide covalenly coupled to PEG-PEI. *J. Control. Release* 2005;**109**:299–316.

60. Zeng, J., X. Wang, and S. Wang. Self-assembled ternary complexes of plasmid DNA, low molecular weight polyethyleneimine and targeting peptide for nonviral gene delivery into neurons. *Biomaterials* 2007;**28**:1443–1451.

61. Rudolph, C., N. Sieverling, U. Schillinger, E. Lesina, C. Plank, A. Thunemann, H. Schonberger, and J. Rosenecker. Thyroid hormone (T3)-modification of polyethyleneglycol (PEG)–polyethyleneimine (PEI) graft copolymers for improved gene delivery to hepatocytes. *Biomaterials* 2007;**28**:1900–1911.

62. Elfinger, M., C. Maucksch, and C. Rudolph. Characterization of lactoferrin as a targeting ligand for nonviral gene delivery to airway epithelial cells. *Biomaterials* 2007;**28**:3448–3455.

63. Moffatt, S., C. Papasakelariou, S. Wiehle, and R. Cristiano. Successful in vivo tumor targeting of prostate-specific membrane antigen with a highly efficient J591/PEI/DNA molecular conjugate. *Gene Ther*. 2006;**13**:761–772.

64. Moffatt, S., S. Wiehle, and R. Cristiano. Tumor-specific gene delivery mediated by a novel peptide-polyethyleneimine-DNA polyplex targeting aminopeptidase N/CD13. *Human Gene Ther*. 2005;**15**:57–67.

65. Kim, E., H. Jeong, I. Park, C. Cho, H. Moon, D. Yu, H. Bom, M. Sohn, and I. Oh. Asialoglycoprotein receptor targeted gene delivery using galactosylated polyethyleneimine-*graft*-poly(ethylene glycol): In vitro and in vivo studies. *J. Control. Release* 2005;**108**:557–567.

66. Cook, S. E., I. K. Park, E. M. Kim, H. J. Jeong, T. G. Park, Y. J. Choi, T. Akaike, and C. S. Cho. Galactosylated polyethylenimine-graft-poly(vinyl pyrrolidone) as a hepatocyte-targeting gene carrier. *J. Control. Release* 2005;**105**:151–163.

67. Fajac, I., G. Thevenot, L. Bedouet, C. Daniel, M. Riquet, M. Merten, C. Figarella, J. Dall'Ava-Santucci, M. Monsigny, and P. Briand. Uptake of plasmid/glycosylated polymer complexes and gene transfer efficiency in differentiated airway epithelial cells. *J. Gene Med*. 2003;**5**:38–48.

68. Grosse, S., G. Thevenot, Y. Aron, E. Duverger, M. Abdelkarim, A. Roche, M. Monsigny, and I. Fajac. In vivo gene delivery in the mouse lung with lactosylated polyethyleneimine, questioning the relevance of in vitro experiments. *J. Control. Release* 2008;**132**:105–112.

69. Lisziewicz, J., J. Trocio, J. Xu, L. Whitman, A. Ryder, N. Bakare, M. G. Lewis, W. Wagner, A. Pistorio, S. Arya, and F. Lori. Control of viral rebound through therapeutic immunization with DermaVir. *AIDS* 2005;**12**:35–43.

70. Lisziewicz, J., J. Trocio, L. Whitman, G. Varga, J. Xu, N. Bakare, P. Erbacher, C. Fox, R. Woodward, P. Markham, S. Arya, J. Behr, and F. Lori. DermaVir: A novel topical vaccine for HIV/AIDS. *J. Invest. Dermatol*. 2005;**124**:160–169.

71. Nguyen, D., S. Raghavan, L. Tashima, E. Lin, S. Fredette, R. Langer, and C. Wang. Enhancement of poly(orthoester) microspheres for DNA vaccine delivery by blending with poly(ethyleneimine). *Biomaterials* 2008;**29**:2783–2793.

72. Turk, M., S. Dincer, and E. Piskin. Smart and cationic poly(NIPA)/PEI block copolymers as nonviral vectors: In vitro and in vivo transfection studies. *J. Tissue Eng. Reg. Med*. 2007;**1**:377–388.

73. Lei, Y. and T. Segura. DNA delivery from matrix metalloprotease degradable poly (ethylene glycol) hydrogels to mouse mesenchymal stem cells. *Biomaterials* 2009;**30**:254–265.

74. Arote, R., T. Kim, Y. Kim, S. Hwang, H. Jiang, H. Song, J. Nah, M. Cho, and C. Cho. A biodegradable poly(ester amine) based on polycaprolactone and polyethyleneimine as a gene carrier. *Biomaterials* 2007;**28**:735–744.

75. Lynn, D., D. Anderson, D. Putnam, and R. Langer. Accelerated discovery of synthetic transfection vectors: Parallel synthesis and screening of a degradable polymer library. *J. Am. Chem. Soc*. 2001;**123**:8155–8166.

76. Lynn, D. and R. Langer. Degradable poly(β-amino esters): Synthesis, characterization and self-assembly with plasmid DNA. *J. Am. Chem. Soc*. 2000;**122**:10761–10768.

77. Green, J., G. Zugates, N. Tedford, Y. Huang, L. Griffith, D. Lauffenburger, J. Sawicki, R. Langer, and D. Anderson. Combinatorial modification of degradable polymers enables transfection of human cells comparable to adenovirus. *Adv. Mater.* 2007;**19**:2836–2842.

78. Tomalia, D., A. Naylor, and W. Goddard. Starburst dendrimers: molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter. *Angew. Chem. Int. Ed. Engl.* 1990;**29**:138–175.

79. Tang, M., C. Redemann, and F. Szoka. In vitro gene delivery by degraded polyamidoamine dendrimers. *Bioconj. Chem.* 1996;**7**:703–714.

80. Mamede, M., T. Saga, T. Ishimori, T. Higashi, N. Sato, H. Kobayashi, W. Brechbiel, and J. Konishi. Hepatocyte targeting of ^{111}In-labeled oligo-DNA with avidin or avidin-dendrimer complex. *J. Control. Release* 2004;**95**:133–141.

81. Malik, R., R. Wiwattanapatapee, R. Klopsch, K. Lorenz, H. Frey, J. Weener, E. Meijer, W. Paulus, and R. Duncan. Dendrimers: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of ^{125}I-labelled polyamidoamine dendrimers in vivo. *J. Control. Release* 2000;**65**:133–148.

82. Huang, R., W. Ke, Y. Liu, C. Jiang, and Y. Pei. The use of lactoferrin as a ligand for targeting the polyamidoamine-based gene delivery system to the brain. *Biomaterials* 2008;**29**:238–246.

83. Huang, R., W. Ke, Y. Qu, J. Zhu, Y. Pei, and C. Jiang. Characterization of lactoferrin receptor in brain endothelial capillary cells and mouse brain. *J. Biomed. Sci.* 2007;**14**:121–128.

84. Okuda, T., S. Kawakami, N. Akimoto, T. Niidome, F. Yamashita, and M. Hashida. PEGylated lysine dendrimers for tumor-selective targeting after intravenous injection in tumor-bearing mice. *J. Control. Release* 2006;**116**:330–336.

85. Ribeiro, S., S. G. Rijpkema, Z. Durrani, and A. T. Florence. PLGA-dendron nanoparticles enhance immunogenicity but not lethal antibody production of a DNA vaccine against anthrax in mice. *Int. J. Pharm.* 2007;**331**:228–232.

86. Russ, V., H. Elfberg, J. Kloeckner, M. Ogris, and E. Wagner. Novel degradable oligoethyleneimine acrylate ester-based pseudodendrimers for in vitro and in vivo gene transfer. *Gene Ther.* 2008;**15**:18–29.

87. Schatzlein, A. G., B. H. Zinselmeyer, A. Elouzi, C. Dufes, Y. T. A. Chim, C. J. Roberts, M. C. Davies, A. Munro, A. I. Gray, and I. F. Uchegbu. Preferential liver gene expression with polypropylenimine dendrimers. *J. Control. Release* 2005;**101**:247–258.

88. Ward, C., M. Pechar, D. Oupicky, K. Ulbrich, and L. Seymour. Modification of pLL/DNA complexes with a multivalent hydrophilic polymer permits folate-mediated targeting in vitro and prolonged plasma circulation in vivo. *J. Gene Med.* 2002;**4**:536–547.

89. Mannisto, P., S. Vanderkerken, V. Toncheva, M. Elomaa, M. Ruponen, E. Schacht, and A. Urtii. Structure-activity relationships of poly(L-lysines): Effects of pegylation and molecular shape on physiochemical and biological properties in gene delivery. *J. Control. Release* 2002;**83**:169–182.

90. Nishikawa, M., Y. Takemura, and M. Hashida. Targeted delivery of plasmid DNA to hepatocytes in vivo: Optimization of the pharmacokinetics of plasmid DNA/

galactosylated poly(L-lysine) complexes by controlling their physiochemical properties. *J. Pharmacol. Exp. Ther.* 1998;**287**:408–415.

91. Brown, M., A. Schatzlein, A. Brownlie, V. Jack, W. Wand, L. Tetley, A. Gray, and I. Uchegbu. Preliminary characterization of novel amino acid based polymeric vesicles as gene and drug delivery agents. *Bioconj. Chem.* 2000;**11**: 880–891.
92. Harada-Shiba, M., K. Yamauchi, A. Harada, I. Takamisawa, K. Shimokado, and K. Kataoka. Polyion complex micelles as vectors in gene therapy pharmacokinetics and in vivo gene transfer. *Gene Ther.* 2002;**9**:407–414.
93. Lee, M., S. Han, K. Ko, J. Koh, J. Park, J. Yoon, and S. Kim. Repression of GAD autoantigen expression in pancreas cells by delivery of antisense plasmid/PEG-*g*-PLL complex. *Mol. Ther.* 2001;**4**:339–346.
94. Unnamalai, N., B. Kang, and W. Lee. Cationic oligopeptide-mediated delivery of dsRNA for post-transcriptional gene silencing in plant cells. *FEBS Let* 2004; **566**:307–310.
95. Moriguchi, R., K. Kogure, H. Akita, S. Futaki, M. Miyagishi, K. Taira, and H. Harashima. A multifunctional envelope-type nanodevice for novel gene delivery of siRNA plasmids. *Pharm. Nanotech.* 2005;**301**:277–285.
96. Hatefi, A., Z. Megeed, and H. Ghandehari. Recombinant polymer-protein fusion: A promising approach towards efficient and targeted gene delivery. *J. Gene Med.* 2006;**8**:468–476.
97. Konstan, M., P. Davis, J. Wagener, K. Hilliard, R. Stern, L. Milgram, T. Kowalczyk, S. Hyatt, T. Fink, C. Gedeon, S. Oette, J. Payne, O. Muhammad, A. Ziady, R. Moen, and M. Cooper. Compacted DNA nanoparticles administered to the nasal mucosa of cystic fibrosis subjects are safe and demonstrate partial to complete cystic fibrosis transmembrane regulator reconstitution. *Hum. Gene Ther.* 2004;**15**:1255–1269.
98. Liu, G., D. Li, M. Pasumarthy, T. Kowalczyk, C. Gedeon, S. Hyatt, J. Payne, T. Miller, P. Brunovskis, T. Fink, O. Muhammad, R. Moen, R. Hanson, and M. Cooper. Nanoparticles of compacted DNA transfect postmitotic cells. *J. Biol. Chem.* 2003;**278**:32578–32586.
99. Ziady, A., C. Gedeon, T. Miller, W. Quan, J. Payne, S. Hyatt, T. Fink, O. Muhammad, S. Oette, T. Kowalczyk, M. Pasumarthy, R. Moen, M. Cooper, and P. Davis. Transfection of airway epithelium by stable PEGylated poly-L-lysine DNA nanoparticles in vivo. *Mol. Ther.* 2003;**8**:936–947.
100. Ziady, A., C. Gedeon, O. Muhammad, V. Stillwell, S. Oette, T. Fink, W. Quan, T. Kowalczyk, S. Hyatt, J. Payne, A. Peischl, J. Seng, R. Moen, M. Cooper, and P. Davis. Minimal toxicity of stabilized compacted DNA nanoparticles in the murine lung. *Mol. Ther.* 2003;**8**:948–956.
101. Itaka, K., S. Ohba, K. Miyata, H. Kawaguchi, K. Nakamura, T. Takato, U. Chung, and K. Kataoka. Bone regeneration by regulated in vivo gene transfer using biocompatible polyplex nanomicelles. *Mol. Ther.* 2007;**15**:1655–1662.
102. Kanayama, N., S. Fukeshima, N. Nishiyama, K. Itaka, W. Jang, K. Miyata, Y. Yamasaki, U. Chung, and K. Kataoka. A PEG-based biocompatible block catiomer with high buffering capacity for the construction of polyplex micelles showing efficient gene transfer toward primary cells. *Chem. Med. Chem.* 2006;**1**:439–444.

103. Mumper, R., J. Wang, J. Claspell, and A. Rolland. Novel polymeric condensing carriers for gene delivery. *Proc. Int. Symp. Control. Release Bioactive Mater.* 1995;**22**:178–179.

104. MacLaughlin, F., R. Mumper, J. Wang, J. Tagliaferri, I. Gill, M. Hinchcliffe, and A. Rolland. Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. *J. Control. Release* 1998;**56**:259–272.

105. Koping-Hoggard, M., K. Varum, M. Issa, S. Danielson, B. Christiensen, B. Stokke, and P. Artursson. Improved chitosan-mediated gene delivery based on easily dissociated chitosan polyplexes of highly defined chitosan oligomers. *Gene Ther.* 2004;**11**:1441–1452.

106. Jiang, H., J. Kwon, Y. Kim, E. Kim, R. Arote, H. Jeong, J. Nah, Y. Choi, T. Akaike, M. Cho, and C. Cho. Galactosylated chitosan-*graft*-polyethyleneimine as a gene carrier for hepatocyte targeting. *Gene Ther.* 2007;**14**:1389–1398.

107. Li, S. and W. Purdy. Cyclodextrins and their applications in analytical chemistry. *Chem. Rev.* 1992;**92**:1457–1470.

108. Davis, M., S. Pun, N. Bellocq, T. Reineke, S. Popielarski, S. Mishra, and J. Heidel. Self assembling nucleic acid delivery vehicles via linear, water soluble, cyclodextrin containing polymers. *Curr. Med. Chem.* 2004;**11**:179–197.

109. Pun, S., N. Bellocq, A. Liu, G. Jensen, T. Machemer, E. Quijano, T. Schluep, S. Wen, H. Engler, J. Heidel, and M. Davis. Cyclodextrin-modified polyethyleneimine polymers for gene delivery. *Bioconj. Chem.* 2004;**15**:831–840.

110. Rolland, A. and R. Mumper. Plasmid delivery to muscle: Recent advances in polymer delivery systems. *Ad. Drug Deliv. Rev.* 1998;**30**:151–172.

111. Hartikka, J., A. Geall, V. Bozoukova, D. Kurniadi, D. Rusalov, J. Enas, J. Yi, A. Nanci, and A. Rolland. Physical characterization and in vivo evaluation of poloxamer-based DNA vaccine formulations. *J. Gene Med.* 2008;**10**:770–782.

112. Roques, C., A. Salmon, M. Y. Fiszman, E. Fattal, and Y. Fromes. Intrapericardial administration of novel DNA formulations based on thermosensitive Poloxamer 407 gel. *Int. J. Pharm.* 2007;**331**:220–223.

113. Zhong, J., B. Eliceiri, D. Stupack, K. Penta, G. Sakamoto, T. Quertermous, M. Coleman, N. Boudrou, and J. Varner. Neovascularization of ischemic tissues by gene delivery of the extracellular matrix protein Del-1. *J. Clin. Invest.* 2003;**112**:30–41.

114. Anwer, K., B. Rhee, and S. Mendiratta. Recent progress in polymeric gene delivery systems. *Crit. Rev. Ther. Drug.* 2003;**20**:249–293.

115. Chang, C., D. Choi, W. Kim, J. Yockman, L. Christensen, Y. Kim, and S. Kim. Non-ionic amphiphilic biodegradable PEG-PLGA-PEG copolymer enhances gene delivery efficiency in rat skeletal muscle. *J. Control. Release* 2007;**118**:245–253.

116. Zentner, G., R. Rathi, C. Shih, J. McRea, M. Seo, H. Oh, B. Rhee, J. Mestecky, Z. Moldoveanu, M. Morgan, and S. Weitman. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Control. Release.* 2001;**72**: 203–215.

117. Ronneberger, B., W. Kao, J. Anderson, and T. Kissel. In vivo biocompatibility study of ABA triblock copolyemers consisting of poly(*L*-lactic-*co*-glycolic acid) A blocks attached to central poly(oxyethylene) B blocks. *J. Biomed. Mater. Res.* 1996;**30**:31–40.

118. Blum, J., and W. M. Salttzman. High loading efficiency and tunable release of plasmid DNA encapsulated in submicron particles fabricated from PLGA conjugated with poly-L-lysine. *J. Control. Release* 2008;**129**:66–72.

119. Csaba, N., A. Sanchez, and M. J. Alonso. PLGA: Poloxamer and PLGA: Poloxamine blend nanostructures as carriers for nasal gene delivery. *J. Control. Release* 2006;113:164–172.

120. Bhavsar, M. D., and M. M. Amiji. Gastrointestinal distribution and in vivo gene transfection studies with nanoparticles-in-microsphere oral system (NiMOS). *J. Control. Release* 2007;**119**:339–348.

121. Grzelinski, M., B. Urban-Klein, T. Martens, K. Lamszus, U. Bakowsky, S. Hobel, F. Czubayko, and A. Aigner. RNA interference-mediated gene silencing of pleiotrophin through polyethyleneimine-complexed small interfering RNAs in vivo exerts antitumoral effects in glioblastoma xenografts. *Hum. Gene Ther*. 2006;**17**:751–766.

122. Urban-Klein, B., S. Werth, S. Abuharbeid, F. Czubayko, and A. Aigner. RNAi-mediated gene-targeting through systemic application of polyethyleneimine (PEI)-complexes siRNA in vivo. *Gene Ther*. 2005;**12**:461–466.

123. Schiffelers, R., A. Ansari, J. Xu, Q. Zhou, Q. Tang, G. Storm, G. Molema, P. Lu, P. Scaria, and M. Woodle. Cancer siRNA therapy by tumor selective delivery with ligand-targeted sterically stabilized nanoparticle. *Nucleic Acids Res*. 2004; **32**:e149.

124. Heidel, J., Z. Yu, J. Liu, S. Rele, Y. Liang, R. Zeidan, D. Kornbrust, and M. Davis. Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. *Proc. Natl. Acad. Sci. U.S.A*. 2007;**104**:5715–5721.

125. Hu-Licskovan, S., J. Heidel, D. Bartlett, M. Davis, and T. Triche. Sequence specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. *Cancer Res*. 2005;**69**:8584–8992.

126. Pun, S. and M. Davis. Development of a nonviral gene delivery vehicle for systemic application. *Bioconj. Chem*. 2002;**13**:630–639.

127. Rozema, D., D. Lewis, D. Wakefield, S. Wong, J. Klein, P. Roesch, S. Bertin, T. Reppen, Q. Chu, A. Blokhin, J. Hagstrom, and J. Wolff. Dynamic polyconjugates for targeted in vivo delvivery of siRNA to hepatocytes. *Proc. Natl. Acad. Sci. U.S.A*. 2007;**104**:12982–12987.

128. Wakefield, D., J. Klein, J. Wolff, and D. Rozema. Membrane activity and transfection ability of amphipathic polycations as a function of alkyl group size. *Bioconj. Chem*. 2005;**16**:1204–1208.

129. Rozema, D., K. Ekena, D. Lewis, A. Loomis, and J. Wolff. Endosomalysis by masking of a membrane-active agent (EMMA) for cytoplasmic release of macromolecules. *Bioconj. Chem*. 2003;**14**:51–57.

130. Kim, S., J. Jeong, S. Lee, S. Kim, and T. Park. Local and systemic delivery of VEGF siRNA using polyelectrolyte compex micelles for effective treatment of cancer. *J. Control. Release* 2008;**129**:107–116.

131. Kim, S., J. Jeong, S. Lee, S. Kim, and T. Park. PEG conjugated VEGF siRNA for anti-angiogenic gene therapy. *J. Control. Release* 2006;**116**:123–129.

132. Matthews, K., J. Kendrick, M. Straughn, M. Barnes, S. Makhija, J. Fewell, K. Anwer, and R. Alvarez.A phase I trial of intraperitoneal EGEN-001, a novel IL-12 gene therapeutic, administered alone or in combination with chemotherapy in patients with recurrent ovarian cancer. *J. Clin. Oncol.* 2008;**26**(May 20 suppl; Abstract 5572).

133. Alvarez, R., M. Barnes, M. Anderson, S. Saddekni, S. Makhija, I. Maya, J. Kendrick, J. Fewell, D. Lewis, and K. Anwer. Progress in clinical development of a novel IL-12 gene therapeutic for the treatment of recurrent ovarian cancer. *Mol. Ther.* 2008;**16**:S63(Abstract 167).

134. Lisziewicz, J., J. Trocio, J. Xu, L. Whitman, A. Ryder, N. Bakare, M. Lewis, W. Wagner, A. Pistorio, S. Arya, and F. Lori. Control of viral rebound through therapeutic immunization with DermaVir. *AIDS* 2005;**19**:35–43.

135. Grossman, P., F. Mendelsohn, T. Henry, J. Hermiller, M. Litt, J. Saucedo, R. Weiss, D. Kandzari, N. Kleiman, R. Anderson, D. Gottlieb, R. Karlsberg, J. Snell, and K. Rocha-Singh. Results from a phase II multicenter, double-blind placebo-controlled study of Del-1 (VLTS-589) for intermittent claudication in subjects with peripheral arterial disease. *Am. Heart J.* 2007;**153**:874–880.

136. Dai, Q., J. Huang, and B. Klitzman. Engineered zinc finger-activating vascular endothelial growth factor transcription factor plasmid DNA induces therapeutic angiogenesis in rabbits with hindlimb ischemia. *Circulation* 2004;**110**:2467–2475.

137. Wloch, M. K., L. R. Smith, S. Boutsaboualoy, L. Reyes, C. Han, J. Kehler, H. D. Smith, L. Selk, R. Nakamura, J. M. Brown, T. Marbury, A. Wald, A. Rolland, D. Kaslow, T. Evans, M. Boeckh. *J Infect Dis.* 2008;**197**:1634–1642.

BIBLIOGRAPHY

Chemin, I., D. Moradpour, S. Wieland, W. Offensperger, E. Walter, J. Behr, and H. Blum. Liver-directed gene transfer: A linear polyethyleneimine derivative mediates highly efficient DNA delivery to primary hepatocytes in vitro and in vivo. *J. Viral Hepatitis* 1998;**5**:369–375.

Choi, Y., F. Liu, J. Choi, S. Kim, and J. Park. Characterization of a targeted gene carrier, lactose-polyethylene glycol-grafted poly-L-lysine and its complex with plasmid DNA. *Hum. Gene Ther.* 1999;**10**:2657–2665.

Choi, Y., F. Liu, J. Park, and S. Kim. Lactose-poly(ethylene glycol)-grafted poly-L-lysine as hepatoma cell-targeted gene carrier. *Bioconj. Chem.* 1998;**9**:708–718.

Hofland, H., C. Masson, S. Iginnla, I. Osetinsky, J. Reddy, C. Lemon, D. Scherman, M. Bessodes, and P. Wils. Folate-targeted gene transfer in vivo. *Mol. Ther.* 2002;**5**:739–744.

Janat-Amsbury, M., J. Yockman, M. Lee, S. Kern, D. Furgeson, M. Bikram, and S. Kim. Combination of local, nonviral IL12 gene therapy and systemic paclitaxel treatment in a metastatic breast cancer model. *Mol. Ther.* 2004;**9**:829–836.

Lim, Y., S. Han, H. Kong, Y. Lee, J. Park, B. Jeong, and S. Kim. Biodegradable polyester, poly[-(4-aminobutyl)-L-glycolic acid], as a non-toxic gene carrier. *Pharm. Res.* 2000;**17**:811–816.

Park, I., T. Kim, Y. Park, B. Shin, E. Choi, E. Chowdhury, T. Akaike, and C. Cho. Galactosylated chitosan-*graft*-poly(ethylene glycol) as hepatocyte-targeting gene carrier. *J. Control. Release*. 2001;**76**:349–362.

Ross, J., P. Chaudhuri, and M. Ratnam. Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications. *Cancer* 1994;**73**:2432–2443.

Slobodkin, G., M. Matar, J. Fewell, and K. Anwer. U.S. Patent Application, US 2006/0093674 A1.

Wilson, J., M. Grossman, C. Wu, N. Chowdhury, G. Wu, and J. Chowdhury. Hepatocyte-directed gene transfer in vivo leads to transient improvement of hypercholesterolemia in low density lipoprotein receptor-deficient rabbits. *J. Biol. Chem*. 1992;**267**:963–967.

Wu, G., J. Wilson, F. Shalaby, M. Grossman, D. Shafritz, and C. Wu. Receptor-mediated gene delivery in vivo. Partial correction of genetic analbuminemia in Nagase rats. *J. Biol. Chem*. 1991;**266**:14338–14342.

CHAPTER 16

BIODEGRADABLE POLYMERS FOR EMERGING CLINICAL USE IN TISSUE ENGINEERING

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16.1 TISSUE ENGINEERING

A large fraction of the total health-care costs worldwide can be attributed to tissue loss or organ failure due to congenital or acquired diseases, accidents, or trauma. These costs are not only of monetary value but, more importantly, of value in human life and quality of life. The current demands for transplant organs and tissues, however, is far outpacing the supply, and all manner of projections indicate that this gap will continue to widen [1]. Thus, there has been an urgent demand for more successful regenerative strategies to repair or replace damaged tissues and organs. Tissue engineering is a thriving new area of multidisciplinary research that has potential to revolutionize the treatment of diseased and damaged tissues or organs. The ability to develop materials that can interface with tissues structurally, mechanically, and biofunctionally is important to the success of regenerative strategies [2]. As a new and multidisciplinary endeavor, tissue engineering holds the promises of (a) eliminating reoperations by using biological substitutes, (b) using biological substitutes to solve problems of implant rejection, transmission of diseases associated with xenografts, and the shortage in organ donations, (c) providing long-term solutions in tissue repair or treatment of diseases, and (d) potentially offering treatments for medical conditions that are currently untreatable [3].

The approaches to construction of any tissue or organ typically rely on three essential components: cells, which will ultimately form the new tissue; scaffolds, designed to maintain the cells in a three-dimensional environment at the implantation site; and signals that guide the gene expression and extracellular matrix (ECM) production of cells during tissue development [4]. There are three major strategies currently being explored for tissue engineering, namely, the use of cells or cell substitutes, the use of cells placed on or within matrices, or the direct implantation of scaffolds (Fig. 16.1). Cell-based tissue engineering strategies allow replacement of only those cells that supply the needed function and permit the manipulation of cells before infusion or implantation. The potential limitations include difficulty to obtain specific cell type in sufficient quantity for reconstruction purposes, failure of cells to maintain their function in the recipient, and immunological rejections. In the second approach, cells are placed on or within matrices or scaffolds, with or without growth factors and grown in vitro. These cells then proliferate, migrate, and differentiate into the specific tissue while secreting the ECM components required for creating

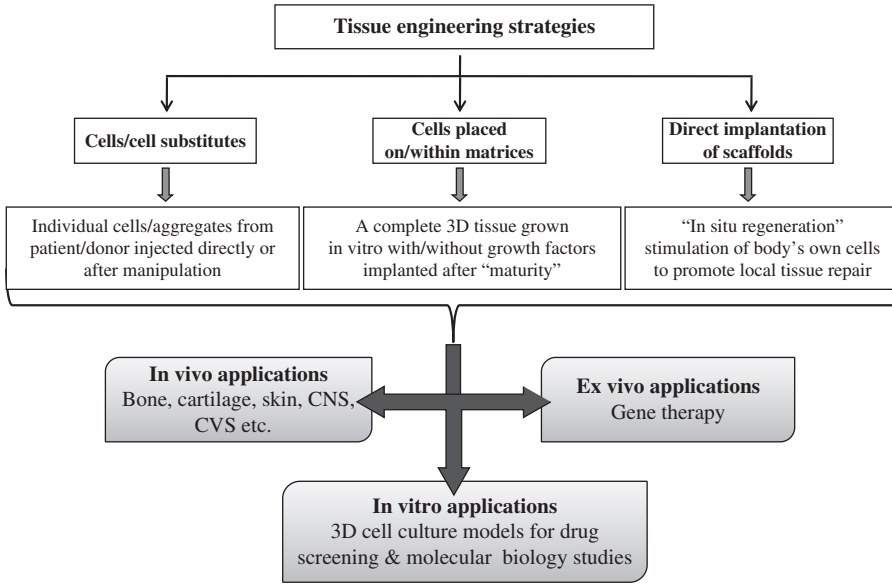

FIGURE 16.1 Various tissue engineering approaches and their clinical applications.

the tissue. The polymer–cell construct thus formed is implanted at the desired site. For in situ regeneration, a scaffold implanted directly into the injured tissue stimulates the body's own cells to promote local tissue repair. Such an approach of guided tissue regeneration involves an autologous reseeding of the decellularized matrix after implantation by the adjacent host tissue through cell migration or circulating stem or progenitor cells [5].

In vitro tissue engineering involves tissue reconstruction in vitro, in factories or laboratories, on a large scale by providing all essential materials. Another way is substitution of organ functions by use of allo or xenogeneic cells. Such engineered organs are called bioartificial organs because they are composed of heterogeneic cells and manmade membranes or porous constructs for immune-isolation to protect the cells from host attack and maintain the cell function. On the other hand, for in vivo tissue engineering, the host living body provides most of the materials necessary for tissue regeneration automatically. Most of the current tissue engineering is performed in vivo with or without biodegradable scaffolds. If the healthy ECM is still available in the body, no artificial scaffold is needed, for example, transplantation of myocardial cells for myocardial infarction therapy [6]. For the regeneration of a large-sized defect, however, it is necessary to use a biodegradable scaffold with/without cell seeding and with/without growth factor depending on application [7].

A number of materials have been used to cater to the diverse needs of tissue regeneration in the form of permanently implanted prostheses, mostly based on

metals and commonly used engineering plastics to a wide range of tissue scaffolds that are mostly based on biostable or biodegradable polymers, either for drug/cell/growth factor delivery or as bioactive or functional support. Biodegradable polymers may be further classified on the basis of their origin, namely, natural and synthetic polymers. Natural polymers have the advantage of having an intrinsic property of environmental responsiveness via degradation, bioactivity, the ability to present receptor-binding ligand to cells, and remodeling by cell-secreted enzymes [8]. They are also generally nontoxic, even at high concentrations, and, therefore, can be readily fabricated into a desired form as a tissue engineering scaffold or used as a growth factor delivery system. However, they suffer from some drawbacks such as immunogenic response, complexities associated with their purification resulting in batch-to-batch variation, restrictions with the versatility of designing devices with specific biomechanical properties, variable rate of in vivo degradation (especially in case of enzymatically degradable polymers) with the site of implantation, depending on availability and concentration of the enzymes and the possibility of disease transmission [9, 10]. Synthetic polymers on the other hand provide the flexibility to tailor mechanical properties and degradation kinetics to suit various applications, are biologically inert, and can be fabricated into various shapes with desired pore morphologic features conducive to tissue growth. Furthermore, synthetic polymers can be designed with chemically functional groups that can induce tissue growth and are available in a wide variety of compositions with readily adjustable properties [10–12].

Owing to the aforementioned features, biodegradable polymers have become indispensible for successful tissue engineering applications. The current review focuses on the biodegradable polymers used in tissue engineering, current advances in the development of need-specific biomaterials, and the clinical applications thereof.

16.1.1 Why Biodegradable Polymers?

Recent times have observed a paradigm shift from use of biostable materials to "biodegradable" materials for use in tissue engineering and other biomedical applications. This is because various tissue engineering applications (therapeutic devices, three-dimensional porous scaffolds, controlled/sustained-release drug delivery vehicles, etc.) demand materials with specific physical, chemical, biological, biomechanical, and degradation properties to provide efficient therapy. Consequently, a wide range of natural or synthetic polymers capable of undergoing degradation by hydrolytic or enzymatic route are being investigated for biomedical applications. A polymer should have certain characteristics for its use as a scaffold in tissue engineering, including biodegradability, biocompatibility, lack of immunogenicity, ease of processability, mechanical strength, biological functionality, and the like. The polymer may be used as such for scaffold fabrication or may be modified before or after scaffold fabrication on a need basis (Fig. 16.2).

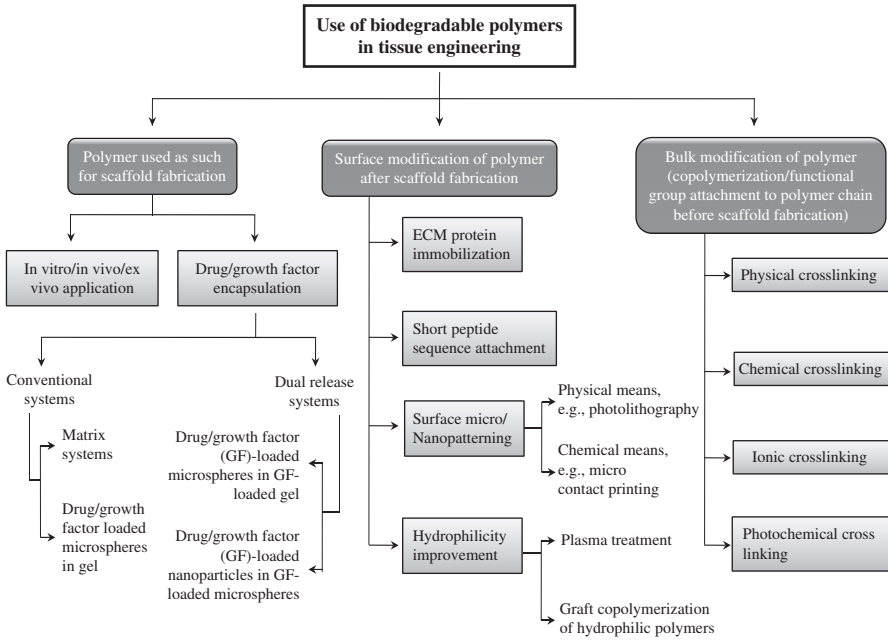

FIGURE 16.2 Strategies for use of biodegradable polymers in tissue engineering.

Biodegradability. Scaffolds may be designed to degrade rapidly as they are replaced by new host tissue or may be chemically crosslinked to slow the rate of degradation or add strength to the scaffold [13]. Degradation rate should be such that the scaffold is bioabsorbed at a predetermined time period and also provide sufficient support until the full regrowth of impaired tissue at the defect site [14]. However, it is always desirable to synchronize the polymer degradation with the replacement by natural tissue produced from cells [15]. Premature degradation of the material combined with lack of timely in vivo development of replacement tissue may result in reduced mechanical strength of an engineered tissue over time, which may lead to its failure. In case of implants, degradability is essential because it eliminates the need for implant removal in a second surgical intervention and provides space for native tissue growth [16].

Various physical and chemical mechanisms have been elucidated for polymeric degradation. Physical degradation mechanisms include sorption, swelling, softening, dissolution, mineralization, extraction, crystallization, decrystallization, stress cracking, fatigue fracture, and impact fracture. Chemical degradation occurs by hydrolysis, oxidation, solvolysis, radiolysis, fracture induced by radical reactions, enzymatic degradation, and so forth [17]. Various parameters may affect the final degradation rate of a given polymeric scaffold, such as polymer properties (molecular weight and its distribution, copolymer ratio,

polymer structure and morphology, crystallinity, glass transition temperature, radiation and chemical treatments, end-group chemistry [18–20], environmental conditions, medium temperature, and pH [21] and scaffold properties (architecture, porosity, surface area, etc.) [22–24]

Polymer degradation rate has an effect on growth factor release, mechanical properties of the scaffold, and on many cellular processes including cell viability, cell growth, tissue regeneration, and host response [25, 26]. The comparative study of fast [poly(lactic-*co*-glycolic) acid (PLGA)] versus slow degrading [poly(ε-caprolactone) (PCL)] three-dimensional scaffolds indicated that fast degradation negatively affects cell viability and migration into the scaffold in vitro and in vivo, the effect being most likely due to significant acidification of the local environment due to polymer degradation [15]. In general, polymer degradation can occur through homogeneous/bulk erosion or heterogeneous/surface erosion. However, it should be mentioned that both are extremes, and erosion mechanism of a polymer device is often characterized by a combination of both. Surface-eroding polymers contain highly labile bonds (polyanhydride) or acid-labile bonds (polyorthoesters) in their polymer main chain, which degrade very fast in contact with water. Thus, degradation rate on the surface is much faster than penetration rate of water into the polymer bulk. In consequence, erosion is limited to the surface and drug release is degradation controlled. This behavior changes if degradation rate is slower than the rate of water diffusion into the device, for example, polyesters. In this case, water penetrates into the bulk and degradation occurs throughout the polymer (bulk erosion). The resulting pores and channels in the matrix influence the rates of polymer degradation and drug release [27].

Biocompatibility and Lack of Immunogenicity. Chemical compositions of polymers used for scaffold fabrication should be such that not only the surface but also the degradation products are biocompatible, causing minimal immune or inflammatory responses, both local and systemic, and with an acceptable toxicity profile. The biocompatibility of a polymer depends on both its chemical structure and the processing method that produces it. During a polymerization process, an initiator, a monomer, and sometimes a catalyst are needed, and these materials often remain in preformed implants even after purification. Residual unreacted monomers or initiators are also a particular concern for in situ forming implants. Therefore, the toxicity and concentration of these substances should be considered when assessing biocompatibility. Removal of these potentially toxic components is usually effected by prolonged rinsing in aqueous solution.

The International Organization for Standardization (ISO) has developed guidelines for biological evaluation of medical devices that apply to biomaterials and tissue engineering systems [28]. The fitness of a biomaterial for a purpose is first determined by studying the chemical, toxicological, physical, electrical, morphological, and mechanical properties. The ISO guidelines for evaluation and testing for material selection include determining (a) materials

required to manufacture the scaffold, (b) intended additives, process contaminants, and residues (e.g., initiator required for a polymerization), (c) leachable substances (important for in situ polymerizations where unreacted monomer is present), (d) properties and characteristics of the final product, and (e) degradation products. Evaluation of degradation products is particularly important for biodegradable materials in tissue engineering. Identification, quantification, and pharmacokinetic studies of the degradation process should be performed [29]. In vitro assays such as Almer blue and MTT assay along with short-term and long-term in vivo implantation methods give a fair idea of the biocompatibility of a polymer. The implant chemistry, degradation, and mechanical strength are analyzed to observe the in vivo response of the biomaterial. The thickness and biochemical composition of the capsule is another marker for measuring inflammatory response. Potential systemic effects may be analyzed through blood and urine sampling and determining the presence of any polymer by-products in organs and tissues. Cellular differentiation studies are particularly important since production of a specific ECM or cell activity is required for tissue development and maintenance.

Biological Functionality. The design criteria for modern tissue engineering scaffolds involve use of materials equipped with molecular cues mimicking certain aspects of structure or function of natural ECM [30]. Biomolecular recognition of materials by cells has been achieved by surface modification of fabricated scaffold or bulk modification of polymer before scaffold fabrication, via chemical or physical methods with bioactive molecules that can incur specific interactions with cell receptors (Fig. 16.2). Surface modification of biomaterials with bioactive molecules such as a native long chain of ECM proteins (e.g., fibronectin, vitronectin, and laminin) as well as short peptide sequences derived from intact ECM proteins (e.g., RGD found in fibronectin, collagen, and vitronectin; REDV found in fibronectin; GTPGPQGIAGQRGVV (P-15) found in collagen) is an attractive approach to develop biomimetic niches that interact biomolecularly with the cells to control their function, guiding the spatially and temporally complex multicellular processes, and facilitating tissue regeneration [31–35]. The use of short cell-binding peptides is, however, advantageous over long-chain native ECM proteins as they are flexible, experience minimal steric effect, have usually lower immunogenicity, can be easily synthesized and purified at relatively low costs, and are more stable than large ECM proteins during the surface modification and sterilization processes [36]. Surface micro/nanopatterning by physical (photolithography) or chemical (microcontact patterning) means and hydrophilicity improvement by plasma treatment or graft copolymerization can also be used to improve the surface properties of polymeric scaffolds for various tissue engineering applications [37]. Bulk modification of polymers can be achieved through physical, chemical, photochemical, and ionic crosslinking of various cell adhesion peptides into three-dimensional polymeric networks [38–40].

Drug/Growth Factor Carrying Ability. Initially, polymeric materials were used only as support materials. However, the current understanding emphasizes the ability to provide additional functionality besides the bare capability to withstand mechanical loads or to possess suitable degradation kinetics. Scaffolds should, among other properties, guide cell adhesion and also provide the means to deliver growth and differentiation factors for long-term support of the proliferating adherent cells, which makes the polymeric carriers traditional drug delivery *matrices* for bioactive molecules. Achieving this "fusion" of cell carrier and release system may be a key factor for the development of new generations of tools and products based on tissue engineering principles. Consequently, today's tissue engineering *scaffolds* can be considered special types of drug/growth factor delivery *matrices*, which additionally possess pores or accessible regions for cell penetration [41]. Hence, factors such as loading capacity, desired drug/growth factor distribution, namely, uniform (for controlled release) or discrete (if the spatial patterning of release is to occur), optimal binding affinity for the drug for achieving desired release kinetics, and drug–polymer compatibility have become important deciding factors when choosing a polymer for a tissue engineering application.

Mechanical Strength. For all body tissues, except bone, the design requirement is such that the polymeric scaffold has to be relatively soft and pliable, yet relatively tough—properties rarely exhibited by materials based on metals or ceramics. Hence, future tissue regeneration scaffolds will have to be based predominantly on biodegradable polymers, and these will eventually become the most widely used biomaterials [42]. A three-dimensional architecture with a desired volume, shape, and mechanical strength sufficient to shield cells from damaging compressive or tensile forces (without inhibiting appropriate biomechanical cues) or matching those of the tissue at the implantation site. The mechanical properties of a tissue scaffold should be similar to those of the surrounding tissues. Though this transition from metals, ceramics, and engineering plastics to biodegradable polymers has been surprisingly slow, in the recent past, a paradigm shift has been observed toward the use of biodegradable polymers for various tissue engineering and biomedical applications. The major driving forces are the mechanical properties and long-term biocompatibility issues with many of the existing permanent implants and many levels of ethical and technical issues associated with revision surgeries [10].

16.2 BIODEGRADABLE POLYMERS USED IN TISSUE ENGINEERING

A wide variety of biodegradable polymers of both natural and synthetic origin have been used in various biomedical applications. Although these biodegradable polymers have been discussed in various sections of this book, we are elaborating only the biodegradable polymers used specifically for tissue engineering applications in the following section.

16.2.1 Natural Polymers

In the natural environment, polysaccharides play a vital role in membranes and intracellular communication, in storage, and protein functions as structural components and as catalysts [43]. Currently, the focus is to mimic the natural milieu; and the natural polymers provide an impressive and practical means for this. Regardless of the tissue/organ involved, natural polymers are applicable in the tissue engineering field to repair/regenerate the tissue/organ. A comprehensive review on use of polymers of natural origin in tissue engineering applications has been presented by Malafaya et al. [44] and Nair and Laurencin [10]. The following section briefly mentions natural polymers such as collagen, gelatin, fibrin, and alginate with major focus on their latest biomedical applications.

Protein-Based Polymers.

Collagen. Collagen is the most abundant protein (30% of all proteins) present in the human body, rendering strength and flexibility to connective tissues such as tendons, bones, cartilage, blood vessels, skin, and other musculoskeletal tissues. It has been extensively explored for an array of tissue engineering and biomedical applications owing to its mechanical, hemostatic, and cell-binding properties; it also exhibits excellent biocompatibility profile and is biodegradable. It is the major component of the ECM and serves as a natural substrate for cell attachment, proliferation, and differentiation. Gelinsky et al. [45] have reported mineralized collagen type-I-nanocomposite-based porous scaffold preseeded with human marrow stromal cells for bone tissue engineering. A Food and Drug Administration (FDA)-approved bilayer skin substitute Integra (Dermal Regeneration Template), is currently on the market for treatment of full thickness or deep partial thickness thermal injury; it is composed of a dermal layer of crosslinked bovine collagen and glycosaminoglycan (GAG) and an epidermal layer of polysiloxane [46]. Orcel and Apligraf are other FDA-approved collagen-based bilayer dressings seeded with live human keratinocytes and fibroblasts for the treatment of chronic ulcers. Hybrid scaffolds of collagen/poly(lactic-*co*-glycolic) acid (PLGA) have been reported for tracheal [47] and articular cartilage tissue engineering [48]. Duragen, is a suture-free, three-dimensional collagen matrix graft developed for spinal dural repair and regeneration has obtained 510(k) clearance from FDA [49]. Similarly, a composite of fibrillar collagen, hydroxyapatite, and tricalcium phosphate (Collagraft) has been approved by FDA for use as a biodegradable synthetic bone graft substitute. YIGSR (Tyr-Ile-Gly-Ser-Arg) peptide-modified collagen gels have been found to improve the adhesion and proliferation of corneal epithelial cells [50]. Several forms of collagen are currently being investigated as scaffolds for cardiovascular, musculoskeletal, and nervous tissue engineering [50].

Gelatin. Gelatin is derived by denaturing collagen and is hence free of antigenicity which is associated with collagen. Depending upon the conditions

(acidic/alkaline) under which collagen is processed, electrically different types of gelatin are obtained with a variety of isoelectric point values ranging from 9.0 to 5.0. It is theoretically possible for gelatin to form polyion complexes with any type of charged biomolecules, although the strength of the interaction depends on the type of biomolecules used. Due to its promising properties, gelatin mainly in the form of microspheres has used in drug delivery for tissue engineering applications targeting several tissues including bone, cartilage, and skin; but others such as adipose tissue have applied gelatin as carrier to deliver an active biomolecule to improve the temporary cell functions. The group of Mikos [51–53] together with Tabata [54, 55] have done vast work with gelatin, commonly using this strategy to incorporate a single biomolecule such as transforming growth factor-β1 (TGF-β1) [51] or in combination with insulin-like growth factor-1 (IGF-1) for dual release [52] as well as the encapsulation of marrow stromal osteoblasts in the surface of the gelatin microspheres [56, 57]. Modified dextran–gelatin hydrogels have shown excellent cell proliferation and network formation of encapsulated umbilical artery smooth muscle cells (SMCs) [58]. There are several commercially available gelatin-based carriers for drug delivery that are being applied in tissue engineering applications [59–61]. The most commonly used ones are Gelfoam commercialized now by Pfizer in United States (formerly Pharmacia and Upjohn), which is an absorbable gelatin sponge also available in powder form by milling the gelatin sponges. Liu et al. [62] have studied nanofibrous gelatin/apatite composite scaffold for bone tissue engineering and reported higher surface area and mechanical strength as compared to Gelfoam and showed enhanced osteogenic differentiation. PCL/gelatin biocomposite scaffolds have been found to exhibit properties most appropriate for nerve tissue engineering and exhibited enhanced nerve differentiation and neurite outgrowth [63].

Fibrin. Fibrin is a biopolymer that is derived from fibrinogen and is involved in the natural blood clotting, fibrinolysis, cellular and matrix interactions, inflammatory response, and wound healing. It is one of the earliest biopolymers used as biomaterials due to the excellent biocompatibility, biodegradability, injectability and the presence of several ECM proteins, such as fibronectin, that favorably affect cell adhesion and proliferation. Several allogenic fibrin sealants such as Tisseel, Evicel, and Crosseal have been approved by FDA for clinical application as hemostats. The most widely used forms of fibrin scaffolds are fibrin hydrogels, fibrin glue, and fibrin microbeads [64]. Due to its injectability and biodegradability, fibrin has also been investigated as a carrier vehicle for bioactive molecules and cells such as keratinocytes [65], urothelium cells [66], tracheal epithelial cells [67], murine embryonic stem cells [68], and mesenchymal progenitor cells [69], and also used to encapsulate chondrocytes for cartilage tissue engineering [70]. Fibrin and fibrin/alginate composites have been studied for chondrogenic differentiation of bone marrow stromal cells (BMSCs). Encapsulated BMSCs in fibrin differentiated into chondrocytes that secreted aggrecan and collagen II and thus the use of fibrin was advocated for

cartilage repair [71]. Bioseed is a fibrin-based product obtained by mixing keratinocytes with fibrin and is used to treat chronic wounds.

Silk Fibroin. Silk represents protein polymer spun into fibers by some *lepidoptera* larvae such as silkworms, spiders, scorpions, mites, and flies [72]. Spider silk is lightweight, extremely strong and elastic, safe, biodegradable, and exhibits mechanical properties comparable to the best synthetic fibers produced by modern technology [73]. Since domesticated spiders are difficult to maintain to produce massive amounts of silk; silk fibroin, a mass-producible natural polymer produced by silkworms, appears to be an attractive alternative. In the medical field, silk has long been used for surgical sutures [74]. Silks are attractive biomaterials for tissue engineering because of their biocompatibility [72, 75], slow degradability [76], and excellent mechanical properties. Degradable silk is a mechanically robust biomaterial that offers a wide range of mechanical and functional properties for biomedical applications including drug delivery [77, 78]. Hino and co-workers have reported a novel type of fibroblast growth factor (FGF) delivery system using fibroin as a scaffold [79]. Three-dimensional porous silk fibroin matrices fabricated by a rapid freeze-drying technique have been reported for tissue engineering applications by Mandal and Kundu [80]. A study of the recovery of Achilles tendon defect of rabbit using silk fibroin has shown feasibility of exploring silk fibroin for tendon engineering [81].

Other Protein-Based Polymers. Elastin and soybean are other attractive protein origin polymers that have been applied to a limited extent in the tissue engineering applications. Elastin is the principal mammalian ECM protein that imparts elasticity to tissues and is a key component of artery, lung, skin, elastic ligament, bladder, and elastic cartilage [82]. It is a highly crosslinked insoluble polymer composed of a number of covalently bonded tropoelastin molecules [83]. Elastin shows minimal interaction with platelets and hence has been evaluated as biological coatings for synthetic vascular grafts [84]. Synthetic elastin microfibers of crosslinked tropoelastin have also been studied for attachment and proliferation of three different primary cell types from elastic tissues and found to support their growth [85]. Elastin-like polypeptides (ELP) are artificial polypeptides composed of the pentapeptide repeats of human tropoelastin. ELPs have been found to have excellent biocompatibility, non-immunogenic properties, and degradation products composed of natural amino acids that are nontoxic. ELPs are also currently being investigated as potential biomaterials for cartilage tissue engineering [86].

Soybean has been the subject of scientific interest due to its richness in proteins (40−50%), lipids (20−30%), and carbohydrates (26−30%). It is a species of legume native that can be processed into three kinds of protein-rich products: soy flour, soy concentrate, and soy isolate, which varies in protein content. Soy protein is abundant, renewable, inexpensive, and biodegradable, making it an attractive source of degradable materials for tissue engineering

uses. Nevertheless, the application of soy-based polymers in this field is still very narrow. The use of soy-based polymers for drug delivery and tissue engineering applications is proposed by the group of Reis et al. [87–90].

Carbohydrate-Based Polymers. Polysaccharides are a class of biopolymers formed from many monosaccharide units joined together by glycosidic linkages. Physical properties of carbohydrates such as solubility, gelation, and surface properties are dictated by the monosaccharide composition, chain shapes, and molecular weight. These macromolecules exhibit good hemocompatibility, are nontoxic, and show unique biological functions ranging from cell signaling to immune recognition. With a few exceptions, they are also more economical in comparison with other biopolymers such as collagen. Polysaccharide-based polymers have been widely proposed as scaffold materials in tissue engineering applications as well as carriers for drug delivery.

Chitosan. Chitosan is a cationic linear polymer obtained from chitin comprising copolymers of $\beta(1\rightarrow4)$-glucosamine and randomly located *N*-acetyl-D-glucosamine. It is the fully or partially deacetylated form of chitin and has attracted much attention in tissue engineering with a wide variety of applications ranging from skin, bone, cartilage, and vascular grafts to substrates for mammalian cell culture. It has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, nontoxic, and biofunctional [91]. The degree of deacetylation of typical commercial chitosan is usually between 70 and 95%, and the molecular weight between 10 and 1000 kDa [92]. Chitosan is also a bioadhesive material. Biological activity of chitosan on bone regeneration has been demonstrated in many reports [93, 94]. Chitosan is structurally similar to GAGs found in extracellular matrices as in native articular cartilage and plays a key role in modulating chondrocytes morphology, differentiation, and function and hence could be explored for cartilage tissue engineering. In addition, chitosan was found to enhance blood coagulation [95] and accelerate wound healing [96, 97]. Thus it can act as an ideal wound dressing as it exhibits a positive charge, film-forming capacity, mild gelation characteristics, and a strong tissue adhesive property. Biomedical applications of chitosan and its derivatives have been reviewed recently by Jayakumar et al. [98] and Kim et al. [99]. Injectable composite hydrogel of chitosan/hyaluronic acid have also been studied for tissue engineering [100]. A chitosan-based bandage is also commercially available on the market (HemCon, HemCon Medical Technologies, Inc., USA) due to its wound healing properties.

Alginate. Alginates are naturally derived polysaccharide block copolymers composed of regions of sequential β-D-mannuronic acid monomers (M blocks), regions of α-L-guluronic acid (G blocks), and regions of interspersed M and G units and have a structural role in giving flexibility and strength to marine plants. Commercial alginates are extracted from brown algae *Laminaria hyperborean*, *Ascophyllum nodosum,* and *Macrocystis* [92]. Bacterial alginates

have also been isolated from *Azotobacter vinelandii* and several *Pseudomonas* species [92]. Alginates undergo reversible gelation in aqueous solution under mild conditions through interaction with divalent cations such as Ca^{2+}. This has led to their wide use as cell transplantation vehicles to grow new tissues, as wound dressings, and also in three-dimensional culture of chondrocytes (Table 16.3). Sequential delivery of growth factor bone morphogenetic proteins (BMP-2 and BMP-7) has been studied using complexed microspheres of poly(4-vinyl pyridine) and alginic acid for bone tissue engineering [101]. Alginate microbeads have also been used to deliver FGF-1 suspended in type I collagen gels and shown to induce neovascularization in a vascular pedicle model of adipose tissue engineering [102]. Several alginate based wound dressings are commercially available, namely, Nu-Derm commercialized by Johnson & Johnson, Curasorb by Covidien, formerly registered as Kendall, and AlgiSite by Smith & Nephew. Another interesting tissue engineering-driven commercial application of alginate is in the form of alginate beads used for plating freshly isolated cells (intervertebral disk, cartilage, bone, synovial tissue, and synovial fluid) marketed by Articular Engineering LCC for research purposes.

Hyaluronan. Hyaluronan/hyaluronic acid is a naturally occurring largest nonsulfated GAG and a major macromolecular component of the intercellular matrix of most connective tissues such as cartilage, vitreous of the human eye, umbilical cord, and synovial fluid [103]. Hyaluronic acid is a linear polysaccharide that consists of alternating disaccharide units of α-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine, linked by $\beta(1\rightarrow3)$ bonds [104]. It is most frequently referred to as hyaluronan due to the fact that it exists in vivo as a polyanion and not in the protonated acid form [103]. It is water soluble and forms highly viscous solutions with unique viscoelastic properties and hence works as an excellent lubricant and shock absorber in synovial fluid. Hyaluronan and its associated networks have many physiological roles that include tissue and matrix water regulation, structural and space-filling properties, lubrication, and a number of macromolecular functions [103]. Tissue engineering applications of hyaluronan has been mainly focused on cartilage, bone and osteochondral tissues, wound healing, and promoting angiogenesis. Hyaluronan is available for several purposes: for lubrication and mechanical support for the joints in osteoarthritis (Nuflexxa from Savient Pharmaceuticals, Inc., USA; Artz from Seikagaku Corporation in Japan), as a viscoelastic gel for surgery and wound healing (Bionect from JSJ Pharmaceuticals, USA), and for implantation of artificial intraocular lens (Healon from OVD from Advanced Medical Optics in the USA, Opegan R from Seikagaku in Japan, Opelead from Shiseido in Japan, and Orthovisc from Anika in the USA). Hyaff commercialized by Fidia in Italy represents a family of benzyl esters of hyaluronic acid with different alcohols and is used as a biomaterial for biomedical applications. Hyaff is biocompatible, biodegradable, and easily processable. A viscous formulation of HA containing fibroblast growth factor (Ossigel) is undergoing

a late-stage clinical trial as a synthetic bone graft to accelerate bone fracture healing.

Chrondroitin Sulfate. Chondroitin sulfate is one of the most important glycosaminoglycan (GAG) and consists of repeating disaccharide units of D-glucuronic acid and *N*-acetyl galatosamine sulfated at either the 4- or 6-position [105]. GAGs are vital constituents of the lubricating fluid of the joints and are also present in cartilage, synovial fluid, bone, and heart valves. With the exception of hyaluronan, these polysaccharides are covalently linked to a protein core, thereby forming proteoglycans. Furthermore, GAGs are practically nonimmunogenic and degrade to nontoxic oligosaccharides. Due to its GAG nature, chondroitin sulfate is an attractive natural origin polymer used particularly in cartilage tissue engineering [106]. Chondroitin sulfate can bind with core protein to produce highly absorbent aggregan, which is a major structure inside cartilage and acts as a shock absorber [105]. Also, in vitro studies suggest that chondroitin sulfate is able to increase matrix component production by human chondrocytes [107]. It can produce sydecan, which is a cell receptor that can interact with adhesion proteins, cells, and the ECM [105]. Furthermore, chondroitin sulfate proteoglycans have a critical role in regeneration and plasticity in the central nervous system as reviewed by Galtrey and Fawcett [108]. Chondroitin sulfate is also a component of the dermal layer of FDA approved skin substitute Integra used for treating burns [109].

Starch. Starch is a natural polysaccharide composed of α-amylose (20–30%) and amylopectin (70–80%). Its biodegradability, abundant availability, and renewable nature make it one of the promising natural polymers for use in tissue engineering applications. Starch per se is extremely difficult to process and is brittle when used without the addition of a plasticizer. Water is the most commonly used plasticizer with starch; at times, low molecular weight alcohols are also employed especially for the production of thermoplastic starches to render starch more processable [110]. Over the years several materials have been blended with starch to improve its processability. Reis and co-workers [111, 112] have proposed starch-based materials (blends of starch with different synthetic polymers, such as ethylene vinyl alcohol, poly(lactic acid) (PLA), cellulose acetate, and poly(ε-caprolactone) (PCL) as materials with potential for biomedical applications, such as scaffolds for bone tissue engineering applications [112, 113], bone cements [114, 115], and as drug delivery systems [112, 116, 117]. Bilayered constructs based on poly(L-lactide) (PLLA) and starch have also been proposed for tissue engineering of osteochondral defects [118]. Starch–PCL microparticles have been reported recently for controlled release of bioactive agents for drug delivery and tissue engineering applications [119].

Polyhydroxyalkanoates. Polyhydroxyalkanoates (PHAs) are biological polyesters produced by microorganisms under unbalanced growth conditions.

PHAs have gained major importance due to their structural diversity and close analogy to plastics, additionally these are also biodegradable and show good biocompatibility. For tissue engineering applications, mainly PHA including poly 3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly 4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx), and poly 3-hydroxyoctanoate (PHO) are available in sufficient quantity for research. Altering the compositions of PHAs allow favorable mechanical properties, biocompatibility, and degradation profiles. Ye et al. [120] have reported PHB/PHBHHx scaffolds as suitable materials to produce neocartilage upon seeding with adipose-derived stem cells (hASCs). PHB foams with high pore connectivity were fabricated, and bioactive glass in micrometer and nanometer size was introduced into the scaffold microstructure. This advanced composite scaffold proved suitable for MG-63 cell attachment and proliferation. When implanted in rat, it exhibited bioactivity, biocompatibility, and bactericidal properties, thus paving the way for the next generation advanced scaffolds for bone tissue engineering [121].

16.2.2 Synthetic Polymers

Poly(α-esters). Poly(α-ester)s represents a class of thermoplastic polymers that have been most explored for tissue engineering applications owing to their immense diversity and synthetic versatility. These have hydrolytically labile aliphatic ester linkages in their backbone and hence are biodegradable. Among the class of poly(α-ester)s, the most extensively investigated polymers are the poly(α-hydroxy acid)s, which include poly(glycolic acid) (PGA) and the stereoisomeric forms of PLA and their copolymers (PLGA) due to their good biocompatibility and controllable degradation rates tailored to specific demands of each tissue type.

Poly(glycolic acid). PGA is a highly crystalline (45–55% crystallinity), hydrophilic, linear aliphatic polyester. It has a glass transition temperature ranging from 35 to 40°C, high melting point (200°C), high tensile strength, and a relatively low solubility in most common organic solvents. Due to its excellent fiber-forming ability, it was initially investigated for developing resorbable sutures. The first biodegradable synthetic suture called DEXON was approved by FDA in 1969. Recently, Wang et al. [122] have studied cell proliferation, osteogenic differentiation, and matrix formation of human umbilical cord mesenchymal stromal cells (hUCMSCs) on three-dimesional (3D) PGA nonwoven mesh scaffolds and found an increase in cell density and differentiation of hUCMSCs along the osteogenic lineage. Composite scaffold of PGA and β-tricalcium phosphate (β-TCP) have exhibited the ability for osteogenesis, mineralization, and biodegradation for repair of critical bone defects in rat femoral medial-epicondyles [123]. However, its high rate of degradation, acidic degradation products, and low solubility limit its biomedical applications.

Poly(lactic acid). PLA contains an extra methyl group that makes it more hydrophobic, more amorphous, and more soluble in organic solvents than PGA. This group also imparts it chiral nature and lactide thus exists in two optically active forms; L-lactide and D-lactide. Poly(L-lactide) (PLLA) is also a crystalline polymer (37% crystallinity), it has a glass transition temperature of 60–65°C, and a melting temperature of approximately 175°C [124]. Poly (L-lactide) is a slow-degrading polymer compared to polyglycolide, has good tensile strength, low extension, and a high modulus (approximately 4.8 GPa), and hence is considered an ideal biomaterial for load-bearing applications, such as orthopedic fixation devices) [10]. Various research and marketed applications of PLA have been reviewed by Nair and Laurencin [10]. An electrospun composite tubular scaffold of PLA/silk fibroin has been reported for tissue engineering blood vessels owing to its biocompatibility, appropriate biomechanical strength, and support of cell growth [125]. Porous PLA/β-TCP composite scaffolds have also been developed and proposed for tissue engineering applications [126].

*Poly(Lactic-co-*Glycolic) Acid. Poly(α-hydroxyesters) have been widely copolymerized to yield polymers with desirable mechanical properties and degradation rates. One such copolymer is PLGA, which has been most extensively explored in drug delivery and tissue engineering applications. In the composition range of 25–75%, PLGA forms amorphous polymers. It has been reported that PLGA 50/50 degrades in approximately 1–2 months, 75/25 in 4–5 months, and 85/15 in 5–6 months [127].

Biodegradable sutures have been one of the earliest and successful application of PLGA: PuraSorb (80LA:20GA), Vicryl (10LA:90GA), Vicryl Rapid (an irradiated version of Vicryl with a faster rate of degradation), and PANACRYL are commercially available. Scaffolds made from PLGA have been used for tissue engineering of various target organs. Commercially available skin substitutes Dermagraft and Transcyte are PLGA based and used for skin regeneration during wound healing. PLGA has also been recently investigated as scaffold for neural tissue regeneration. Plasma polymerized allylamine treated PLGA scaffold particles when injected into the lesion cavity along with neural stem cells, integrated efficiently with the host tissue forming a primitive neural tissue, thus presenting an advanced method to enhance brain repair after stroke [128]. Also, micropatterned PLGA films conjugated with laminin and collagen have shown positive results in early-stage neurite outgrowth and elongation [129]. Hydroxyapatite-PLGA particularly electrospun PLGA fibers and nano-HA, is one of the most studied composite scaffolds for bone tissue engineering and has shown promising results due to its appropriate mechanical strength and tailored degradation rates [130, 131]. PLGA has been used to develop microtubular orientation-structured blood vessel and in vitro experiments showed good cell proliferation and anchorage along the direction of microtubes [132].

Poly(Caprolactone). PCL is a semicrystalline polyester of great research interest owing to its ease of processability and solubility in a wide range of organic solvents. It has a low melting point (55–60°C) and glass transition temperature (− 60°C) along with the ability to form miscible blends with a wide range of polymers [10]. Due to its slow degradation and biocompatibility, it offers an attractive material for long-term biomedical and drug delivery applications. Our lab has also reported surface-modified PCL microspheres as scaffold for tissue engineering [18, 133]. PCL electrospun nanofibers have also been reported as a scaffold for tissue engineering applications. Several copolymers of PCL have been reported with improved properties and faster degradation rates. Fibers of copolymer of PGA and PCL are commercially available as monofilament suture (Monoacryl). PCL/polyethylenimine (PEI) blend electrospun nanofibers have been investigated for tissue engineering applications [134]. Poly-L-lactic acid (PLLA) fibers embedded in a porous poly (caprolactone) matrix have been reported for guided cell–material interaction of marrow stromal cells and human osteoblasts (HOB) for bone tissue engineering [135].

Polydioxanone. Polydioxanone (PDO) is colorless, crystalline, biodegradable synthetic polyester that is used for biomedical applications, particularly in the preparation of surgical sutures. Polydioxanone suture (also called PDS) is a synthetic absorbable monofilament suture. In addition to sutures, PDS has also been investigated for several orthopedic applications as fixation screws for small bone and osteochondral fragments (Orthosorb Absorbable Pins) [136]. Other biomedical applications include orthopedics, plastic surgery, drug delivery, cardiovascular applications, and tissue engineering.

Polyorthoesters. Polyorthoesters (POE) are amorphous hydrophobic polymers containing hydrolytically labile, acid-sensitive, backbone linkages. These were developed by the ALZA Corporation (Alzamer) as hydrophobic surface-eroding polymers particularly for drug delivery applications. By using diols with varying levels of chain flexibility, the rate of degradation for these polymers, pH sensitivity, and glass transition temperatures can be controlled. Till date, four different classes of polyorthoesters have been developed (POE I, II, III, and IV). Few orthopedic applications of this class of polymers have been explored but major use of POEs has been limited to drug delivery systems [137].

Polyanhydrides. Polyanhydrides are a class of hydrolytically unstable surface-eroding polymers that are either aliphatic, aromatic, or a combination of the two [138, 139]. To obtain polyanhydrides with high mechanical strength for load-bearing applications, poly(anhydride-*co*-imides) have also been designed and investigated for bone tissue engineering, have been reported to be osteocompatible, and supported endosteal bone growth [140]. A copolymer of 1 : 1 sebacic acid and erucic acid dimer has been found to be useful as a

potential delivery vehicle for gentamicin (Septacin) in the treatment of osteomyelitis. A novel polyanhydride blend has been used to fabricate nerve guidance conduits to align and support regenerating cells, and these, when implanted subcutaneously in rats initiated fibrin matrix production and angiogenesis and were noncytotxic [141]. Readers are recommended to refer to literature reviews for details of hydroxy-fatty-acid-based polyanhydride [142] and poly(ester-anhydride) drug delivery systems [143, 144].

Polyurethanes. Polyurethanes (PURs) are generally prepared by the polycondensation reaction of diisocyanates with alcohols and amines. Biostable polyurethanes have been extensively investigated as materials for the preparation of long-term medical implants, especially cardiac pacemakers and vascular grafts due to their excellent biocompatibility, mechanical properties, and their synthetic versatility [10]. Attempts have been made to develop biodegradable polyurethanes. Biodegradable polyurethanes have recently been investigated as candidate materials for bone-regenerative medicine. Polyurethanes for biomedical engineering have been reviewed by Gunatillake and Meijs [145] and Guelcher [146]. Human-bone-derived cells (HBDCs) were seeded and cultured on polyurethane scaffolds in a bioreactor for 14 days and implanted subcutaneously into Severe Combined Immunodeficiency (SCID) mice for 4 and 13 weeks. It was also found that HBDCs implanted as a component of tissue-engineered product survived and retained their ability to produce the specific human bone ECM, which resulted in higher mechanical properties of the harvested explants when preseeded with HBDCs [147]. Polyurethane scaffolds have also been investigated for localized delivery of platelet-derived growth factor (PDGF) in rat skin excisional wounds where a biphasic release was seen for PDGF, and PUR/PDGF scaffolds showed almost complete wound healing with reepithelization at day 14 after implantation [148]. Biodegradable segmented L-tyrosine polyurethanes (LTUs) have been developed using a tyrosine-based chain extender desaminotyrosine-tyrosyl-hexyl (DTH) ester and proposed for biomedical applications as tissue engineering scaffolds [149].

PEG-Based Polymers. Polyethylene glycol (PEG) is a biocompatible and nontoxic polymer that has been mainly used to modify other polymers since it is known to reduce protein adsorption and modify polymer conformation. PEG hydrogels have been studied for biomedical applications in tissue engineering and delivery of growth factors [150, 151]. Enzymatically degradable PEG hydrogel scaffolds have shown an ability to direct cell differentiation of mesenchymal stem cells to smooth muscle cells and circumvent a major problem related to scar formation during bladder reconstruction [152].

There have been concerns regarding use of PLA as tissue engineering scaffolds due to its hydrophobic surface and dimensional shrinkage during degradation. Hence, PEG–PLA copolymers have been explored for biomedical applications. Differentiation of marrow stromal cell to the osteoblastic phenotype was evaluated on PEG–PLA scaffolds compared to PLA, PGA, and

polystyrene scaffolds, marked improvement was seen as measured by two-fold increase in alkaline phosphatase activity, and mineralization [153]. Blends of electrospun PEG–PLA have also shown positive response toward the biological activities of seeded human dermal fibroblasts (HDFs) and enhanced cell growth within fibrous mats [154]. Strehin et al. [155] have recently reported a pH-sensitive chondroitin sulfate–PEG tissue adhesive and hydrogel with several potential biomedical applications specifically in wound healing and regenerative medicine. The hydrogel supported the cell viability and produced minimal inflammatory response when implanted subcutaneously in a rat model.

Pluronic F-127 is a commercial name for a copolymer of polyethylene oxide and polypropylene oxide. It is a thermosensitive, biocompatible hydrogel and is FDA approved for use in humans [156] and also investigated for cartilage and lung tissue engineering [157–159]. Vashi et al. [156] developed a three-dimensional culture system using bone-marrow-derived mesenchymal stem cells (BM-MSCs) and Pluronic F-127 hydrogel scaffold and found BM-MSCs differentiated into adipocytes in Pluronic F-127 in the presence of adipogenic stimuli over a period of 2 weeks, with some differentiation present even in the absence of such stimuli. Recently, a biomimetic hydrogel has also been developed using Pluronic F-127 and fibrinogen and reported to be biocompatible and aiding cell-signaling through fibrinogen backbone [160].

Polyfumarates. Poly(propylene)fumarate (PPF) is a linear polyester whose repeating units contain two ester bonds and one unsaturated carbon–carbon double bond. It undergoes bulk erosion via hydrolysis of its ester bonds, and the degradation products formed are primarily fumaric acid and propylene glycol. The double bonds in PPF allow the polymer to be crosslinked thermally or by photoinitiator into a solid, polymeric network. These networks are mechanically strong, biocompatible, and biodegradable [161, 162]. Polyfumarates suffer a limitation particularly with respect to bone tissue engineering, that is, lack of mechanical strength due to flexible C–O–C region in its backbone. Hence, several strategies have been devised that include incorporation of ceramics [162, 163] or nanoparticles [164] in them. Several studies have proved the potential of crosslinked PPF alone or its composites for bone tissue engineering. Composites of PPF with ceramics such as tricalcium phosphate or calcium sulfate create high-strength matrices and hence have been reported to be suitable for orthopedic applications [162, 163].

Other polyfumarate-based polymers, namely poly(caprolactone fumarate) and poly(ethyleneglycol fumarate), have also been developed into injectable systems and termed as "self-crosslink," as no crosslinking agent is required, but a photoinitiator and accelerator are required [165]. Mikos and co-workers have done considerable work on fumarates and reported their significance in tissue engineering [51–53, 56, 57, 166]. Their group has designed novel oligo[poly (ethylene glycol) fumarate] (OPF) hydrogel composites containing embedded chondrocytes and TGF-β1-loaded gelatin microparticles for cartilage tissue engineering [53].

Poly(amino acids). Poly(L-glutamic acid) is composed of naturally occurring L-glutamic acid residues linked together through amide bonds. It is degraded into monomeric L-glutamic acid by lysosomal enzymes, thus making it an ideal candidate as biodegradable biomaterial. The polymer is highly charged at physiological pH and has been identified as a unique gene/plasmid delivery vehicle [167]. Chang et al. [106] have recently studied poly(γ-glutamic acid)-graft-chondroitin sulfate-blend-poly(ε-caprolactone) (γ-PGA-*g*-CS/PCL) composite biomaterial as a scaffold for cartilage tissue engineering and reported excellent biodegradation and biocompatibility for chondrocytes and its potential in tissue engineering as temporary substitutes for articular cartilage regeneration.

Poly(aspartic acid) (PAA) is synthesized from aspartic acid by thermal polymerization. PAA is a highly water-soluble ionic polymer with a carboxylate content much higher than poly(glutamic acid) [10]. Chemically modified PAA are also being considered as potential biomaterials. PAA has also been used to modify poly(D,L-lactide) (PDLLA) films to enhance the cell affinity and found to enhance the interactions between osteoblasts and PDLLA films [168].

Poly(L-lysine) is a small polypeptide of the essential amino acid L-lysine. Recently, the poly-L-lysine-coated PLGA microspheres containing retinoic acid have been explored for nerve tissue engineering. Embryonic carcinoma cells were seeded on them and found to exhibit differentiation into neural cells [169].

16.3 MAJOR APPLICATIONS OF BIODEGRADABLE POLYMERS IN TISSUE ENGINEERING

16.3.1 Scaffolds to Support Cellular Activities and Induce Tissue Regeneration

Scaffolds are central components of many tissue engineering strategies because they provide an architectural context in which ECM, cell–cell, and growth factor interactions combine to generate regenerative niches. There is a significant challenge in the design and manufacture of scaffolds that possess both: a highly porous structure and the ability to control the release kinetics of growth factors over the period of tissue regeneration [137]. Biodegradable polymeric scaffolds for tissue engineering have received much attention since they provide a temporal and spatial environment for cellular growth and tissue growth [14].

Three-dimensional culture on scaffolds has a long history, starting with the culture of chick embryo heart tissue on silk veil, followed by the introduction of sponge matrices for the culture of tissue [170]. In 1969, it was observed that bone formed when pieces of a synthetic sponge made out of polyhydroxyethyl methacrylate (poly-HEMA) were implanted into the skin of young pigs [171]. Since then, the idea of using bioengineered three-dimensional scaffolds for in vitro cell culture as well as for in vivo tissue replacement has received increasing

attention and is today the most promising approach to mimic the complex three-dimensional cellular structure of living tissues [172].

Three-dimensional cell culture matrices/scaffolds can overcome monolayer culture limitations for clinical-scale cell expansion, such as lack of structural architecture, finite material selections, surface area, and unique ECM for each cell type and thus support cell growth, organization, and differentiation on or within their structure [109]. Various cell culture matrices currently being explored include hydrogels, microparticles, fibers, self-assembling peptides, three-dimensional porous scaffolds in the form of meshes, spheres, foams, and sponges [18, 173–179]. Various techniques have been used for fabricating biodegradable polymers into three-dimensional porous scaffolds. The conventional methods include fiber felts, fiber bonding, melt molding, solvent casting/particulate leaching, membrane lamination, gas foaming/particulate leaching, emulsion freeze-drying, hydrocarbon templating, thermally induced phase separation, and high-pressure processing [14, 179–185]. Electrospinning has also been utilized in producing a nanofibrous 3-D matrix. Complex scaffold architecture designs generated using hierarchical image-based or computer-aided design (CAD) techniques cannot readily be built using conventional techniques. Instead, scaffold architectures must be built using layer-by-layer manufacturing processes known collectively as solid freeform fabrication (SFF) or rapid prototyping technologies. SFF systems as categorized by the processing technique include laser-based processing systems, printing-based systems, and nozzle-based systems. Laser-based systems include the stereolithography system, which photopolymerizes a liquid and the selective laser sintering (SLS) system, which sinters powdered material. In each system, material is swept over a build platform that is lowered for each layer. Printing-based systems include three-dimensional printing, which prints a chemical binder onto a powder bed, and wax printing machine, which prints two types of wax materials in sequence. Nozzle-based systems process material either thermally or chemically as it passes through a nozzle. The fused deposition modeler prints a thin filament of material that is heated through a nozzle. The Bioplotter, the only commercial machine developed to print biological cells as well as a range of biomaterials, prints material that is processed either thermally or chemically [186–191].

16.3.2 Delivery Carriers for Drugs or Growth Factors

Though scaffolds play an important role as support matrices, they are often unable to create the exact/correct microenvironment during the engineered tissue development to promote the accurate in vitro tissue development. The emerging and promising next generation of engineered tissues is relying on producing scaffolds with an informational function, for example, material containing growth factor sequence that facilitates cell attachment, proliferation, and differentiation that is far better than noninformational polymers. The use of growth factors has been considered as a way to manipulate not only the

host healing response at the site of injury to facilitate the tissue repair but also to manipulate and improve the in vitro tissue growth in order to produce more biofunctional engineered tissues. Various chemoattractants, growth factors, and cytokines have been used to draw desired cell types into structure growth factors, induce cell proliferation to regenerate tissue, and to induce tissue-specific cell functions, respectively. Hence, the strategy is to mimic the matrix and provide the necessary information or signaling for cell attachment, proliferation, and differentiation to meet the requirement of dynamic reciprocity for tissue engineering.

Classically, single proteins have been delivered as either bolus injections into the site of disease or by systemic administration. This strategy is limited because the inherent instability of many proteins in vivo requires very high levels of protein for a measurable effect, and the potential exists for uncontrolled activities at distant sites [192]. Currently, various delivery systems used in tissue-engineered devices incorporate growth factors either directly in the scaffold (dispersed/dissolved) or after fabrication, and release occurs as the scaffold degrades to induce tissue regeneration. Conventionally, these delivery systems are of matrix or reservoir type. Further, the release may occur by a diffusion-controlled mechanism, erosion mechanism, or a combination of both. Factors affecting the release include those related to scaffold (pore size and tortuosity), polymer (type, molecular weight, rate, and mechanism of degradation) and growth factor (solubility, rate of diffusion through the pores of the scaffold) [193]. Depending on device and application, the growth factor type(s), dosage, release pattern (constant, pulsatile, and time programmed), spatial distribution and kinetics of release, and duration of delivery need to be optimized. Successful delivery of growth factor(s) requires targeting responsive cells, at the required pharmacological concentration, while maintaining the stability of the active form of the growth factor(s). Cell responsiveness is determined by their level of growth factor receptor expression and would clearly determine the effectiveness of an appropriately delivered factor.

The limited success of current efforts may be related not only to mode of growth factor delivery but also to the requirements for multiple signals to drive the regeneration process to completion [194]. The lack of delivery vehicles that allow for a localized and controlled delivery of more than a single factor is a major constraint on delivering appropriate combinations of factors for successful tissue engineering. Dual delivery of growth factors, namely vascular endothelial growth factor (VEGF)-165 (as an initiator of angiogenesis) and platelet-derived growth factor (PDGF)-BB (for promotion of maturation of blood vessels), each with distinct kinetics, from a single, structural polymer scaffold were reported for the first time by Richardson et al. [194]. Holland et al. [52] reported an injectable, biodegradable scaffold for simultaneous delivery of IGF-1 and TGF-β1 to injured cartilage tissue in a controlled manner using gelatin microparticles encapsulated in water-soluble polymer, OPF. Table 16.1 lists a few commercially available products where polymeric carriers have been used for delivery of various drugs and growth factors.

TABLE 16.1 Biodegradable Polymer-Based Products as Drug Carriers in Tissue Engineering[a]

Polymer	Product	Description and Uses	Manufacturer	Status
Multiblock copolymer of CL, LA, GA, PEG	SynBiosys	Drug delivery coatings for small and medium sized biologically active molecules	InnoCore Technologies BV	M
Poly(ester amide) blend	CAMEO	Site-specific delivery of small hydrophobic drugs and peptides	Devax (Irvine, CA)	Patent
Polyanhydride	Septacin	Delivery vehicle made of 1 : 1 sebacic acid and erucic acid dimer for gentamicin for treatment of osteomyletis	Chinoin (Mexico)	M
Collagen	Sulmycin Implant-Schwamm	Gentamicin delivery for chronic osteomyelitis therapy	Aesca Pharma GmbH, 1120 Wien	M
	COLLATAMP, Collatamp G, Collatamp EG, Sulmycin Implant, Garamycin Schwamm, Duracoll, Garacoll, Garacol, Gentacoll, Gentacol, Cronocol	Lyophilized collagen implant impregnated with the aminoglycoside antibiotic gentamicin; used in local hemostasis of capillary, parenchymatous, and seeping hemorrhages in areas with a high risk of infection; wound healing	Eusa Pharma (Europe) Ltd.	M
	Septocoll	Resorbable equine fleece used as hemostatic incorporating two gentamicin salts having different solubilities	Biomet, Inc.	M
	INFUSE	Collagen matrix for use with rhBMP-2 used in Lumbar inter body spinal fusion, dental applications	Medtronic Sofamor Danek, USA	M, 2007

(*Continued*)

TABLE 16.1 (Continued)

Polymer	Product	Description and Uses	Manufacturer	Status
	Collagraft	Collagen matrix for use with rhBMP-2 matrix, HA and TCP used for acute long bone fractures and osseous defects	Collagen Corp.	M, 1994
	OP-1 Putty	Recombinant human osteogenic protein (rhOP-1), Type I bovine bone collagen matrix and carboxymethylcellulose sodium (CMC); used in revision posterolateral (intertransverse) lumbar spinal fusion	Stryker Biotech, USA	M, 2004
	OP-1 implant	Genetically engineered OP1 protein (rhBMP-7) in Type 1 bovine collagen carrier; recalcitrant long bone nonunions	Stryker Biotech, USA	M
	Trinam	Local drug delivery device (termed EG001) for VEGF gene by an adenoviral vector; hemodialysis graft access surgery	Ark Therapeutics Oy, Finland	Phase III
SIBS	TAXUS Express2 coronary stent system	Paclitaxel-eluting polymer-coated stainless steel stent for atherosclerosis treatment	Boston Scientific Corporation, USA	M, 2004
PEVA and PBMA	CYPHER coronary stent	Sirolimus-eluting polymer-coated stainless steel stent for atherosclerosis treatment	Cordis Corporation, Florida	M, 2003

[a]Abbreviations: SIBS, styrene–isobutylene–styrene triblock copolymer; PEVA, poly(ethylene-*co*-vinyl acetate); PBMA, poly(*n*-butyl methacrylate).

Recently, gene delivery in the form of naked DNA, plasmid DNA, small inhibitory ribonucleic acid (SiRNA) releasing scaffolds has been applied as a versatile and promising approach for manipulating the local environment for directing cell function in various tissue engineering applications. The first papers to address the issue of difficulty in delivering and sustaining release of peptide-based growth factors rationalized that cells grown on a polymer scaffold could be transfected with growth-factor-encoding plasmid that was released from the scaffold as it degraded. The transfected cells could then synthesize their own growth factors, leading to enhanced viability and proliferation [195]. Plasmid DNA was incorporated into porous scaffolds fabricated from PLGA polyesters that varied in their molecular weight and lactic/glycolic acid ratios (to modulate the rate of polymer degradation). The mechanical properties of the scaffold can also influence the efficiency of gene transfer to seeded cells. DNA transfer to cells in culture can also be improved by DNA delivery from the cell growth substrate. Synthetic gene delivery platforms typically encompass three length scales—nano, micro, and macro—depending on what is the desired cell type, anatomical site, or clinical application. Nanoscale delivery vectors are generally polycationic polymers or lipids that self-assemble with the polyanionic nucleic acids to form polyelectrolyte complexes that are internalized by endocytic pathways in somatic cell lineages. Microscale delivery systems usually consist of DNA entrapped within a polymer matrix for DNA vaccination or local DNA delivery. Macroscale systems are two-dimensional or three-dimensional scaffolds designed to deliver DNA to a population of cells proximal to the scaffold surface, for tissue engineering, and other applications [196].

16.3.3 Biologically Functional Device Components

Biodegradable polymers have also been used as components of biologically functional devices. Some of the applications include their use in multifunctional devices as sensing/stimulating elements (conducting polymers) [197], as non-thrombogenic surfaces [198] or diffusional barriers, as a temporary barrier to protect a space of tissue regeneration from the host, and as bioartificial organs [199–201]. Table 16.2 presents a list of commercially available functional devices based on biodegradable polymers.

When a body defect is generated, the defect space will be soon filled with the fibrous tissue produced by fibroblasts, which are ubiquitously present in the body and can rapidly proliferate. Once this ingrowth of fibrous connective tissue into the space takes place, more tissue repair or regeneration cannot be expected. To prevent tissue ingrowth, biomedical materials called "barrier membranes" are used. The objective of membranes is to make space for tissue regeneration and prevent the undesirable tissue ingrowth, permiting repair of the defective tissue. The examples include guided channel for broken peripheral nerve fibers and guided regeneration of lost periodontal tissues and alveolar bone [202, 203].

TABLE 16.2 Biodegradable Polymer-Based Functional Devices in Surgical Applications

Product	Description	Indication	Manufacturer	Status
Adhesion Barriers				
Degrapol	Porous poly(ester urethane) scaffold	Tissue engineering applications	Via Nerviano, Italy	R&D
REPEL-CV	Film composed of poly-lactic acid and polyethylene glycol	Temporary barrier within the chest cavity that prevents adhesions from forming	SyntheMed, Inc.	M, 2009
AMPLATZER septal occluder	Expandable double disk made from wire mesh and polyester fabric	Used in multiple hole Atrial Septal Defects	AGA Medical Corporation	M, 2001
GYNECARE INTERGEL	Thick liquid (gel) made of sodium hyaluronate and iron	Used during gynecological surgery to separate and protect tissues as they heal, preventing adhesions	Lifecore Biomedical, USA	M, 2001
Seprafilm adhesion barrier	Hyaluronic acid and carboxymethylcellulose	Reduces adhesions in abdominopelvic laparotomy	Genzyme Biosurgery, Cambridge, MA	M
ADEPT	Icodextrin solution (4%)	Reduces adhesion formation following gynecologic adhesiolysis	Baxter, USA	M

(*Continued*)

TABLE 16.2 (Continued)

Product	Description	Indication	Manufacturer	Status
Sealants				
CoSeal surgical sealant	Polyethylene glycols (PEGs)	Used in peripheral vascular reconstructions to achieve adjunctive hemostasis by mechanically sealing areas of leakage	Cohesion Technologies, Inc, USA	M, 2001
Gelfoam	Purified pork skin gelatin granules	Hemostat in surgical procedures	Pharmacia & Upjohn Company, USA	M
TISSEEL	Fibrin sealant	Adjunct to hemostasis in surgeries involving cardiopulmonary bypass and treatment of splenic injuries due to blunt or penetrating trauma to the abdomen; an adjunct for the closure of colostomies	Baxter Healthcare Corp	M
EVICEL	Fibrin sealant (human)	An adjunct to hemostasis for use in patients undergoing liver or vascular surgery	OMRIX Biopharmaceuticals Ltd, Israel	M, 2008
Crosseal	Fibrin sealant (human)	An adjunct to hemostasis for use in patients undergoing liver or vascular surgery	OMRIX Biopharmaceuticals Ltd., Israel	M
HemCon	Chitosan	Hemostatic bandage	HemCon Medical Technologies Inc., USA	M

Another major application for use of biodegradable polymers is their use as adhesion barriers. An ideal adhesion prevention barrier is a material, agent, or substance that would prevent drying, necrosis, or inflammation of the tissue. It should prevent tissue surfaces from contacting, prevent bleeding from tissue surfaces, and prevent blood clot formation from sticking tissue planes together. The material should not provide a matrix for fibroblasts to infiltrate and bridge the gap between tissue surfaces. Hydrophilic polymers such as hyaluronic acid, carboxymethylcellulose, and dextran are used in the form of dilute solutions for reducing adhesion in various surgical interventions. Recently, Repel-CV, a film composed of PLA and PEG has been commercialized by SyntheMed, Inc. for use as a temporary adhesion barrier. PEGs have also been used in peripheral vascular reconstructions to achieve adjunctive hemostasis by mechanically sealing areas of leakage, for example, CoSeal. A self-polymerizing sealant consisting of PEG ester and trilysine amine solutions has recently been approved by the FDA as a spine sealant following dura mater surgery.

Engineered organs made by substitution of organ functions by the use of allo- or xenogeneic cells are called bioartificial organs because they are composed of heterogeneic cells and manmade membranes or porous constructs for immunoisolation to protect the cells from host attack and maintain the cell function. Liver, kidney, and pancreas are target organs that have attracted much attention of researchers on bioartificial organs. Recent successes in harvesting and expanding cells in vitro and the development of biologically active scaffolds may allow the creation of functioning renal units that can be applied for partial or, eventually, full replacement of organ function.

16.4 CLINICAL APPLICATIONS

16.4.1 Skin and Cosmetic Surgery

Skin-engineered products have applications in full thickness burns, chronic wounds, aesthetic surgery, and cosmetic dermatology. Extensive burns cause the most serious injury to the human skin. The deep skin wounds of a large area must be permanently covered as soon as possible in order to optimize the healing and maximize survival. To achieve this, various skin substitutes in the form of hydrogels, membranes, sponges, fibrous meshes, and the like have been proposed to cover full-thickness skin defects. These may be fabricated using synthetic or natural biopolymers such as collagen, elastin, chitosan, polyglactin, and biodegradable polyesters [204]. Collagen sponges, dressings, acellular collagen matrix from a human cadaver, and drugs (antibiotics such as gentamicin and local anesthetics, e.g., lidocaine) containing collagen products are also available for wound dressing, surgery, and cosmetic dermatology (Table 16.3).

The concept of drug and growth factor loading into the scaffold or carrier gel have improved the therapy pattern by reducing infection at the site of injury, for

TABLE 16.3 Biodegradable Polymer-Based Products for Skin Tissue Engineering[a]

Product	Details (Cells)	Indication	Manufacturer	Status
Collagen				
APLIGRAF	Bovine collagen bilayer dressing (AF, K)	Diabetic and venous static ulcers	Organogenesis Inc., USA	M
OrCel	Bovine Type 1 collagen sponge (AF (dermal), K (epidermal))	Diabetic and venous stasis ulcers	Forticell Bioscience Inc., USA	M
FortaFlex Technology, FortaGen, FortaPerm, CuffPatch, BioSTAR, FortaDerm Antimicrobial	Bioengineered collagen for guided tissue regeneration	Wound closure	Organogenesis Inc., USA	M
Integra	Dermal layer: crosslinked bovine collagen and GAG, epidermal layer: polysiloxane	Full-thickness wounds	Integra LifeSciences Corporation, USA	M, 2002
Promogran	Dressing collagen and oxidized regenerated cellulose	Diabetic and venous static ulcers	Johnson & Johnson Medical, UK	M
Biobrane	Acellular collagen matrices obtained from chemically processed human cadavers	Biosynthetic wound dressing	Smith & Nephew, UK	M
Alloderm	Acellular matrices obtained from chemically processed human cadavers	Hernia repair and breast reconstruction	LifeCell Corporation	M
TransCyte	Nylon mesh coated with porcine dermal collagen and bonded to a polymer membrane (silicone) [AF (human)]	Full-thickness wounds	Smith & Nephew, UK	M
CosmoDerm 1& 2, CosmoPlast	Human collagen containing lidocaine	Aesthetic surgery, cosmetic dermatology	Inamed Corporation, USA	M, 2003
ArteFill	Polymethylmethacrylate beads, collagen, and lidocaine	Aesthetic surgery, cosmetic dermatology	Artes Medical, Inc.	M, 2006

(*Continued*)

TABLE 16.3 (Continued)

Product	Details (Cells)	Indication	Manufacturer	Status
Hyaluronic acid				
Hyalograft 3D	HA ester biopolymer scaffold (AF)	Nonhealing ulcers, burns (dermal graft)	Fidia Advanced Biopolymers, Italy	M
HYAFF	Esterified derivatives like ethyl/benzyl esters	Wound dressing application	Fidia Advanced Biopolymers, Italy	M
Laserskin	HA ester biopolymer scaffold with orderly arrays of laser-drilled microperforations (AF)	Skin wounds, ulcers (epidermal graft)	Fidia Advanced Biopolymers, Italy	M
Perlane Injectable Gel	Gel particles of HA	Aesthetic surgery, cosmetic dermatology	Medicis Aesthetics Holdings Inc., USA	M, 2007
Cosmetic Tissue Augmentation Product	HA gel with 0.3% lidocaine	Aesthetic surgery, cosmetic dermatology	Anika Therapeutics, Inc., USA	M, 2006
Juvéderm 24HV, Juvéderm 30 and Juvéderm 30HV Gel	HA gel	Aesthetic surgery, cosmetic dermatology	Inamed Corporation, USA	M, 2006
Restylane Injectable Gel	HA gel	Aesthetic surgery, cosmetic dermatology	Medicis Aesthetics Holdings Inc., USA	M, 2005
Hylaform	HA gel	Dermal filler	Genzyme Corporation, USA	M, 2004
TISSUEtech autograft system	HA ester biopolymer scaffold (AF, K)	Burns, chronic ulcers, loss of skin tissue	Fidia Advanced Biopolymers, Italy	M

(*Continued*)

TABLE 16.3 (Continued)

Product	Details (Cells)	Indication	Manufacturer	Status
PLGA				
Dermagraft	PLGA-degradable polymer (AF)	Treatment of full-thickness diabetic and venous stasis ulcers	Advanced Tissue Sciences/ Smith and Nephew, UK	M
Vicryl Mesh	PLGA-degradable polymer (scaffolding material for Dermagraft)	Skin replacement materials and duramater substitutes	Ethicon, Inc., Johnson & Johnson	M
Sculptra, Sculptra Aesthetic	Injectable microparticles of poly-L-lactic acid	Restoration or correction of facial fat loss or lipoatrophy in people with HIV	Dermik Laboratories; Sanofi-Aventis USA	M, 2004
Fibrin				
Bio-Seed M, Bio-Seed S	Fibrin-based gel-like biomatrix (Autologous oral mucosal cells, K)	Defects of the oral mucosa, aesthetic surgery, cosmetic dermatology	BioTissue Technologies, Germany	M
Acu-Dress	Fibrin to be combined with Ethicon's Integra template (Autologous epidermal sheet)	Burns; plastic reconstructive surgery and other wound conditions	IsoTis SA, CH/NL	Phase I
Polysaccharide Based				
Chitech	Films and applications partly coated with bioactive compounds	Wound healing	Medicarb, Sweden	Phase II
Nu-Derm	Alginate	Wound dressing	Johnson & Johnson, USA	M
Curasorb	Calcium alginate	Diabetic and venous static ulcers	Covidien AG	M
AlgiSite* M	Calcium alginate	Diabetic and venous static ulcers	Smith & Nephew, Inc. USA	M

[a]Abbreviations: AF, allogenic fibroblasts; K, keratinocytes; GAG, glycosaminoglycan; HA, hyaluronic acid.

example, Collatamp. Collagen, hyaluronic acid, and fibrin-based gels are also used as carriers for the fibroblast cells so that wound healing at the injury site can be accelerated. Collagen itself has very poor dimensional stability due to its fast degradation when being contacted with body fluid. Therefore, collagens are often incorporated into some other biopolymers for improved strength and tailored degradation rates of the resultant blends or composites. Besides collagen, another biopolymer and synthetic biodegradable polymers such as chitosan, PLGA, and hyaluronic acid are also widely used in skin tissue engineering. Mainly, collagen, chitosan, chitosan-*g*-caprolactone [204], PLGA [205], poly(ethyleneglycol terephthalate)–poly(butylenes terephthalate) (PEGT/PBT) [206] are being explored in various forms. Biomaterials used as skin grafts play a role in providing epidermal cover, dermal replacement, and epidermal/dermal replacement. Contraction of skin grafts [207], sterilization, neovascularization of tissue-engineered skin, and fibrosis of the skin grafts are some of the major challenges for the skin tissue engineering [208].

16.4.2 Bone, Dental, Oral, and Craniofacial Applications

Bone is a dynamic and highly vascularized tissue that continues to remodel throughout the life of an individual and poses high regenerative capacity. Despite this, large bone defects, as observed after bone tumor resections and severe nonunion fractures, lack the template for an orchestrated regeneration and require surgical intervention [209]. Bone graft materials are used in a variety of clinical orthopedic procedures, including spinal fusion, total joint replacement, trauma, delayed unions, nonunions, oral and craniofacial, and other void and defect-filling applications.

The drive to create synthetic alternatives to conventional bone grafts has led to the development of several clinically available implants. Table 16.4 shows a list of some of the more common synthetic bone replacement materials. Bone replacement implants vary in composition, which include ceramics, polymers, and natural materials such as collagen. The main types of bone grafts currently used in the clinical setup may be broadly classified as biological (autografts, allografts, and xenografts) or synthetic, based on their origin. An autograft is a section of bone taken from the patient's own body, whereas an allograft is taken from a cadaver. Autologous iliac crest bone graft (ICBG) is considered as the gold standard for bone repair. Xenografts are bone tissues obtained from other species. This method of grafting provides the defect site with structural stability and natural osteogenic behavior. However, both types of grafts are limited by certain uncontrollable factors. For autografts, the key limitation is donor site morbidity in which the remaining tissue at the harvest site is damaged by removal of the graft. It may be manifested as infections, seromas, hematomas, herniation, vascular and neurological injuries, and donor site fractures. Other considerations include the limited amount of bone available for harvesting and unpredictable resorption characteristics of the graft [210, 211]. A limitation of some allografts and xenografts has been the immunogenic

TABLE 16.4 Biodegradable Polymer-Based Products for Bone Tissue Engineering

Product	Description	Indication	Manufacturer	Status
Collagen				
HEALOS	Hydroxyapatite-coated collagen microfibers	Bone repair	DePuy Spine, Inc., USA	M
Hyaluronic Acid				
Ossigel	Injectable formulation of basic fibroblast growth factor (bFGF) with a hyaluronic acid (HA) gel	Bone fracture healing	Orquest, Johnson & Johnson	M
Synthetic Polymer				
IMMIX Extenders and Immix CB94	Porous PLAGA particles (75/25)	Bone-void filler	Osteobiologics, Inc.	510(K)
OPLA	Porous poly(lactic-*co*-glycolic acid)	3D porous (90%) scaffolds	Becton–Dickinson (BD) Inc., USA	M
Biofix	Polyglycolic acid	Bone internal fixation device, Pins	Bioscience, Inc., USA	M
OrthoSorb pins	Polydioxanone (PDS)	Fixation screws for small bone and osteochondral fragments	DePuy Ace	M
Acufex bioresorbable screws	Polyglyconates (block *co*-polymers of trimethylene carbonate and glycolides)	Orthopedic tacks and screws	Smith & Nephew, Inc. USA	M
Alginate				
Biora AB	Enamel matrix proteins in propylene glycol alginate	Wound healing after periodontal surgery	Straumann, Norway	M

response to the foreign tissue of the graft [212]. The tissue is often rejected by the body and is subject to an inflammatory reaction. These grafts are also capable of transmitting diseases.

Hence, a need has arisen for developing new bone regenerative strategies where synthetic bone materials such as calcium phosphate, hydroxyapatite, and polymers are being used for the treatment. These tissue-engineered products are bone fillers, bone grafting materials, and cements that primarily include osteogenic, osteoconductive, and osteoinductive elements for rapid bone healing [213]. Polymers of natural source, such as collagen, hyaluronic acid, chitosan [214], and alginates [59] are widely used and provide biological informational guidance to cells thus favoring cell attachment and promoting chemotactic response. Apart from this, synthetic polymers such as PCL [215], PLGA [216], PLA, PGA, polyfumarates, and PEG have been explored in various alternative forms as drug delivery carriers and as scaffolds for bone tissue engineering. Additionally, composite materials, which involve use of inorganic-organic composites aiming to mimic the composite nature of the real bone, are also widely explored as they combine the toughness of the polymer phase with the compressive strength of an inorganic one to generate bioactive materials with improved mechanical properties and degradation profile [62, 123, 161, 210].

Hydrogel, nanocomposites, biofunctionalized porous scaffolds such as fibers, and polymeric microparticles are used as carrier systems. Collagen is widely used as the component for bone tissue engineering. In past decades the concepts of using bioactive scaffold and loading bioactive agents such as growth factors, gene vector, and loading the cells such as MSCs and differentiated cells within the scaffold are preferred for enhanced bone healing. TGF-β1, BMP-2, BMP-4, BMP-7, FGF-2, and PDGF are some of the commonly used growth factor loads in the carrier systems for bone formation and dental treatment [217, 218]. BMP stimulates endochondral ossification and chondrogenesis of mesenchymal stem cells, and IGF strongly stimulates proliferation and chemotactic migration of many cell populations and plays an important role in bone metabolism. Growth factors overall affect the bone regeneration and its healing, hence loaded in the carrier system. Cells that are responsible for tissue regeneration may be either delivered via biomaterial carriers or recruited in vivo by signaling molecules. Readers may refer to the cited reviews for a detailed overview of polymeric biomaterials used in bone repair [219].

There is a special challenge for the regeneration of cartilage, bone, muscle, tendons, cranial sutures, temporomandibular joints (TMJ), salivary glands, periodontium, and teeth due to the complexity of dental, oral, and craniofacial (DOC) structures [217]. Oral dental applications may be further divided into tooth replacement and repair [220], oral and maxillofacial surgery [221], periodontal tissue engineering [222], and small bone void-filling applications. Conventional dental implants are metal based. Similarly, many tooth fillings still use mercury-based amalgams. The possibility that repair of dental hard tissues (dentine and enamel) might involve the use of cells to remineralize

the damage to teeth naturally, is an exciting prospect, which will involve the role of stem-cell-based tissue engineering using polymeric scaffolds for whole-tooth replacement and repair of dental disease [220]. Gene-based therapies for regeneration of dental and periodontal tissue regeneration are also used [223, 224].

16.4.3 Cartilage

Cartilage, a predominantly avascular, aneural, and alymphatic tissue, is composed of sparsely distributed chondrocytes embedded within a dense ECM. This ECM is composed primarily of type II collagen and proteoglycans, that provide the tissue with sufficient mechanical properties for function in vivo and have limited capacity for self-repair [225]. Two possible approaches for cartilage tissue regeneration have been developed—preparation of cells that are subsequently injected into the lesion (with or without scaffold) allowing regeneration to occur in vivo [termed autologous chondrocyte implantation (ACI)] and tissue reconstruction in vitro whereby a ready-to-use graft is transplanted into the defect. ACI has become the dominant clinical cell-based therapy for the repair of cartilage lesions over the past decade. In this technique, expanded articular chondrocytes are implanted under a periosteal flap after surgical debridement of the lesion. ACI has demonstrated excellent short to midterm repair, although the evaluation of long-term repair remains somewhat controversial. The second approach aims to produce neocartilaginous tissue combining cells with various biomaterials, bioreactor systems, and growth factor cocktails [226].

At present, tissue-engineered cartilage products that target defects of stressed cartilage such as in joints or intervertebral disks are considered more important commercially (Table 16.5). Such defects can be due to injury, trauma, osteoarthritis, or rheumatoid arthritis. Established forms of therapy for cartilage damage in joints are arthroscopic surgery to smooth the surface of the damaged cartilage area; surgical procedures, such as microfracture, drilling, abrasion, in order to let bone marrow cells infiltrate the defect, resulting in the formation of fibrous cartilage tissue; analgesic therapy; full or partial artificial joint prostheses, often after years of progredient joint defects. Artificial joints generally last 10–15 years and revision surgery is problematic, and joint replacement therapy is recommended mainly for patients over the age of 50 [227].

As earlier discussed, collagen and hyaluronic-acid-based products are more widely used clinically, when compared to other polymers in tissue engineering. Cartilage tissue has an abundance of collagen type II and hyaluronic acid, hence these are used most commonly as the injectable carrier gels for the delivery of the cells (i.e., chondrocytes and mesenchymal stem cells) to the site of defects and gel alone is also used as the visco supplements to relieve pain due to osteoarthritis [226] (Table 16.5). Particulate carrier systems and gels were considerably explored in the last decade as an injectable system, majorly due to the larger surface-to-volume ratio and simultaneous achievement of localized

TABLE 16.5 Biodegradable Polymer-Based Products for Cartilage Tissue Engineering[a]

Product	Details	Cell	Indication	Manufacturer	Status
Collagen Based					
Cartilink-1	Collagen membrane	AC	Cartilage repair, ACI	Interface Biotech A/S, Denmark	Phase 4
Cartilink-2	Type I/type II bilayer collagen scaffold	AC	Cartilage repair, ACI	Interface Biotech A/S	M
Cartilink-3	ASEED scaffold fixed to cartilage by fibrin glue	AC	Cartilage repair, ACI	Interface Biotech A/S, Denmark	M
Ca-ReS	Collagen matrix	AC	Cartilage repair, ACI	Ars Arthro AG, Germany	M
ARTRO cell	Collagen matrix (ChondroGide)	AC	ACI, cartilage repair, relatively large (2–10 cm^2) isolated chondral (Outerbridge grade III or IV) or osteochondral defects	Ormed, Germany	M
Artifit	Synthetic meniscal implant	—	Partial menisectomy, lateral ore medial	Ormed, Germany	M
VeriCart	Collagen scaffold	—	Cartilage repair	Histogenics Corporation, USA	Phase 1
Chondrogel	Self-polymerizing four-armed thiol PEG (10K), a four-armed NHS-PEG (I0K), and methylated collagen	—	Meniscal repair	Angiotech Biomaterials Corporation, CA	Phase 1
Matrix-induced AC I (MACI)	Porcine type I/III collagen membrane	AC	Cartilage repair, ACI	Genzyme Biosurgery, Cambridge, MA	M

(*Continued*)

TABLE 16.5 (Continued)

Product	Details	Cell	Indication	Manufacturer	Status
Hyaluronic Acid Based					
Hyalograft C	Tridimensional matrix composed of hyaluronic acid ester derivative	AC	Cartilage repair	Fidia Advanced Biomaterials, Italy	M
HYA-JECT, ARTROJECT	Hayluronic acid	—	Cartilage repair	Ormed, Germany	M
Synvisc	Viscous hyaluronic acid solutions	—	Synovial fluid substitute to relieve pain and improve joint mobility in OA patients	Genzyme Biosurgery, Cambridge, MA	M, 2009
Nuflexxa	Sodium hyaluronate solution (1% w/v)	—	Synovial fluid substitute to relieve pain and improve joint mobility in OA patients	Savient Pharmaceuticals, Inc., USA	M, 2004
Orthovisc	Sodium hyaluronate solution	—	Synovial fluid substitute to relieve pain and improve joint mobility in OA patients	Anika Therapeutics, Inc.	M, 2004
Supartz Dispo, Artz	Sodium hyaluronate solution	—	Synovial fluid substitute to relieve pain and improve joint mobility in OA patients	Seikagaku Corporation, Japan	M, 2001
Alginate					
Chondrogel	Calcium alginate	AC	Vesico-urethral reflux disease, pediatric congenital ureter defects, cartilage repair	Curis, USA	phase III
Synthetic Polymer					
ASEED Scaffold	MPEG-PLGA 3D porous film with thickness equal to human cartilage		Scaffold for cartilage repair in Cartilink-3	Interface Biotech A/S, Denmark	M

[a]Abbreviations: AC, autologous chondrocytes; OA, osteoarthritis; ACI, autologous chondrocyte implantation.

and controlled delivery of drug and growth factors [228, 229]. Readers may refer to the mentioned citations for details of various polymers, cells and, scaffolds explored in research and understanding of the osteoarthritis pathology conditions [225, 226, 230, 231].

16.4.4 Vascular and Cardiovascular: Heart Valves, Stents, and Blood Vessels

Heart valves, vessel grafts, and cell grafts (into the heart muscle after myocardial infarction) are major tissue-engineered products used in the cardiovascular treatment. Atherosclerosis and cardiovascular disease are the most common causes of morbidity and mortality worldwide. Such vascular and coronary diseases are treated surgically using bypass procedures, whereby grafts of principally autogenous tissue, for example, internal mammary artery or saphenous vein, are used. The patency rates of internal mammary artery are high at 10 years, whereas 50% of saphenous vein grafts become occluded after 10 years owing to gradual deterioration and neointima formation. In 30–50% of bypass patients, all viable autologous tissue has already been used; under these circumstances, synthetic prostheses are utilized.

Major applications of biodegradable polymers in cardiovascular tissue engineering involve use of various hemostatic agents (e.g., CoStasis, Cohesion Technologies, Inc., USA), artificial heart valves (e.g., Mitroflow Aortic Pericardial Heart Valve, CarboMedics, Inc. Austin, Texas), and vascular grafts (e.g., Vascugel, Pervasis Therapeutics, Inc., USA). Polymers also play a major role as drug delivery devices in atherosclerosis treatment in the form of drug eluting stents (TAXUS, CYPHER) (Table 16.6). In peripheral surgery, woven polyethylene tetraphthlate (Dacron) and expanded polytetrafluoroethylene (ePTFE) are used, although for coronary artery bypass ePTFE alone is suitable. One-stage or two-stage seeded ePTFE in coronary artery bypass grafts, a 91% patency rate at 2.5 years (4 mm) and 65% patency at 9 years (5 mm) have been demonstrated in studies of endothelial cells (EC); ePTFE graft coated with synthetic peptide RGD (arginine–glycine–aspartic acid) or fibrin are used for the same. Human stem and progenitor cells have been isolated and grown in the grafts. Progenitor cells have potential therapeutic uses owing to their ability for self-renewal, a high proliferation capacity and potential to differentiate [232]. Research is also going to grow the heart muscle cells for cardiac regeneration [233]. Masuda et al. [234] reported the use of myocardial cell sheet for the repair to a damaged heart. Potential of stem cells in cardiac tissue regeneration is described by Wu et al. [235].

16.4.5 Neural Tissue Engineering

The complexity of the nervous system allows for information to be received and transmitted through the body. As a result, brain, spinal cord, and peripheral nerve tissue pose unique challenges when designing drug delivery scaffolds to

TABLE 16.6 Biodegradable Polymer-Based Products for Cardiovascular Tissue Engineering

Product	Details	Indication	Manufacturer	Status
Collagen Based				
CoStasis/ DynaStat Surgical Hemostat	Bovine microfibrillar collagen, bovine thrombin combined with autologous plasma	Surgical hemostat	Cohesion Technologies, Inc., USA	M, 2000
Gelatin Based				
FloSeal Hemostatic Matrix	Bovine-derived gelatin granules and thrombin	Hemostatic agent in surgical procedures (other than in ophthalmic) for the control of operative and postoperative bleeding	Baxter Healthcare Corporation, USA	M
Vascugel	Gelatin matrix with allogeneic endothelial cells to deliver growth regulatory compounds directly to the site of vascular injury	Restenosis following coronary artery bypass or angioplasty and to prevent thrombosis of AV fistula or AV graft failure	Pervasis Therapeutics, Inc., USA	Orphan Drug, 2009
Synthetic Polymer Based				
Mitroflow Aortic Pericardial Heart Valve	Bovine pericardium preserved with glutaraldehyde and sewn onto a polyester-covered polymer stent	Replacement of diseased, damaged, or malfunctioning native or prosthetic aortic valves	CarboMedics, Inc. Austin, USA	M, 2007

serve as replacements for injured or diseased tissue. The regeneration capability of the human adult nervous system is often limited. As a result, patients who have injuries or traumas to the nervous system often suffer from the loss of sensory or motor function and experience neuropathic pains. In order to facilitate nerve regeneration, many therapeutic approaches have been attempted. In the peripheral nervous system (PNS), direct end-to-end surgical reconnection is a common method of treatment for nerve transection injuries, when the injury gap is small. Nerve autografts are considered as the gold standard for bridging larger nerve defect gaps. However, tissue-engineered scaffolds may serve as an alternative choice for implantation to facilitate neural repair [236] (Table 16.7). Many requirements must be met when designing such scaffolds, including creating a permissible, biocompatible environment that allows for cell infiltration and restoration of neuronal connections lost to injury. The scaffolds should also deliver appropriate cues for promoting nerve regeneration in a controlled, localized manner. By following this guidance, engineered tissues can be produced that promote regeneration while becoming fully integrated into the existing healthy tissue [237]. Nanofibers have also been explored for neural tissue repairs due to their high surface-to-volume ratio. Fibers fabricated with collagen, gelatin, laminin, chitosan, PCL, PLA, PLGA, PHB, PHBV, poly(acrylonitrile-*co*-methylacrylate) (PAN-MA), copolymer of methyl methacrylate (MMA) and acrylic acid (AA) (PMMAAA), PDS, and polyamide have been explored for the neural tissue engineering [236].

16.4.6 Tissue-Engineered Organs

Despite high medical needs, tissue engineering of complete organs is far from being available. Important scientific-technical hurdles must be overcome, for example, vascularization of tissue-engineered organs, controlled three-dimensional structures, and coordinated action of different cell types. The concept of "organ printing," that is, computer-aided, jet-based three-dimensional tissue engineering of organs, has been proposed as a possible means to achieve this goal [227]. There are few organs such as urinary bladder, kidney, heart valves and heart muscle, liver, and pancreas that are being developed based on the tissue engineering approach (Table 16.8). Encapsulated pancreatic islets have been implanted in patients for the treatment of diabetes. BetaRx and DIABECELL are two such products that are currently under clinical trials for the treatment of diabetes. Liver assist systems (HepatAssist 2000 System) and Bioartificial Liver Support System (BLSS) containing encapsulated hepatocytes are under clinical investigation to provide extracorporeal support to patients with liver failure. A kidney support system with encapsulated urothelial cells is in development for the treatment of patients with kidney failure [238] and a bioartificial bladder (Neobladder, Curis) based on PGA has been developed as a replacement engineered organ.

TABLE 16.7 Biodegradable Polymer-Based Products for Neural Tissue Engineering

Product	Details	Indication	Manufacturer	Status
Collagen Based				
NeuraGen nerve guide	Absorbable collagen tube as a nerve guide	Forms an interface between the nerve and the surrounding tissue and creates a conduit for axonal growth across a nerve gap, spinal cord injury	Integra LifeSciences Corporation, USA	M
NeuraWrap nerve protector	Absorbable collagen implant	A nonconstricting encasement for injured peripheral nerves for protection of the neural environment	Integra LifeSciences Corporation, USA	M
DuraGen Dural graft	Suture-free 3D collagen matrix for dural closure	Onlay graft for dural defects, acts as a matrix for repair of dura mater	Integra LifeSciences Corporation, USA	M
Synthetic Polymer Based				
DuraSeal Spine Sealant System	Polyethylene glycol (PEG) ester and trilysine amine solutions	Used following dura mater surgery to prevent CSF leakage along dural sutures	Covidien, USA	M, 2009

TABLE 16.8 Biodegradable Polymer-Based Tissue Engineered Organs

Product	Details	Indication	Manufacturer	Status
BetaRx	Encapsulated human insulin producing cells	Diabetes	VivoRx Inc., USA	Phase I/II clinical trials
DIABECELL	Encapsulated porcine insulin producing cells	Type 1 diabetes	Living Cell Technologies Limited	Phase I/IIa clinical trial
Fac8Cell	A liver product aimed at producing factor 8, a blood clotting agent	Cellular therapy for the treatment of hemophilia	Living Cell Technologies Limited	Preclinical stage
NTCell	Choroid plexus cell product	For treatment of nerve degenerative disorders, e.g., Parkinson's disease, Alzheimer's disease, Huntington's disease and stroke	Living Cell Technologies Limited	Preclinical stage
NeoBladder	Biodegradable polyglycolic acid polymer scaffold	Replacement or augmentation bladder for patients with trauma, surgery for cancer, or congenital defects, using urothelial and smooth muscle cells	Curis, USA	Preclinical stage
Liver Dialysis UnitTM (formaly Biologic-DT)	Noncellular (charcoal) membrane separated hemodialysis unit	Liver assist device	HemoTherapies (formerly HemoCleanse), USA	FDA approved , Multicenter
Molecular Adsorbent Recycling System (MARS)	Human albumin hollow fiber bioreactor	Liver assist device	Teraklin, Germany	I/II/II CE-approved Multicenter

(*Continued*)

TABLE 16.8 (Continued)

Product	Details	Indication	Manufacturer	Status
Extracorporeal Liver Assist	Hollow fiber membrane bioreactor based on immortalized human cells	Liver assist device	Vitagen, USA	I/II Multicenter
HepatAssist 2000 System	Hollow fiber membrane bioreactor based on porcine hepatocytes	Liver assist device	Circe Biomedical, USA	II Multicenter completed
Bioartificial Liver Support System (BLSS)	Hollow fiber membrane bioreactor based on primary porcine hepatocytes	Liver assist device	Excorp Medical, Inc., USA	I/II Multicenter
Modular Extracorporeal Liver System (MELS)	Hollow fiber membrane bioreactor based on human hepatocytes	Liver assist device	Hybrid Organ, Germany	I/II Multicenter
LIVERX2000 System	Hollow fiber membrane bioreactor based on primary porcine hepatocytes	Liver assist device	Algenix, Inc., USA	I planned

16.4.7 Surgical Applications

Surgical aids are devices used during surgery such as an adhesive or sealant, in the form of screws and sutures, or as adhesion barriers. Various biodegradable polymers have been used for a wide range of surgical applications. Biodegradable sutures have been one of the earliest and successful applications of polyesters such as PLGA and PLA. PLGA-based sutures include PuraSorb (80LA:20GA), Vicryl (10LA:90GA), Vicryl Rapid (an irradiated version of Vicryl with a faster rate of degradation), PANACRYL, and so forth. Sutures made up of PLA (Progrip) and PDS are also commonly used as surgical aids (Table 16.9). Polyester mesh (Parietex) is used for tissue repair. Various commercially available polymeric functional devices are discussed in Section 16.3.3 (Table 16.2).

16.5 ADVANCES IN DISCOVERY OF NEED-SPECIFIC POLYMERIC BIOMATERIALS AND BIOMATERIAL DESIGN

Despite the advances in the field of tissue engineering, the major challenges still remain unresolved as lab-grown tissues still exhibit lack of functional and biomechanical stability and vascularization needed for transplantation. Recent advances in the field of stem cell technology have opened a new arena in the development of regenerative medicine [239]. A major drawback of traditionally used polymeric scaffolds is that, while providing architectural support for neotissue development, they do not adequately mimic the complex interactions between cells and ECM that promote functional tissue regeneration. Hence, the development of "smart" bioactive biomaterials that actively participate in the formation of functional tissue is paramount for improvement in the clinical outcomes of current regenerative strategies [240]. Currently, material design aims to regulate tissue regeneration by different mechanisms, such as controlling specific cell-binding interactions, releasing growth factors, degrading at a controlled rate, and responding to environmental cues [30].

Virtual polymer libraries are an extraordinary means to explore a wide range of new polymer compositions in a time- and cost-effective fashion. Briefly, virtual polymer libraries are large collections of polymer structures created using various molecular modeling tools. The model structures are then used to derive predictions on polymer properties, thereby creating a rational way to select a smaller subset of these virtual polymers for actual synthesis and exploration. This approach, commonly used in drug discovery, is only now being explored as a tool in biomaterials design.

In this context, biomaterials scientists can learn a lot from the field of drug discovery where two powerful techniques, molecular similarity–diversity analysis and quantitative structure–property relationship models enable the prediction of target properties for a library of compounds, thereby accelerating and optimizing the discovery process. The real challenges in the adaptation of

TABLE 16.9 Biodegradable Polymer-Based Products for Surgical Applications

Polymer	Product	Indication	Manufacturer	Status
Collagen Based				
Macroporous polyester mesh with absorbable collagen film	Parietex	Tissue repair	Coviden, USA	M
Collagen (>90%)	Restore Orthobiologic Soft Tissue Implant	Rotator cuff repair	DePuy Orthopaedics, Inc., USA	M
Crosslinked porcine dermal collagen implant	Permacol	Hernia and abdominal wall repair	Coviden, USA	M
Polyester Based				
PGA	Dexon	Synthetic absorbable braided and monofilament surgical sutures used for general soft tissue approximation and/or ligation, including use in ophthalmic procedures	Coviden Syneture, USA	M
PLGA	Resomers LR708	Bioresorbable implant material	Boehringer Ingelheim Pharma KG, Germany	M
	Vicryl	Multifilament suture	Ethicon, Inc., Johnson and Johnson	M
	Vicryl Rapid	Irradiated version of the suture to increase the rate of degradation	Ethicon, Inc., Johnson & Johnson	M

(*Continued*)

TABLE 16.9 (Continued)

Polymer	Product	Indication	Manufacturer	Status
PLGA	Lactomer	Absorbable surgical sutures	United States Surgical Corporation	M
	AbsorbaTack	Single-use 5mm fixation device for fixation of prosthetic material, such as hernia mesh, to soft tissue	Coviden, USA	M
	Vicryl Mesh, polyglactin 910 woven mesh	Suture reinforcements, hernia repair, used as a buttress to provide temporary support during healing	Ethicon, Inc., Johnson & Johnson	M
Polydioxanone	PDS	Monofilament suture	Ethicon, Inc., Johnson & Johnson	M
	PDS Plus	Antibacterial sutures, kill bacteria and inhibit bacterial colonization of the suture	Ethicon, Inc., Johnson & Johnson	M
Polyglyconates	MONOCRYL	Flexible monofilament suture materials	Davis and Geck, Danbury, CT	M
PLLA	Full Thread Bio Interference Screws	Fixation device for bone-patellar tendon-bone (BTB) and soft tissue grafts	Arthrex, USA	M
	Clearfix Meniscal Darts	Meniscal repair, ACL reconstruction	Innovasive Devices	M
	BioStinger	Meniscal stingers for meniscus repair, ACL reconstruction	Linvatec Largo, FL	M
	BioSyn	Suture material	Coviden Syneture, USA	M
	ProGrip Self-Fixating mesh, with monofilaments of PET and PLA	Standard open patch technique for hernia repair	Coviden, USA	M

(*Continued*)

TABLE 16.9 (Continued)

Polymer	Product	Indication	Manufacturer	Status
PLLA	MAXON and MAXON CV	Pediatric heart wall repair	Coviden Syneture, USA	M
Glycolide (60%), dioxanone (14%), and trimethylene carbonate (26%)	Endo-Fix	Maxon Tissue Fixation Screws for Bone-Tendon-Bone graft fixation	Smith & Nephew Surgical Pty Ltd, Australia	M
Copolymer of glycolic acid and trimethylene carbonate	Resomer X	Bioresorbable implant material	Boehringer Ingelheim Pharma KG, Germany	M
X - Poly(dioxanone)	Resomer LC 703 S	Bioresorbable implant material	Boehringer Ingelheim Pharma KG, Germany	M

these techniques in a wide range of material design programs are the lack of knowledge of many materials scientists of the powerful high-throughput and modeling techniques already available for drug discovery, and the high cost of acquiring the necessary high-throughput instrumentation and computational resources in the laboratory [42].

Further, basic understanding of the three-dimensional structure of existing biological molecules is being applied to a "bottom-up" approach to generate new, self-assembling supramolecular architectures [241]. Smart peptide amphiphiles, which can be induced to self-assemble by changes in concentration, pH, or the level of divalent cations or can be modified to present bioactive sequences such as RGD, YIGSR to cells via nanofiber gels, or as coatings on conventional tissue engineering scaffolds have been developed [242, 243]. Genetic engineering, advances in chemical synthesis, and exploitation of peptide and oligonucleotide self-assembly have allowed tissue engineers to use a bottom-up approach in combining multiple properties to tailor materials for specific applications. Current trends suggest that biomaterial development will continue to create more lifelike multifunctional materials that are able to simultaneously provide complex biological signals (chemical, structural, and mechanical), replace mechanical function, and respond to environmental stimuli. A continuing challenge for this approach will be to find ways of exploiting these sophisticated tools without unduly complicating large-scale production for clinical research [244].

Another challenge is to design an interface so that the implant can be securely attached and is compatible with cell growth but also allows removal if infection or poor performance is experienced. Dongan Wang and colleagues [245] recently proposed an exciting new concept based on a "glue" capable of binding the proteins present in the existing cartilage to the materials used for tissue reconstruction or regeneration. They functionalized chondroitin sulfate, which is one of the components of native cartilage, with two distinct organic moieties: methacrylate and aldehyde groups. Aldehydes could form a covalent bond with the native cartilage tissue (presumably reacting with the amine groups of the collagen) forming an adhesive layer, and at the same time this adhesive could bind to the biomaterial, as the methacrylate groups participated in the polymerization reaction used to solidify the biomaterial once it is introduced in the tissue defect. This study is an excellent example of the high level of sophistication that can be achieved in tissue engineering and introduces the perspective that native tissue is to be considered as a partner in determining the fate of new tissues [246].

A successful strategy to develop true human replacement parts will require convergence of the recent advances in tissue, matrix, growth factor, and developmental biology with technological breakthroughs in tissue informatics, bioinformatics, high-throughput combinatorial and computational methods in biomaterials design, and stem cell technologies [1247].

16.6 CONCLUSION

It is expected that the field of biomaterials will continue to expand. However, there are a number of challenges that must be overcome. Advances in the isolation, expansion, and storage of embryonic and adult pluripotent stem cells, the development of bioactive scaffolds for efficient delivery of cell-based therapeutics, and the application of genetic engineering will help to conquer the current limitations of regenerative medicine. The development of high-throughput biological and biochemical screens for evaluation and development of novel materials and growth factor composites is also necessary.

Further, there is a need to develop new polymeric materials that can reproduce the effects of biological structures. The adoption of combinatorial and computational approaches in biomaterials design can potentially address challenges in design of need-specific materials by accelerating the discovery of new biomaterials and by increasing the diversity of promising polymer structures. With the recent advancements and further developments, the scientific community is aiming to develop the tissue substitute that will fulfill everyone's clinical needs.

REFERENCES

1. Anonymous. Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1997–2006 ed., Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation: Rockville, MD; 2007.
2. Griffith, L. G. and G. Naughton. Tissue engineering—current challenges and expanding opportunities. *Science* 2002;**295**(5557):1009–1014.
3. Wang, M. Materials selection and scaffold fabrication for tissue engineering in orthopaedics. In Advanced Bioimaging Technologies in Assessment of the Quality of Bone and Scaffold Materials. L. Qin, H. K. Genant, J. F. Griffith, and K. S. Leung..(eds.) Springer: New York, 2007. pp. 259–288.
4. Flanagan, T. C. and A. Pandit. Living artificial heart valve alternatives: A review. *Eur. Cell Mater.* 2003;**6**:28–45.
5. Stock, U. A. and K. Schenke-Layland. Performance of decellularized xenogeneic tissue in heart valve replacement. *Biomaterials* 2006;**27**(1):1–2.
6. Forrester, J. S., M. J. Price, and R. R. Makkar. Stem cell repair of infarcted myocardium: An overview for clinicians. *Circulation* 2003;**108**(9):1139–1145.
7. Tabata, Y. Significant role of cell scaffolding and DDS technology in tissue regeneration: Tissue engineering strategies. *Int. Cong. Ser.* 2005;**1284**:257–265.
8. Dang, J. M. and K. W. Leong. Natural polymers for gene delivery and tissue engineering. *Adv. Drug Deliv. Rev.* 2006;**58**(4):487–499.
9. Krebs, N. Advances in cardiac tissue engineering and cardiac tissue replacement modalities. *J. Undergrad. Res.* 2003;**2**(1):41–45.

10. Nair, L. S. and C. T. Laurencin. Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* 2007;**32**(8–9):762–798.
11. Gunatillake, P. A. and R. Adhikari. Biodegradable synthetic polymers for tissue engineering. *Eur. Cell Mater.* 2003;**5**:1–16.
12. Pillai, O. and R. Panchagnula. Polymers in drug delivery. *Curr. Opin. Chem. Biol.* 2001;**5**(4):447–451.
13. Gilbert, T. W., A. M. Stewart-Akers, and S. F. Badylak. A quantitative method for evaluating the degradation of biologic scaffold materials. *Biomaterials* 2007;**28**(2): 147–150.
14. Chung, H. J. and T. G. Park. Surface engineered and drug releasing pre-fabricated scaffolds for tissue engineering. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):249–262.
15. Sung, H. J., C. Meredith, C. Johnson, and Z. S. Galis. The effect of scaffold degradation rate on three-dimensional cell growth and angiogenesis. *Biomaterials* 2004;**25**(26):5735–5742.
16. Park, H., J. S. Temenoff, and A. G. Mikos. Biodegradable orthopedic implants. In F. Bronner, M. C. Farach-Carson, and A. G. Mikos(Eds.). *Engineering of Functional Skeletal Tissues*, Springer: London, 2007.
17. Chandra, R. and R. Rustgi. Biodegradable polymers. *Prog. Polym. Sci.* 1998;**23**(7): 1273–1335.
18. Garkhal, K., S. Verma, S. Jonnalagadda, and N. Kumar. Fast degradable poly (L-lactide-*co*-caprolactone) microspheres for tissue engineering: Synthesis, characterization, and degradation behavior. *J. Polym. Sci.: Part A: Polym. Chem.* 2007;**45**: 2755–2764.
19. Chen, D., H. Chen, J. Bei, and S. Wang. Morphology and biodegradation of microspheres of polyester–polyether block copolymer based on polycaprolactone/ polylactide/poly(ethylene oxide). *Polym. Int.* 2000;**49**:269–276.
20. Dong, C. M., Y Z. Guo, K. Y. Qiu, Z. W. Gu, and X. D. Feng. In vitro degradation and controlled release behavior of D,L-PLGA50 and PCL-B-D,L-PLGA50 copolymer microspheres. *J. Control. Release* 2005;**107**(1):53–64.
21. Grayson, A. C., M. J. Cima, and R. Langer. Size and temperature effects on poly (lactic-*co*-glycolic acid) degradation and microreservoir device performance. *Biomaterials* 2005;**26**(14):2137–2145.
22. Mittal, A., P. Negi, K. Garkhal, S. Verma, and N. Kumar. Integration of porosity and biofunctionalization to form 3D scaffold: Cell culture studies and in vitro degradation. *Biomed. Mater.* 2010;**5**(4):045001
23. Cao, Y., G. Mitchell, A. Messina, L. Price, E. Thompson, A. Penington, W. Morrison, A. O'Connor, G. Stevens, and J. Cooper-White. The influence of architecture on degradation and tissue ingrowth into three-dimensional poly(lactic-*co*-glycolic acid) scaffolds in vitro and in vivo. *Biomaterials* 2006;**27**(14):2854–2864.
24. Wu, L. and J. Ding. In vitro degradation of three-dimensional porous poly(D,L-lactide-*co*-glycolide) scaffolds for tissue engineering. *Biomaterials* 2004;**25**(27):5821–5830.
25. Verma, S. and N. Kumar. Effect of biomimetic 3D environment and degradation behavior of injectable bone filler: An in vitro evaluation. *Mater. Sci. Eng: C* 2010;**30** (8):1118–1128.
26. Mikos, A. G., L. V. McIntire, J. M. Anderson, and J. E. Babensee. Host response to tissue engineered devices. *Adv. Drug Deliv. Rev.* 1998;**33**(1–2):111–139.

27. Schliecker, G., C. Schmidt, S. Fuchs, and T. Kissel. Characterization and *in vitro* degradation of poly(2,3-(1,4-diethyl tartrate)-*co*-2,3-isopropyliden tartrate). *J. Control. Release* 2004;**98**(1):11–23.
28. ISO. *Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing*. International Organization for Standardization: Geneva, 1997.
29. Northup, S. J. Safety evaluation of medical devices: U.S. Food and Drug Administration and International Standards Organization Guidelines. *Int. J. Toxicol.* 1999;**18**(4):275–283.
30. Lutolf, M. P. and J. A. Hubbell. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nature Biotechnol.* 2005;**23**(1):47–55.
31. Garkhal, K., A. Mittal, S. Verma, and N. Kumar. P-15 functionalized porous microspheres as biomimetic habitats for bone tissue engineering applications. *Polym. Adv. Technol.* 2011;**22**(1):190–198.
32. Pegueroles, M., C. Aparicio, M. Bosio, E. Engel, F. J. Gil, J. A. Planell, and G. Altankov. Spatial organization of osteoblast fibronectin matrix on titanium surfaces: Effects of roughness, chemical heterogeneity and surface energy. *Acta Biomater.* 2010;**6**(1):291–301.
33. Neiva, R. F., Y. P. Tsao, R. Eber, J. Shotwell, E. Billy, and H.-L. Wang. Effects of a putty-form hydroxyapatite matrix combined with the synthetic cell-binding peptide P-15 on alveolar ridge preservation. *J. Periodontol.* 2008;**79**:291–299.
34. Milburn, C., J. Chen, Y. Cao, G. M. Oparinde, M. O. Adeoye, A. Beye, and W. O. Soboyejo. Investigation of effects of argentine–glycine–aspartate (RGD) and nano-scale titanium coatings on cell spreading and adhesion. *Mater. Sci. Eng.: C* 2009;**29**(1):306–314.
35. Andukuri, A., W. P. Minor, M. Kushwaha, J. M. Anderson, and H. W. Jun. Effect of endothelium mimicking self-assembled nanomatrices on cell adhesion and spreading of human endothelial cells and smooth muscle cells. *Nanomedicine* 2010;**6**(2): 289–297.
36. Shin, H., S. Jo, and A. G. Mikos. Biomimetic materials for tissue engineering. *Biomaterials* 2003;**24**(24):4353–4364.
37. Gadegaard, N., E. Martines, M. O. Riehle, K. Seunarine, and C. D. W. Wilkinson. Applications of nano-patterning to tissue engineering. *Microelect. Eng.* 2006;**83** (4–9):1577–1581.
38. Charulatha, V. and A. Rajaram. Influence of different crosslinking treatments on the physical properties of collagen membranes. *Biomaterials* 2003;**24**(5):759–767.
39. Hennink, W. E. and C. F. V. Nostrum. Novel crosslinking methods to design hydrogels. *Adv. Drug Deliv. Rev.* 2002;**54**(1):13–36.
40. Elvin, C. M., A. G. Brownlee, M. G. Huson, T. A. Tebb, M. Kim, R. E. Lyons, T. Vuocolo, N. E. Liyou, T. C. Hughes, J. A. M. Ramshaw, and J. A. Werkmeiste. The development of photochemically crosslinked native fibrinogen as a rapidly formed and mechanically strong surgical tissue sealant. *Biomaterials* 2009;**30**(11):2059–2065.
41. Tessmar, J. K. and A. M. Gopferich. Matrices and scaffolds for protein delivery in tissue engineering. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):274–291.
42. Kohn, J. New approaches to biomaterials design. *Nature Mater.* 2004;**3**(11): 745–747.

43. Yu, L., K. Dean, and L. Li. Polymer blends and composites from renewable resources. *Prog. Polym. Sci.* 2006;**31**:576–602.
44. Malafaya, P. B., G. A. Silva, and R. L. Reis. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug Deliv. Rev.* 2007;**59**:207–233.
45. Gelinsky, M., P. B. Welzel, P. Simon, A. Bernhardt, and U. König. Porous three-dimensional scaffolds made of mineralised collagen: Preparation and properties of a biomimetic nanocomposite material for tissue engineering of bone. *Chem. Eng. J.* 2008;**137**:84–96.
46. Thornton, J. F. and R. J. Rohrich. Dermal substitute (Integra) for open nasal wounds. *Plastic Reconst. Surg.* 2005;**116**:677.
47. Wu, W., X. Feng, T. Mao, X. Feng, H. W. Ouyang, G. Zhao, and F. Chen. Engineering of human tracheal tissue with collagen-enforced poly-lactic-glycolic acid non-woven mesh: A preliminary study in nude mice. *Br. J. Oral Maxillofac. Surg.* 2007;**45**:272–278.
48. Dai, W., N. Kawazoe, X. Lin, J. Dong, and G. Chen. The influence of structural design of PLGA/collagen hybrid scaffolds in cartilage tissue engineering. *Biomaterials* 2010;**31**(8):2141–2152.
49. Narotham, P. K., S. Jose, N. Nathoo, C. Taylon, and Y. Vora. Collagen matrix (DuraGen) in dural repair: Analysis of a new modified technique. *Spine* 2004;**29**:2861–2867.
50. Duan, X., C. McLaughlin, M. Griffith, and H. Sheardown. Biofunctionalization of collagen for improved biological response: Scaffolds for corneal tissue engineering. *Biomaterials* 2007;**28**:78–88.
51. Holland, T. A., J. K. V. Tessmar, Y. Tabata, and A. G. Mikos. Transforming growth factor-beta1 release from oligo(poly(ethylene glycol) fumarate) hydrogels in conditions that model the cartilage wound healing environment. *J. Control. Release*. 2004;**94**:101–114.
52. Holland, T. A., Y. Tabata, and A. G. Mikos. Dual growth factor delivery from degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. *J. Control. Release* 2005;**101**(1–3):111–125.
53. Park, H., J. S. Temenoff, T. A. Holland, Y. Tabata, and A. G. Mikos. Delivery of TGF-beta1 and chondrocytes via injectable, biodegradable hydrogels for cartilage tissue engineering applications. *Biomaterials* 2005;**26**:7095–7103.
54. Okamoto, T., Y. Yamamoto, M. Gotoh, C. L. Huang, T. Nakamura, Y. Shimizu, T. Tabata, and H. Yokomise. Slow release of bone morphogenetic protein 2 from a gelatin sponge to promote regeneration of tracheal cartilage in a canine model. *J. Thorac. Cardiovasc. Surg.* 2004;**127**:329–334.
55. Kimura, Y., M. Ozeki, T. Inamoto, and Y. Tabata. Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor. *Biomaterials* 2003;**24**:2513–2521.
56. Payne, R. G., M. J. Yaszemski, A. W. Yasko, and A. G. Mikos. Development of an injectable, in situ crosslinkable, degradable polymeric carrier for osteogenic cell populations. Part 1. Encapsulation of marrow stromal osteoblasts in surface crosslinked gelatin microparticles. *Biomaterials* 2002;**23**:4359–4371.

57. Payne, R. G., J. S. McGonigle, M. J. Yaszemski, A.W. Yasko, and A. G. Mikos. Development of an injectable, in situ crosslinkable, degradable polymeric carrier for osteogenic cell populations. Part 2. Viability of encapsulated marrow stromal osteoblasts cultured on crosslinking poly(propylene fumarate). *Biomaterials* 2002;**23**:4373–4380.
58. Liu, Y. and M. B. Chan-Park. A biomimetic hydrogel based on methacrylated dextran-graft-lysine and gelatin for 3D smooth muscle cell culture. *Biomaterials* 2010;**31**(6):1158–1170.
59. Awad, H. A., M. O. Wickham, H. Leddy, J. M. Gimble, and F. Guilak. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials* 2004;**25**:3211–3222.
60. Ponticiello, M. S., R. M. Schinagl, S. Kadiyala, and F. P. Barry. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. *J. Biomed. Mater. Res.* 2000;**52**:246–255.
61. Malda, J., E. Kreijveld, J. S. Temenoff, C. A. Van Blitterswijk, and J. Riesle. Expansion of human nasal chondrocytes on macroporous microcarriers enhances redifferentiation. *Biomaterials* 2003;**24**:5153–5161.
62. Liu, X., L. A. Smith, J. Hu, and P. X. Maa. Biomimetic nanofibrous gelatin/apatite composite scaffolds for bone tissue engineering. *Biomaterials* 2009;**30**:2252–2258.
63. Ghasemi-Mobarakeh, L., M. P. Prabhakarana, M. Morshed, M. H. Nasr-Esfahani, and S. Ramakrishna. Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials* 2008;**29**(34):4532–4539.
64. Ahmed, T. A., E. V. Dare, and M. Hincke. Fibrin: A versatile scaffold for tissue engineering applications. *Tissue Eng. Part B* 2008;**14**(2):199–215.
65. Wechselberger, G., R. Russell, M. Neumeister, T. Schoeller, H. Piza-Katzer, and C. Rainer. Successful transplantation of three tissue-engineered cell types using capsule induction technique and fibrin glue as a delivery vehicle. *Plastic Reconst. Surg.* 2002;**110**:123–129.
66. Wechselberger, G., T. Schoeller, A. Stenzl, M. Ninkovic, S. Lille, and R. C. Russell. Fibrin glue as a delivery vehicle for autologous urothelial cell transplantation onto a prefabricated pouch. *J. Urol.* 1998;**160**(2):583–586.
67. Rainer, C., G. Wechselberger, T. Bauer, M. W. Neumeister, S. Lille, A. Mowlavi, H. Piza, and T. Schoeller. Transplantation of tracheal epithelial cells onto a prefabricated capsule pouch with fibrin glue as a delivery vehicle. *J. Thor. Cardiovas. Surg.* 2001;**121**(6):1187–1193.
68. Willerth, S. M., K. J. Arendas, D. I. Gottlieb, and S. E. Sakiyama-Elbert. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. *Biomaterials* 2006;**27**:5990–6003.
69. Schantz, J. T., A. Brandwood, D. Hutmacher, H. Khor, and K. Bittner. Osteogenic differentiation of mesenchymal progenitor cells in computer designed fibrin-polymer-ceramic scaffolds manufactured by fused deposition modeling. *J. Mater. Sci. Mater. Med.* 2005;**16**:807–819.
70. Eyrich, D., F. Brandl, B. Appel, H. Wiese, G. Maier, M. Wenzel, R. Staudenmaier, A. Goepferich, and T. Blunk. Long-term stable fibrin gels for cartilage engineering. *Biomaterials* 2007;**28**:55–65.

71. Ho, S. T. B., S. M. Cool, J. H. Hui, and D. W. Hutmacher. The influence of fibrin based hydrogels on the chondrogenic differentiation of human bone marrow stromal cells. *Biomaterials* 2010;**31**:38–47.

72. Altman, G. H., F. Diaz, C. Jakuba, T. Calabro, R. Horan, J. Chen, H. Lu, J. Richmond, and D. L. Kaplan. Silk-based biomaterials. *Biomaterials*. 2003;**24**: 401–416.

73. Hinman, M. B., J. A. Jones, and R. V. Lewis. Synthetic spider silk: A modular fiber. *Trends Biotechnol*. 2000;**18**:374–379.

74. Tamada, Y. New process to form a silk fibroin porous 3-D structure. *Biomacromolecules* 2005;**6**:3100–3106.

75. Pra, I. D., G. Freddi, J. Minic, and U. Armato. De novo engineering of reitcular connective tissue in vivo by silk fibroin nonwoven materials. *Biomaterials* 2005;**26**:1987–1999.

76. Horan, R. L., K. Antle, A. L. Collette, Y. Wang, J. Huang, and J. E. Moreau. In vitro degradation of silk fibroin. *Biomaterials* 2005;**26**:3385–3393.

77. Hofmann, S., C. Foo, F. Rossetti, M. Textor, G. Vunjak-Novakovic, D. L. Kaplan, H. P. Merkle, and L. Meinel. Silk fibroin as an organic polymer for controlled drug delivery. *J. Control. Release* 2006;**111**:219–227.

78. Sofia, S., M. B. MacCarthy, G. Gronowiz, and D. L. Kaplan. Functionalized silk based biomaterials. *J. Biomed. Mater. Res*. 2001;**54**:139–148.

79. Hino, R., M. Tomito, and K. Yoshizato. The generation of germline transgenic silkworms for the production of biologically active recombinant fusion proteins of fibroin and human basic fibroblast growth factor. *Biomaterials* 2006;**27**:5715–5724.

80. Mandal, B. B., and S. C. Kundu. Cell proliferation and migration in silk fibroin 3D scaffolds. *Biomaterials* 2009;**30**(15):2956–2965.

81. Fang, Q., D. Chen, Z. Yang, and M. Li. In vitro and in vivo research on using Antheraea pernyi silk fibroin as tissue engineering tendon scaffolds. *Mater. Sci. Eng.: C* 2009;1527–1534.

82. Patel, A., B. Fine, M. Sandig, and K. Mequanint. Elastin biosynthesis: The missing link in tissue-engineered blood vessels. *Cardiovasc. Res*. 2006;**71**:40–49.

83. Mithieux, S. M., and J. E. J. Rasko, and A. S. Weiss. Synthetic elastin hydrogels derived from massive elastic assemblies of selforganized human protein monomers. *Biomaterials* 2004;**25**:4921–4927.

84. Woodhouse, K. A., P. Klement, V. Chen, M. B. Gorbet, and F. W. Keeley. Investigation of recombinant human elastin polypeptides as non-thrombogenic coatings. *Biomaterials* 2004;**25**:4543–4553.

85. Nivison-Smith, L., J. Rnjak, and A. S. Weiss. Synthetic human elastin microfibers: Stable cross-linked tropoelastin and cell interactive constructs for tissue engineering applications. *Acta Biomater*. 2010;**6**:354–359.

86. Betre, H., S. R. Ong, F. Guilak, A. Chilkoti, B. Fermor, and L. A. Setton. Chondrocytic differentiation of human adipose derived adult stem cells in elastin-like polypeptide. *Biomaterials* 2006;**27**:91–99.

87. Silva, S. S., J. M. Oliveira, J. A. Mano, and R. L. Reis. Physicochemical characterization of novel chitosan-soy protein/TEOS porous hybrids for tissue engineering applications. *Mater. Sci. Forum* 2006;1000–1004.

88. Vaz, C. M., P. F. N. M. V. Doeveren, R. L. Reis, and A. M. Cunha. Development and design of double-layer co-injection moulded soy protein based drug delivery devices. *Polymer* 2003;**44**:5983–5992.

89. Vaz, C. M., P. F. V. Doeveren, R. L. Reis, and A. M. Cunha. Soy matrix drug delivery systems obtained by melt-processing techniques. *Biomacromolecules*, 2003;1520–1529.

90. Vaz, C. M., M. Fossen, R. F. V. Tuil, L. A. D. Graaf, R. L. Reis, and A. M. Cunha. Casein and soybean protein-based thermoplastics and composites as alternative biodegradable polymers for biomedical applications. *J. Biomed. Mater. Res*. 2003;**65A**:60–70.

91. Khor, E., and L. Y. Lim. Implantable applications of chitin and chitosan. *Biomaterials* 2003;**24**:2339–2349.

92. George, M., and T. E. Abraham. Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan—a review. *J. Control. Release* 2006;**114**:1–14.

93. Martino, A. D., M. Sittinger, and M. V. Risbud. Chitosan: A versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials* 2005;**26**:5983–5990.

94. Lee, J. Y., S. H. Nam, S. Y. Im, Y. J. Park, Y. M. Lee, Y. J. Seol, C. P. Chung, and S. J. Lee. Enhanced bone formation by controlled growth factor delivery from chitosan-based biomatetials. *J. Control. Release* 2002;**78**:187–197.

95. Okamoto, Y., R. Yano, K. Miyatake, I. Tomohiro, Y. Shigemasa, and S. Minami. Effects of chitin and chitosan on blood coagulation. *Carbohydr. Polym*. 2003;**53**:337–342.

96. Kweon, D. K., S. B. Song, and Y. Y. Park. Preparation of water-soluble chitosan/heparin complex and its application as wound healing accelerator. *Biomaterials* 2003;**24**:1595–1601.

97. Ueno, H., T. Mori, and T. Fujinaga. Topical formulations and wound healing applications of chitosan. *Adv. Drug Deliv. Rev*. 2001;**52**:105–115.

98. Jayakumar, R., M. Prabaharan, S. V. Nair, and H. Tamura. Novel chitin and chitosan nanofibers in biomedical applications. *Biotech. Adv*. 2010;**28**:142–150.

99. Kim, I., S. Seo, H. Moon, M. Yoo, I. Park, B. Kim, and C. Cho. Chitosan and its derivatives for tissue engineering applications. *Biotech. Adv*. 2008;**26**:1–21.

100. Tan, H., C. R. Chu, K. A. Payne, and K. G. Marra. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. *Biomaterials* 2009;**30**(13):2499–2506.

101. Basmanav, F. B., G. J. Koseb, and V. Hasirci. Sequential growth factor delivery from complexed microspheres for bone tissue engineering. *Biomaterials* 2008;**29**:4195–4204.

102. Moya, M. L., M. H. Cheng, J. Huang, M. E. Francis-Sedlak, S. I. Kao, E. C. Opara, and E. M. Brey. The effect of FGF-1 loaded alginate microbeads on neovascularization and adipogenesis in a vascular pedicle model of adipose tissue engineering. *Biomaterials* 2010;**31**(10):2816–2826.

103. Liao, Y. H., S. A. Jones, B. Forbes, G. P. Martin, and M. B. Brown. Hyaluronan: Pharmaceutical characterization and drug delivery. *Drug Deliv*. 2005;**12**: 327–342.

104. Laurent, T. C., U. B. G. Laurent, and J. R. E. Fraser. Functions of hyaluronan. *Ann. Rheum. Dis.* 1995;**54**: 429–432.

105. Lee, C. T., P. H. Kung, and Y. D. Lee. Preparation of poly(vinyl alcohol)-chondroitin sulfate hydrogel as matrices in tissue engineering. *Carbohydr. Polym.* 2005;**61**:348–354.

106. Chang, K.-Y., L.-W. Cheng, G. H. Ho, Y.-P. Huang, and Y.-D. Lee. Fabrication and characterization of poly(β-glutamic acid)-graft-chondroitin sulfate/polycaprolactone porous scaffolds for cartilage tissue engineering. *Acta Biomater.* 2009;**5** (6):1937–1947.

107. Bassleer, C. T., J. P. A. Combal, S. Bougaret, and M. Malaise. Effects of chondroitin sulfate and interleukin-1β on human articular chondrocytes cultivated in clusters. *Osteoarthr. Cartil.* 1998;**6**:196–204.

108. Galtrey, C. M., and J. W. Fawcett. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. *Brain Res. Rev.* 2007;**54**:1–18.

109. Lee, J., M. J. Cuddihy, and N. A. Kotov. Three-dimensional cell culture matrices: State of the art. *Tissue Eng.: Part B* 2008;**14**(1):61–86.

110. Poutanen, K. and P. Forssell. Modification of starch properties with plasticizers. *Trends Polym. Sci.* 1996;**4**:128–132.

111. Reis, R. L., and A. M. Cunha. Characterization of two biodegradable polymers of potential application within the biomaterials field. *J. Mater. Sci. Mater. Med.* 1995;**6**:786–792.

112. Gomes, M. E., A. S. Ribeiro, P. B. Malafaya, R. L. Reis, and A. M. Cunha. A new approach based on injection moulding to produce biodegradable starch-based polymeric scaffolds: Morphology, mechanical and degradation behaviour. *Biomaterials* 2001;**22**:883–889.

113. Gomes, M. E., J. S. Godinho, D. Tchalamov, A. M. Cunha, and R. L. Reis. Alternative tissue engineering scaffolds based on starch: Processing methodologies, morphology, degradation and mechanical properties. *Mater. Sci. Eng.* 2002;**20**:19–26.

114. Espigares, I., C. Elvira, J. E. Mano, B. Vazquez, J. S. Roman, and R. L. Reis. New partially degradable and bioactive acrylic bone cements based on starch blends and ceramic fillers. *Biomaterials* 2002;**23**:1883–1895.

115. Boesel, L. F., and R. L. Reis. The effect of water uptake on the behaviour of hydrophilic cements in confined environments. *Biomaterials* 2006;**27**:5627–5633.

116. Silva, G. A., F. J. Costa, N. M. Neves, O. P. Coutinho, A. C. P. Dias, and R. L. Reis. Entrapment ability and release profile of corticosteroids from starch-based particles. *J. Biomed. Mater. Res.* 2005;**73**:234–243.

117. Malafaya, P. B., F. Stappers, and R. L. Reis. Starch-based microspheres produced by emulsion crosslinking with a potential media dependent responsive behavior to be used as drug delivery carriers. *J. Mater. Sci.: Mater. Med.* 2006;**17**:371–377.

118. Ghosh, S., J. C. Viana, R. L. Reis, and J. F. Mano. Bi-layered constructs based on poly(L-lactic acid) and starch for tissue engineering of osteochondral defects. *Mater. Sci. Eng.: C* 2008;**28**(1):80–86.

119. Balmayor, E. R., K. Tuzlakoglu, H. S. Azevedo, and R. L. Reis. Preparation and characterization of starch-poly-ε-caprolactone microparticles incorporating

bioactive agents for drug delivery and tissue engineering applications. *Acta Biomater*. 2009;**5**(4):1035–1045.

120. Ye, C., P. Hu, M. Ma, Y. Xiang, R. Liu, and X. Shang. PHB/PHBHHx scaffolds and human adipose-derived stem cells for cartilage tissue engineering. *Biomaterials* 2009;**30**(26):4401–4406.
121. Misra, S. K., T. I. Ansari, S. P. Valappil, D. Mohn, S. E. Philip, W. J. Stark, I. Roy, J. C. Knowles, V. Salih, and A. R. Boccaccini. Poly(3-hydroxybutyrate) multifunctional composite scaffolds for tissue engineering applications. *Biomaterials* 2010;**31**(10):2806–2915.
122. Wang, L., N. H. Dormer, L. Bonewald, and M. S. Detamore. Osteogenic differentiation of human umbilical cord mesenchymal stromal cells in polyglycolic acid scaffolds. *Tissue Eng.: Part A* 2010;**16**(6):1937–1948.
123. Cao, H. and N. Kuboyama. A biodegradable porous composite scaffold of PGA/beta-TCP for bone tissue engineering. *Bone* 2010;**46**(2):386–395.
124. Middleton, J. C. and A. J. Tipton. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials* 2000;**21**:2335–2346.
125. Wang, S., Y. Zhang, H. Wang, G. Yin, and Z. Dong. Fabrication and properties of the electrospun polylactide/silk fibroin-gelatin composite tubular scaffold. *Biomacromolecules* 2009;**10**:2240–2244.
126. Haaparanta, A. M., S. Haimi, V. Ellä, N. Hopper, S. Miettinen, R. Suuronen, and M. Kellomäki. Porous polylactide/beta-tricalcium phosphate composite scaffolds for tissue engineering applications. *J. Tissue. Eng. Regen. Med.* 2010;**4**(5):366–373.
127. Miller, R. A., I. M. Brady, and D. E. Cutright. Degradation rates of oral resorbable implants (polylactates and polyglycolates): Rate modification with changes in PLA/PGA copolymer ratios. *J. Biomed. Mater. Res.* 1977;**11**:711–719.
128. Bible, E., D. Y. S. Chau, M. R. Alexander, J. Price, K. M. Shakesheff, and M. Modo. The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles. *Biomaterials* 2009;**30**(16):2985–2994.
129. Yao, L., S. Wang, W. Cui, R. Sherlock, C. O'Connell, G. Damodaran, A. Gorman, A. Windebank, and A. Pandit. Effect of functionalized micropatterned PLGA on guided neurite growth. *Acta Biomater*. 2009;**5**:580–588.
130. Ngiam, M., S. Liao, A. J. Patil, Z. Cheng, C. K. Chan, and S. Ramakrishna. The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. *Bone* 2009;**45**:4–16.
131. Jose, M. V., V. Thomas, K. T. Johnson, D. R. Dean, and E. Nyairo. Aligned PLGA/HA nanofibrous nanocomposite scaffolds for bone tissue engineering. *Acta Biomater*. 2009;**5**:305–315.
132. Hu, X., H. Shen, F. Yang, J. Bei, and S. Wang. Preparation and cell affinity of microtubular orientation-structured PLGA(70/30) blood vessel scaffold. *Biomaterials* 2008;**29**(21):3128–3136.
133. Garkhal, K., S. Verma, K. Tikoo, and N. Kumar. Surface modified poly(L-lactide-*co*-epsilon-caprolactone) microspheres as scaffold for tissue engineering. *J. Biomed. Mater. Res. A* 2007;**82**(3):747–756.
134. Kim, J. H., P. H. Choung, I. Y. Kim, K. T. Lim, H. M. Son, Y. H. Choung, C. S. Cho, and J. H. Chung. Electrospun nanofibers composed of poly(ε-caprolactone)

and polyethylenimine for tissue engineering applications. *Mater. Sci. Eng.: C* 2009;**29**:1725–1731.

135. Guarino, V., F. Causa, P. Taddei, M. D. Foggia, G. Ciapetti, D. Martini, C. Fagnano, N. Baldini, and L. Ambrosio. Polylactic acid fibre-reinforced polycaprolactone scaffolds for bone tissue engineering. *Biomaterials* 2008;**29**: 3662–3670.
136. Prior, T. D., D. L. Grace, J. B. MacLean, P. W. Allen, P. G. Chapman, and A. Day. Correction of hallux abductus valgus by Mitchell's metatarsal osteotomy: Comparing standard fixation methods with absorbable polydioxanone pins. *Foot* 1997;**7**:121–125.
137. Sokolsky-Papkov, M., K. Agashi, A. Olaye, K. Shakesheff, and A.J. Domb. Polymer carriers for drug delivery in tissue engineering. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):187–206.
138. Jain, J. P., S. Modi, A. J. Domb, and N. Kumar. Role of polyanhydrides as localized drug carriers. *J. Control. Release* 2005;**103**:541–563.
139. Jain, J. P., D. Chitkara, and N. Kumar. Polyanhydrides as localized drug delivery carrier: An update. *Expert Opin. Drug Deliv.* 2008;**5**(8):889–907.
140. Ibim, S. E. U., M. Attawia, V. R. Shastri, S. F. El-Amin, and R. Bronson. Preliminary in vivo report on the osteocompatibility of poly(anhydride-co-imides) evaluated in a tibial model. *J. Biomed. Mater. Res.* 1998;**43**:374–379.
141. Griffin, J., A. Carbone, R. Delgado-Rivera, S. Meiners, and K. E. Uhrich. Design and evaluation of novel polyanhydride blends as nerve guidance conduits. *Acta Biomater* 2010;**6**(6):1917–1924.
142. Jain, J. P., S. Modi, and N. Kumar. Hydroxy fatty acid based polyanhydride as drug delivery system: Synthesis, characterization, *in vitro* degradation, drug release, and biocompatibility. *J. Biomed. Mater. Res. A* 2008;**84**:740-752.
143. Modi, S., J. P. Jain, and N. Kumar. Synthesis, characterization, and degradation of poly(ester-anhydride) for particulate delivery. *Israel J. Chem.* 2005;**45**: 401–409.
144. Modi, S., J. P. Jain, A. J. Domb, and N. Kumar. Copolymers of pharmaceutical grade lactic acid and sebacic acid: Drug release behavior and biocompatibility. *Eur. J. Pharm. Biopharm.* 2006;**64**:277–286.
145. Gunatillake, P. A., and G. F. Meijs. Polyurethanes in biomedical engineering. In Encyclopedia of Materials: Science and Technology: K. H. J. Buschow, R. W. Cahn, M. C. Flemings, B.J. Ilschner, F. J. Kramer, S. Mahajan, and P. Veyssière, (Eds.). Elsevier: Oxford, 2001, pp. 7746–7752.
146. Guelcher, S. A. Biodegradable polyurethanes: Synthesis and applications in regenerative medicine. *Tissue Eng.: Part B Rev.* 2008;**14**:3–17.
147. Woźniak, P., M. Bil, J. Ryszkowska, P. Wychowański, E. Wróbel, A. Ratajska, G. Hoser, J. Przybylski, K. J. Kurzydłowski, and M. Lewandowska-Szumieł. Candidate bone-tissue-engineered product based on human-bone-derived cells and polyurethane scaffold. *Acta Biomater.* 2010;**6**(7):2484–2493.
148. Li, B., J. M. Davidson, and S. A. Guelcher. The effect of the local delivery of platelet-derived growth factor from reactive two-component polyurethane scaffolds on the healing in rat skin excisional wounds. *Biomaterials* 2009;**30**(20): 3486–3494.

149. Shah, P. N., R. L. Manthe, S. T. Lopina, and Y. H. Yun. Electrospinning of l-tyrosine polyurethanes for potential biomedical applications. *Polymer* 2009; **50** (10):2281–2289.

150. Saito, N., T. Okada, H. Horiuchi, H. Ota, J. Takahashi, N. Murakami, M. Nawata, S. Kojima, K. Nozaki, and K. Takaoka. Local bone formation by injection of recombinant human bone morphogenetic protein-2 contained in polymer carriers. *Bone* 2003;**32**:381–386.

151. Toyoda, H., H. Terai, R. Sasaoka, K. Oda, and K. Takaoka. Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist. *Bone* 2005;**37**:555–562.

152. Adelöw, C., T. Segura, J. A. Hubbell, and P. Frey. The effect of enzymatically degradable poly(ethylene glycol) hydrogels on smooth muscle cell phenotype. *Biomaterials* 2008;**29**:314–326.

153. Lieb, E., J. Tessmar, M. Hacker, C. Fischbach, D. Rose, T. Blunk, A. G. Mikos, A. Gopferich, and M. B. Schulz. Poly(D,L-lactic acid)-poly(ethylene glycol) monomethyl ether diblock copolymers control adhesion and osteoblastic differentiation of marrow stromal cells. *Tissue Eng*. 2003;**9**:71–84.

154. Cui, W., X. Zhu, Y. Yang, X. Li, and Y. Jin. Evaluation of electrospun fibrous scaffolds of poly(DL-lactide) and poly(ethylene glycol) for skin tissue engineering. *Mater. Sci. Eng.: C* 2009;**29**:1869–1876.

155. Strehin, I., Z. Nahas, K. Arora, T. Nguyen, and J. Elisseeff. A versatile pH sensitive chondroitin sulfate–PEG tissue adhesive and hydrogel. *Biomaterials* 2010;**31**(10):2788–2797.

156. Vashi, A. V., E. Keramidaris, K. M. Abberton, W. A. Morrison, J. L. Wilson, A. J. O'Connor, J. J. Cooper-White, and E. W. Thompson. Adipose differentiation of bone marrow-derived mesenchymal stem cells using Pluronic F-127 hydrogel in vitro. *Biomaterials* 2008;**29**:573–579.

157. Ruszymah, B. H., K. Chua, M. A. Latif, F. N. Hussein, and A. B. Saim. Formation of in vivo tissue engineered human hyaline cartilage in the shape of a trachea with internal support. *Int. J. Pediatr. Otorhinolaryngol*. 2005;**69**: 1489–1495.

158. Liu, Y., F. Chen, W. Liu, L. Cui, Q. Shang, and W. Xia. Repairing large porcine full-thickness defects of articular cartilage using autologous chondrocyte-engineered cartilage. *Tissue Eng*. 2002;**8**:709–721.

159. Cortiella, J., J. E. Nichols, K. Kojima, I. J. Bonassar, P. Dargon, and A. K. Roy. Tissue-engineered lung: An in vivo and in vitro comparison of polyglycolic acid and Pluronic F-127 hydrogel/somatic lung progenitor cell constructs to support tissue growth. *Tissue Eng*. 2006;**12**:1213–1225.

160. Shachaf, Y., M. Gonen-Wadmany, and D. Seliktar. The biocompatibility of Pluronic® F127 fibrinogen-based hydrogels. *Biomaterials* 2010;**31**(10):2836–2847.

161. Yaszemski, M. J., R. G. Payne, W. C. Hayes, R. Langer, and A. G. Mikos. In vitro degradation of a poly(propylene fumarate)-based composite materials. *Biomaterials* 1996;**17**:2120–2130.

162. Peter, S. J., L. Lu, D. J. Kim, and A. G. Mikos. Marrow stromal osteoblast function on a poly(propylene fumarate)/-tricalcium phosphate biodegradable orthopaedic composite. *Biomaterials* 2000;**21**:1207–1213.

163. Temenoff, J. S. and A. G. Mikos. Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials* 2000;**2**:2405–2412.

164. Horch, R. A., N. Shahid, A. S. Mistry, M. D. Timmer, A. G. Mikos, and A. R. Barron. Nanoreinforcement of poly(propylene fumarate)-based networks with surface modified alumoxane nanoparticles for bone tissue engineering. *Biomacromolecules* 2004;**5**(5):1990–1998.

165. Jabbari, E., S. F. Wang, L. C. Lu, J. A. Gruetzmacher, S. Ameenuddin, I. E. Hefferan, B. L. Currier, A. J. Windebank, and M. J. Yaszemski. Synthesis, material properties, and biocompatibility of a novel self-cross-linkable poly (caprolactone fumarate) as an injectable tissue engineering scaffold. *Biomaterials* 2005;**6**:2503–2511.

166. Payne, R. G., J. S. McGonigle, M. J. Yaszemski, A. W. Yasko, and A. G. Mikos. Development of an injectable, in situ crosslinkable, degradable polymeric carrier for osteogenic cell populations. Part 3. Proliferation and differentiation of encapsulated marrow stromal osteoblasts cultured on crosslinking poly(propylene fumarate). *Biomaterials* 2002;**23**:4381–4387.

167. Nicol, F., M. Wong, F. C. MacLaughlin, J. Perrard, E. Wilson, and J. L. Nordstrom. L-glutamate, an anionic polymer, enhances transgene expression for plasmids delivered by intramuscular injection with *in vivo* electrophoration. *Gene Ther*. 2002;**9**:1351–1358.

168. Cai, K., K. Yao, X. Hou, Y. Wang, Y. Hou, Z. Yang, X. Li, and H. Xie. Improvement of the functions of osteoblasts seeded on modified poly(D,L-lactic acid) with poly(aspartic acid). *J. Biomed. Mater. Res*. 2002;**62**:283–291.

169. Nojehdehian, H., F. Moztarzadeh, H. Baharvand, H. Nazarian, and M. Tahriri. Preparation and surface characterization of poly-l-lysine-coated PLGA microsphere scaffolds containing retinoic acid for nerve tissue engineering: In vitro study. *Colloids Surf. B Biointerf*. 2009;**73**:23–29.

170. Hoffman, R. M. To do tissue culture in two or three dimensions? That is the question. *Stem Cells* 1993;**11**(2):105–111.

171. Winter, G. D. and B. J. Simpson. Heterotopic bone formed in a synthetic sponge in the skin of young pigs. *Nature* 1969;**223**(5201):88–90.

172. Liebmann-Vinson, A., J. J. Hemperly, R. D. Guarino, C. A. Spargo, and M. A. Heidaran. Bioactive extracellular matrices: Biological and biochemical evaluation. In *Tissue Engineering and Biodegradable Equivalents Scientific and Clinical Applications*. K. U. Lewandrowski, D. L. Wise, D. J. Trantolo, J. D. Gresser, M. J. Yaszemski, and D. E. Altobelli, (Eds.). Marcel Dekker: New York, 2002.

173. Yates, K. E., F. Allemann, and J. Glowacki. Phenotypic analysis of bovine chondrocytes cultured in 3D collagen sponges: Effect of serum substitutes. *Cell Tissue Bank* 2005;**6**(1):45–54.

174. Haider, M., J. Cappello, H. Ghandehari, and K. W. Leong. In vitro chondrogenesis of mesenchymal stem cells in recombinant silk-elastinlike hydrogels. *Pharm. Res*. 2008;**25**(3):692–699.

175. Moutos, F. T., L. E. Freed, and F. Guilak. A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. *Nature Mater*. 2007;**6**:162–167.

176. Gabler, F., S. Frauenschuh, J. Ringe, C. Brochhausen, P. Gotz, C. J. Kirkpatrick, M. Sittinger, H. Schubert, and R. Zehbe. Emulsion-based synthesis of PLGA-microspheres for the in vitro expansion of porcine chondrocytes. *Biomol. Eng.* 2007;**24**(5):515–520.

177. Wahl, D. A., E. Sachlos, C. Liu, and J. T. Czernuszka. Controlling the processing of collagen-hydroxyapatite scaffolds for bone tissue engineering. *J. Mater. Sci. Mater. Med.* 2007;**18**(2):201–209.

178. Holmes, T. C. Novel peptide-based biomaterial scaffolds for tissue engineering. *Trends Biotechnol.* 2002;**20**(1):16–21.

179. Kim, T. K., J. J. Yoon, D. S. Lee, and T. G. Park. Gas foamed open porous biodegradable polymeric microspheres. *Biomaterials* 2006;**27**(2):152–159.

180. Yang, S., K. F. Leong, Z. Du, and C. K. Chua. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Eng.* 2001;**7**(6):679–689.

181. Yang, S., K. F. Leong, Z. Du, and C. K. Chua. The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. *Tissue Eng.* 2002; **8**(1):1–11.

182. Nam, Y. S., J. J. Yoon, and T. J. Park. A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive. *J. Biomed. Mater. Res.* 2000;**53**(1):1–7.

183. Hou, Q., D. W. Grijpma, and J. Feijen. Porous polymeric structures for tissue engineering prepared by a coagulation, compression moulding and salt leaching technique. *Biomaterials* 2003;**24**(11):1937–1947.

184. Oh, S. H., S. G. Kang, and J. H. Lee. Degradation behavior of hydrophilized PLGA scaffolds prepared by melt-molding particulate-leaching method: comparison with control hydrophobic one. *J. Mater. Sci. Mater. Med.* 2006; **17**(2):131–137.

185. Kim, S. E., J. H. Park, Y. W. Cho, H. Chung, S. Y. Jeong, E. B. Lee, and I. C. Kwon. Porous chitosan scaffold containing microspheres loaded with transforming growth factor-β1: Implications for cartilage tissue engineering. *J. Control. Release* 2003;**91**(3):365–374.

186. Choi, J. S., S. J. Lee, G. J. Christ, A. Atala, and J. J. Yoo. The influence of electrospun aligned poly(varepsilon-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. *Biomaterials* 2008;**29** (19):2899–2906.

187. Vozzi, G., C. Flaim, A. Ahluwalia, and S. Bhatia. Fabrication of PLGA scaffolds using soft lithography and microsyringe deposition. *Biomaterials* 2003;**24**(14): 2533–2540.

188. Hollister, S. J. Porous scaffold design for tissue engineering. *Nature Mater.* 2005;**4**:518–524.

189. Williams, J. M., A. Adewunmi, R. M. Schek, C. L. Flanagan, P. H. Krebsbach, S. E. Feinberg, S. J. Hollister, and S. Das. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* 2005;**26**(23):4817–4827.

190. Moroni, L. and C. A. V. Blitterswijk. Converge and regenerate. *Nature Mater.* 2006;**5**:437–438.

191. Sun, W., B. Starly, A. Darling, and C. Gomez. Computer-aided tissue engineering: Application to biomimetic modelling and design of tissue scaffolds. *Biotech. Appl. Biochem.* 2004;**39**(Pt 1):49–58.

192. Yancopoulos, G. D., S. Davis, N. W. Gale, J. S. Rudge, S. J. Wiegand, and J. Holash. Vascular-specific growth factors and blood vessel formation. *Nature* 2000;**407**(6801):242–248.

193. Babensee, J. E., L. V. McIntire, and A. G. Mikos. Growth factor delivery for tissue engineering. *Pharm. Res.* 2000;**17**(5):497–504.

194. Richardson, T. P., M. C. Peters, A. B. Ennett, and D. J. Mooney. Polymeric system for dual growth factor delivery. *Nature Biotechnol.* 2001;**19**(11):1029–1034.

195. Shea, L. D., E. Smiley, J. Bonadio, and D. J. Mooney. DNA delivery from polymer matrices for tissue engineering. *Nature Biotechnol.* 1999;**17**(6):551–554.

196. Putnam, D. Polymers for gene delivery across length scales. *Nature Mater.* 2006; **5**(6):439–451.

197. Guimard, N. K., N. Gomez, and C. E. Schmidt. Conducting polymers in biomedical engineering. *Prog. Polym. Sci.* 2007;**32**(8–9):876–921.

198. Gong, F., X. Cheng, S. Wang, Y. Zhao, Y. Gao, and H. Cai. Heparin-immobilized polymers as non-inflammatory and non-thrombogenic coating materials for arsenic trioxide eluting stents. *Acta Biomater.* 2010;**6**(2):534–546.

199. Friend, J. R. and W.-S. Hu. Engineering a bioartificial liver support. *Frontiers Tissue Eng.* 1998;678–695.

200. Mayer, J., E. Karamuk, T. Akaike, and E. Wintermantel. Matrices for tissue engineering-scaffold structure for a bioartificial liver support system. *J. Control. Release* 2000;**64**(1–3):81–90.

201. Stamatialis, D. F., B. J. Papenburg, M. Gironés, S. Saiful, S. N. M. Bettahalli, S. Schmitmeier, and M. Wessling. Medical applications of membranes: Drug delivery, artificial organs and tissue engineering. *J. Memb. Sci.* 2008;**308**(1–2):1–34.

202. Nieminen, T., I. Kallela, J. Keränen, I. Hiidenheimo, H. Kainulainen, E. Wuolijoki, and I. Rantala. In vivo and in vitro degradation of a novel bioactive guided tissue regeneration membrane. *Int. J. Oral. Maxillofacial. Surg.* 2006;**35** (8):727–732.

203. Lu, Q., A. Simionescu, and N. Vyavahare. Novel capillary channel fiber scaffolds for guided tissue engineering. *Acta Biomater.* 2005;**1**(6):607–614.

204. Wan, Y., H. Wu, B. Xiao, X. Cao, and S. Dalai. Chitosan-*g*-polycaprolactone copolymer fibrous mesh scaffolds and their related properties. *Polym. Adv. Technol.* 2009;**20**:795–801.

205. Kumbar, S. G., S. P. Nukavarapu, R. James, I. S. Nair, and C. T. Laurencin. Electrospun poly(lactic acid-co-glycolic acid) scaffolds for skin tissue engineering. *Biomaterials* 2008;**29**(30):4100–4107.

206. Ghalbzouri, A. E., E. N. Lamme, C. V. Blitterswijk, J. Koopman, and M. Ponec. The use of PEGT/PBT as a dermal scaffold for skin tissue engineering. *Biomaterials* 2004;**25**:2987–2996.

207. Harrison, C. A. and S. MacNeil. The mechanism of skin graft contraction: An update on current research and potential future therapies. *Burns* 2008; **34**(2):153–163.

208. MacNeil, S. Biomaterials for tissue engineering of skin. *Materialstoday* 2008;**11**:26–35.

209. Stevens, M. M. Biomateials for bone tissue engineering. *Materialstoday* 2008;**11**:18–25.

210. Hedberg, E. L., A. Tang, R. S. Crowther, D. H. Carney, and A. G. Mikos. Controlled release of an osteogenic peptide from injectable biodegradable polymeric composites. *J. Control. Release* 2009;**84**(3):137–150.

211. Arrington, E. D., W. J. Smith, H. G. Chambers, A. L. Bucknell, and N. A. Davino. Complications of iliac crest bone graft harvesting. *Clin. Orthop. Relat. Res.* 1996;**329**:300–309.

212. Lee, S. H. and H. Shin. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5) :339–359.

213. Silva, G. A., O. P. Coutinho, P. Ducheyne, and R. L. Reis. Materials in particulate form for tissue engineering. 2. Applications in bone. *J. Tissue Eng. Regen. Med.* 2007;**1**:97–109.

214. Zhang, Y., B. Shi, C. Li, Y. Wang, Y. Chen, W. Zhang, T. Luo, and X. Cheng. The synergetic bone-forming effects of combinations of growth factors expressed by adenovirus vectors on chitosan/collagen scaffolds. *J. Control. Release* 2009;**136** (3):172–178.

215. Porter, J. R., A. Henson, and K. C. Popat. Biodegradable poly(epsilon-caprolactone) nanowires for bone tissue engineering applications. *Biomaterials* 2009;**30**(5): 780–788.

216. Shi, X., Y. Wang, L. Ren, W. Huang, and D. A. Wang. A protein/antibiotic releasing poly(lactic-co-glycolic acid)/lecithin scaffold for bone repair applications. *Int. J. Pharm.* 2009;**373**(1–2):85–92.

217. Moioli, E. K., P. A. Clark, X. Xin, S. Lal, and J. J. Mao. Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering. *Adv. Drug Deliv. Rev.* 2007;**59**(4–5):308–324.

218. Kaigler, D., J. A. Cirelli, and W. V. Giannobile. Growth factor delivery for oral and periodontal tissue engineering. *Expert Opin. Drug Deliv.* 2006;**3**:647–662.

219. Puppi, D., F. Chiellini, A. M. Piras, and E. Chiellini. Polymeric materials for bone and cartilage repair. *Prog. Polym. Sci.* 2008;**33**(12):1119–1198.

220. Smith, A. J. and P. T. Sharpe. Biological tooth replacement and repair. In *Principles of Tissue Engineering*, 3rd ed.: R. Lanza, R. Langer, and J. Vacanti, (Eds.). Academic Press: Burlington, 2007, pp. 1067–1077.

221. Young, S., K. A. Athanasiou, A. G. Mikos, and ME.-K. Wong. Oral and maxillofacial surgery. In *Principles of Tissue Engineering*, 3rd ed. R. Lanza, R. Langer, and J. Vacanti, (Eds.). Academic Press: Burlington, 2007, pp. 1079–1094.

222. Zhang, H., H. K. Fong, W. V. Giannobile, and M. J. Somerman. Periodontal tissue engineering. In *Principles of Tissue Engineering*, 3rd ed. R. Lanza, R. Langer, J. Vacanti, (Eds.). Academic Press: Burlington, 2007, pp. 1095–1109.

223. Edwards, P. C. and J. M. Mason. Gene-enhanced tissue engineering for dental hard tissue regeneration: (2) Dentin-pulp and periodontal regeneration. *Head Face Med.* 2006;**2**:16.

224. Edwards, P. C. and J. M. Mason. Gene-enhanced tissue engineering for dental hard tissue regeneration: (1) Overview and practical considerations. *Head Face Med*. 2006;**2**:12.

225. Chung, C. and J. A. Burdick. Engineering cartilage tissue. *Adv. Drug Deliv. Rev*. 2008;**60**:243–262.

226. Nesic, D. R. Whiteside, M. Brittberg, D. Wendt, I. Martin, and P. Mainil-Varlet. Cartilage tissue engineering for degenerative joint disease. *Adv. Drug Deliv. Rev*. 2006;**58**:300–322.

227. Hüsing, B., B. Bührlen, and S. Gaisser. *Human Tissue Engineered Products—Today's Markets and Future Prospects*. Fraunhofer Institute for Systems and Innovation Research: Karlsruhe, Germany, pp. 1–129.

228. Elisseeff, J., W. McIntosh, K. Fu, T. Blunk, and R. Langer. Controlled-release of IGF-I and TGF-P 1 in a photopolymerizing hydrogel for cartilage tissue engineering. *J. Orthop. Res*. 2001;**19**:1098–1104.

229. Hedberg, E. L., C. L. Shih, L. A. Solchaga, A. I. Caplan, and A. G. Mikos. Controlled release of hyaluronan oligomers from biodegradable polymeric microparticle carriers. *J. Control. Release* 2004;**100**:257–266.

230. Aigner, T., A. Sachse, P. M. Gebhard, and H. I. Roach. Osteoarthritis: Pathobiology—targets and ways for therapeutic intervention. *Adv. Drug Deliv. Rev*. 2006;**58**: 128–149.

231. Aigner, T. and J. Stove. Collagen—major component of the physiological cartilage matrix, major target of cartilage degeneration, major tools in cartilage repair. *Adv. Drug Deliv. Rev*. 2003;**55**:1569–1593.

232. Sales, K. M., H. J. Salacinski, N. Alobaid, M. Mikhail, V. Balakrishnan, and A. M. Seifalian. Advancing vascular tissue engineering: The role of stem cell technology. *Trends Biotechnol*. 2005;**23**:461–467.

233. Leora, J., Y. Amsalema, and S. Cohen. Cells, scaffolds, and molecules for myocardial tissue engineering. *Pharmacol. Therap*. 2005;**105**:151–163.

234. Masuda, S., T. Shimizu, M. Yamato, and T. Okano. Cell sheet engineering for heart tissue repair. *Adv. Drug Deliv. Rev*. 2008;**60**:277–285.

235. Wu, K. H., X. M. Moa, Y. L. Liu, Y. S. Zhang, and Z. C. Han. Stem cells for tissue engineering of myocardial constructs. *Ageing Res. Rev*. 2007;**6**:289–301.

236. Cao, H., T. Liu, and S. Y. Chew. The application of nanofibrous scaffolds in neural tissue engineering. *Adv. Drug Deliv. Rev*. 2009;**61**(12):1055–1064.

237. Willerth, S. M. and S. E. Sakiyama-Elbert. Approaches to neural tissue engineering using scaffolds for drug delivery. *Adv. Drug Deliv. Rev*. 2007;**59**(4–5):325–338.

238. Humes, H. D., S. M. MacKay, A. J. Funke, and D. A. Buffington. Tissue engineering of a bioartificial renal tubule assist device: In vitro transport and metabolic characteristics. *Kidney Int*. 1999;**55**:2502–2514.

239. Bianco, P. and P. G. Robey. Stem cells in tissue engineering. *Nature* 2001;**414**:118–121.

240. Furth, M. E., A. Atala, and M. E. V. Dyke. Smart biomaterials design for tissue engineering and regenerative medicine. *Biomaterials* 2007;**28**:5068–5073.

241. Zhao, X. and S. Zhang. Fabrication of molecular materials using peptide construction motifs. *Trends Biotechnol*. 2004;**22**(9):470–476.

242. Guler, M. O., L. Hsu, S. Soukasene, D. A. Harrington, J. F. Hulvat, and S. I. Stupp. Presentation of RGDS epitopes on self-assembled nanofibers of branched peptide amphiphiles. *Biomacromolecules* 2006;**7**(6):1855–1863.

243. Hartgerink, J. D., E. Beniash, and S. I. Stupp. Peptide-amphiphile nanofibers: A versatile scaffold for the preparation of self-assembling materials. *Proc. Natl. Acad. Sci. U.S.A.* 2002;**99**(8):5133–5138.

244. Chan, G. and D. J. Mooney. New materials for tissue engineering: Towards greater control over the biological response. *Trends Biotechnol.* 2008;**26** (7):382–392.

245. Wang, D. A., S. Varghese, B. Sharma, I. Strehin, S. Fermanian, J. Gorham, D. H. Fairbrother, B. Cascio, and J. H. Elisseeff. Multifunctional chondroitin sulphate for cartilage tissue-biomaterial integration. *Nature Mater* 2007;**6**(5):385–392.

246. Mooney, D. J. and E. A. Silva. A glue for biomaterials. *Nature Mater.* 2007;**6**:327–328.

247. Heidaran, M. A. Tissue engineering: A biological solution for tissue damage, loss or end stage organ failure. *Iran. Biomed. J.* 2000;**4**:1–5.

CHAPTER 17

INJECTABLE POLYMERS

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CONTENTS

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17.1 INTRODUCTION

One of the main challenges associated with clinical applicability of new biomaterials is the requirement for rapid and innocuous implantation. Traditional implantable materials have required surgeries that greatly increase the tissue trauma and risk of infection and overall morbidity [1, 2]. Additionally, the surgical technique itself may lead to inflammation that can negatively affect outcomes and tissue–material integration [3]. As a result, interest has been focused on biopolymers that can be injected into the body in a minimally invasive manner while retaining their abilities to repair or regenerate damaged tissues. These in situ forming injectable systems have the additional advantage of being able to completely fill irregular and less accessible defects, resulting in close tissue–biomaterial integration and improved outcomes [4–6].

Injectable systems have been designed for both tissue engineering and drug delivery applications. In one case, drug-loaded hydrogels or microspheres are injected into the patient to achieve sustained local or systemic delivery. In tissue engineering, a biomaterial is loaded with cells or bioactive agents and injected into a tissue defect. This implanted cell–biomaterial combination will then interact with the surrounding environment in order to form a functional replacement for tissue lost to disease or trauma.

While injectable systems have many advantages over other biomaterial implants, they also pose some challenges to achieve full efficacy. The monomer that is injected must exhibit low toxicity to encapsulated cells, and the polymerization method must not cause chemical or heat damage to the surrounding tissue. This constraint sharply limits the chemistries and techniques available for in situ scaffold solidification. In situ forming injectable systems also often result in materials with weaker mechanical properties than might be otherwise possible with pre-formed implantable materials. The mechanically weak implants possible with most in situ polymerization technologies have restricted much of these injectable polymers to the tissue engineering of soft tissues including cartilage, intervertebral disk, dermal fillers, and ophthalmic materials [6–9]. Nevertheless, injectable systems have also been used as temporary fillers for defects in harder tissues such as bone [10–13].

There exist two major classes of injectable polymers in clinical use today. Naturally occurring biological polymers such as collagens, glycosaminoglycans (GAGs), and other extracellular matrix (ECM) molecules have been used extensively in injectable materials [14]. These materials have the advantages of exhibiting significant biological functionality and activity and are readily biodegradable. However, they are derived from biological sources, which raises significant concerns of immunogenicity, homogeneity, and difficulty in their control and modification. The potential for disease transmission, while very low, must also be recognized.

On the other extreme are entirely synthetic injectable polymers. There are a wide variety of such materials, including such diverse polymers as poly(ethylene glycol) (PEG), poly(*N*-isopropylacrylamide) (NIPAAm), and suspensions of

solid poly(lactide-*co*-glycolide) (PLG) microspheres. These polymers can be easily modified to produce a variety of tightly controlled synthetic materials for injection [15, 16]. Due to the extremely flexible nature of synthetic polymer chemistry, these can be modified to form solids or gels in situ using a variety of triggers: pH, ionic strength, light, and temperature. Pitfalls to the use of synthetic polymers revolve around their relatively weak biological functionality, biodegradability, and concerns about the toxicity of unpolymerized monomers and leaching of chemicals necessary for polymerization.

More recently, researchers have begun to develop biosynthetic composite materials to capitalize on the advantages of both systems [4, 16, 17]. These materials include covalent and noncovalent interactions between a synthetic polymer backbone and biologically active molecules (often extracellular matrix components or relevant proteins). This combination has resulted in injectable materials with well-controlled physical properties that exhibit significant biological functionality and better biocompatibility.

A number of promising injectable materials have been developed for clinical use, and many more are in development. This chapter will examine injectable polymers currently in clinical use or clinical development. First, we will examine the underlying chemistry and design of in situ forming materials. Additional design parameters affecting material properties and functionality will be discussed. We will conclude with a thorough overview of common injectable materials in the clinic and their uses, with an eye to highlighting future trends in the field.

17.2 CHEMISTRY OF IN SITU FORMING MATERIALS

Frequently, there exists a mismatch between the desired material properties of an injectable polymer during injection and its long-term properties at the implant site. Thus, much of the material's design associated with injectable polymers focuses on how to change the injected material in situ into a crosslinked solid or gel. This in situ crosslinking is carried out by a number of triggers, the most popular being photoactivation, temperature, pH, or ionic strength. This section will briefly examine the major chemistries involved in each crosslinking method as well as potential drawbacks.

17.2.1 Photoinitiated Biomaterials

In situ crosslinking of an injected material can be accomplished using a light source and photoinitator that results in free radical formation. A standard free radical-driven polymerization of appropriately functionalized monomers results, frequently using (meth)acrylate or other unsaturated derivatives [16, 18–20]. The speed and degree of crosslinking can be tailored with varying light wavelengths and photoinitiator choice. Commonly used photoinitiation systems use ultraviolet or visible light and a variety of photoinitiators [20–22].

A number of studies have shown that transdermal photopolymerization of injected materials is possible, though this is highly contingent on the tissue thickness and wavelength of light chosen [23]. Generally, red and infrared wavelengths ($\sim$600–1000 nm) will penetrate much deeper into tissue than blue or ultraviolet [24, 25]. While deeper penetration and faster polymerization times can be obtained by using higher intensity light sources, tissue heating and damage may result. Alternatively, light can be applied directly to the injected material using minimally invasive instrumentation. Concerns exist about the biocompatibility of various photoinitation systems, as many are cytotoxic at high doses. Nevertheless, a number of low-concentration photoinitiation systems have been developed that exhibit minimal toxicity and still result in efficient crosslinking [21].

17.2.2 Thermogelling Biomaterials

As opposed to covalently crosslinked hydrogels, some injectable polymers spontaneously and reversibly form a gel upon heating, the opposite of what normally occurs with polymers. This curious behavior of thermally responsive polymers is due to lower critical solution temperature (LCST) behavior found in a specific class of biomaterials [19, 26, 27]. Generally, these materials include both hydrophobic and hydrophilic regions, such as the well-known block copolymer poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO), commonly known as Pluronics or poloxamers [26]. Upon an increase in temperature, the more hydrophobic PPO blocks aggregate and expel their associated water, forming a solid gel. This behavior is due to an energetic balance between the enthalpy associated with gelation and the entropy of free water molecules compared to PPO-associated water. An increase in temperature favors the energetic contribution of free water entropy, resulting in gel formation. Appropriately tweaking the size and hydrophilicity of the polymer blocks as well as the polymer concentration in solution can yield an LCST transition near body temperature. Thus, upon injection into the body, the liquid polymer solution can solidify without other crosslinking agents. This property has been used extensively for cell encapsulation due to its mild conditions and reversible nature. Commonly used thermally responsive polymers use the aforementioned poloxamers, poly(*N*-isopropylacrylamide) (NIPAAm), methylcellulose, and chitosan [27, 28]. While these gels tend to avoid the toxicity concerns of photopolymerization, they do not contain covalent crosslinks and are therefore significantly weaker.

17.2.3 Ionic Crosslinking of Biomaterials

Some biomaterials can be reversibly gelled using divalent cations such as Ca^{2+}. The cations form ionic bridges between two negatively charged groups on a polymer, effectively crosslinking the material. These ionic crosslinks can be broken using chelating agents such as citrate. Alginate, which contains

negatively charged carboxylate groups on its guluronate residues, is the most commonly used polymer in ionic crosslinking [29]. Alginate is a long-chain polysaccharide isolated from the cell walls of brown algae. It is composed of a complex combination of glycosidically linked mannuronate and guluronate residues; the guluronate residues contribute to the ionic crosslinking [30].

The degree of crosslinking and the subsequent gel properties can be modified by a variety of methods. Increasing calcium and alginate concentration can result in faster gelation and stronger mechanical properties [31, 32]. The composition of the alginate itself can also affect gelation. Increasing the ratio of guluronate to mannuronate and the molecular weight will result in stronger gels [30, 33]. As binding of calcium to guluronate residues is cooperative, longer blocks of guluronate residues will also yield stronger crosslinking [33, 34]. While this theoretically allows for very exact control over degree of crosslinking, the biological origin of alginate makes it difficult to obtain homogeneous and consistent samples.

17.2.4 Other In Situ Crosslinking Methodologies

A variety of other strategies exist for in situ crosslinking of injectable biomaterials. Several systems utilize spontaneous chemical reactions between two monomer solutions that are mixed together immediately prior to injection. Fibrinogen and thrombin are a typical example of this technique; upon mixing, thrombin converts soluble fibrinogen into a crosslinked fibrin network within seconds [35]. Another example of this strategy is the use of the spontaneous reaction of *N*-hydroxysuccinimide (NHS)-conjugated GAG molecules and amine-functionalized synthetic monomers. Not only does this reaction form a crosslinked network upon mixing, but the NHS moieties can also bind to amines in the surrounding tissue to improve tissue–material integration and adhesion [36].

Injectable biomaterials can also form networks through self-assembly. Upon mixing of monomers or an environmental trigger (osmolarity, pH, light, etc.), the materials spontaneously form a network structure through noncovalent interactions. Self-assembling systems are many and varied. Synthetic peptide amphiphiles that self-assemble through hydrophobic interactions have been used extensively to form nanofibrous scaffolds [37–39]. Other groups have used peptide/protein–ECM interactions to drive self-assembly, such as interactions between the polysaccharide heparin and polymer-conjugated heparin binding peptides [40, 41]. Peptides that mimic ECM proteins such as elastin and silk have been observed to self-associate upon a temperature change [42]. Self-assembled networks are readily reversible upon a change in buffer or extracellular fluid conditions, and their physical properties can be easily tailored by changing the concentrations, hydrophilicities, and molecular weights of their components.

Lastly, some injectable materials do not exhibit significant in situ forming properties. These materials frequently consist of a highly viscous “carrier”

solution containing within it microscale crosslinked particles such as microspheres or processed pieces of larger crosslinked gels. This approach has the advantages of not requiring complex and potentially cytotoxic crosslinking methodologies and requires no external stimuli after injection. Unsurprisingly, though, these injectable materials tend to have much shorter residence times at the tissue of interest, and may have compromised physical and biological properties as a result of their processing for injection.

17.3 DESIGN CONSIDERATIONS FOR INJECTABLE MATERIALS

While much of the design of injectable materials focuses on scaffold formation after injection, a number of other criteria must be considered in material selection and optimization. These considerations are determined by both clinical needs and biologically driven design for optimal results. While the specific concerns for each tissue and material are quite varied, a number of fundamental criteria are fairly universal. These include the handling characteristics of the material during preparation and injection, the mechanics and degradability of the scaffold in vivo, potential cytotoxicity and tissue damage associated with polymerization, and the immune response to the material.

17.3.1 Handling Characteristics

Injectable polymers have strict handling requirements in order to be useful clinically. Most clinical applications require that the material be viscous prior to injection in order to facilitate easier and more precise placement of the injected material in vivo. At the same time, the viscous solution must be easily injected through a small diameter needle. As a result, many injectable polymer solutions have been designed to include shear thinning properties. These materials have initially high viscosity, which decreases with increasing rate of shear. During injection, these materials will decrease viscosity, allowing for easier flow, but will exhibit high-viscosity behavior and limit material "creep" in vivo [43, 44]. When injection is followed by in situ crosslinking, the high viscosity enables the crosslinking to take effect before significant amounts of injected material is lost to the surrounding tissue. Additionally, shear thinning behavior can limit cell settling in cell-laden injections. Many long-chain polymer and biopolymer solutions exhibit shear thinning properties. These properties can be understood as a result of physical entanglement of polymer strands. At higher shear rates, the time scale of chain reentanglement is slower than the shear rate, resulting in lower resistance to flow. Thus, shear thinning properties can often be added to an injectable material by supplementing the solution with certain high-molecular-weight polymers.

Some injected materials have more specific rheological requirements. Orthopedic injectable biomaterials ideally exhibit characteristics similar to Bingham fluids. These fluids behave similarly to pastes or mayonnaise; they are a rigid

solid at low shear stresses until a "yield stress" is reached, where they will then deform as a viscous fluid [45]. The advantage of such a material design is that the material will stay in place between injection and crosslinking and can be appropriately "molded" by the physician prior to polymerization. While shear thinning fluids will still flow at low shear rates, albeit slowly, Bingham fluids will act as solids under such conditions. This yields great improvements in the geometrical requirements for orthopedic surgeries; knees can be operated on in such a way that defects that are not horizontal can still be filled with the biomaterial. As of yet, there are no clinical products exhibiting this behavior.

Materials such as fibrin glue that assemble spontaneously by the reaction or binding between two components must also consider mixing in their design. To achieve a homogeneous final product, the unreacted components must be mixed quickly and efficiently. This process is even more difficult given the viscosity of most polymer solutions. Mixing has been addressed through the use of specially designed mixing chambers in dispensing syringes. Additionally, the speed of the reaction can be carefully tailored by changing buffer conditions (pH, ionic strength, etc.) to enable adequate mixing time while still polymerizing quickly enough in situ.

17.3.2 Mechanics and Degradation

Until now, we have only addressed design criteria associated with the injection and crosslinking of the material. Once crosslinking is completed, injectable materials may have a range of mechanical and degradative properties. These properties should be tailored for the tissue and application in question.

Generally, injectable polymer networks will have fairly weak mechanical properties; Young's moduli range from tens to hundreds of kilopascals. Materials can be strengthened by increasing polymer concentration and decreasing molecular weight between crosslinks. Additionally, materials have been significantly strengthened by the addition of composite materials, including encapsulated microspheres and fibers [46, 47]. Addition of long-chain polymers that are not incorporated into the network (such as unmodified ECM molecules) can increase the strength of the construct through physical entanglement but may also interfere with the crosslinking process.

Degradation of injected materials is closely tied to mechanical properties. As a material degrades, it may become weaker (in bulk degradation regimes), and a stronger network may degrade more slowly. Degradation can occur passively through hydrolysis or by an active proteolytic process. Hydrolytic degradation occurs with rates ranging from days to months or years for a variety of synthetic polymers, including polyesters such as poly(lactide-*co*-glycolide) (PLGA), poly (caprolactone) (PCL), and various poly(anhydrides). Hydrolytically degradable materials cannot respond appreciably to cellular cues, so the degradation rate should be carefully tuned to match the observed rate of tissue synthesis.

Proteolytically degradable materials may include ECM molecules that are susceptible to degradation by cell-secreted matrix metalloproteinases (MMPs)

and other proteases. Researchers have also isolated MMP-sensitive peptide sequences that have been synthesized and incorporated into polymer scaffolds [48]. These MMP-sensitive synthetic networks allow the cells to selectively degrade the scaffold while retaining much of the control afforded by synthetic scaffolds. In a similar vein, the degradation rate of crosslinked ECM scaffolds has been tuned by including hydrolytically degradable polymers such as PLGA or PCL between the crosslinks to supplement its endogenous proteolytic degradation [49].

Recently developed biomaterials have also raised the possibility of selectively photodegradable materials. While not normally hydrolytically or proteolytically degradable, these materials contain bonds that can be selectively cleaved upon exposure to light. This can be a useful clinical tool to specifically control the time and degree of degradation to match a therapeutic need. Furthermore, photolabile bonds can be used to control release of pendant functional groups from a polymer network after implantation [50].

Degradation of injectable materials also plays a role in cellular migration and infiltration inside a scaffold. Cellular motility within a scaffold is largely dependent upon pore size, cell attachment moieties, and mechanics. Migration within a scaffold can be adjusted using varying degradation rates and sensitivity to proteolysis [48]. This can also prove useful for encouraging or discouraging infiltration of the host's cells into the scaffold.

17.3.3 Cytotoxicity and Tissue Damage

As with any implantable material, injectable materials must be carefully designed and tested to reduce or eliminate toxicity and tissue damage. This is particularly relevant for in situ crosslinking materials, as both the uncrosslinked polymers and the crosslinking process may damage cells and tissues in the area. Polymers that may be relatively benign in crosslinked form can cause cell death in monomeric or macromeric form. Additionally, reactive functional groups on uncrosslinked polymers may react with proteins and other cellular components, damaging both encapsulated cells and the surrounding tissue. These concerns can be minimized by reducing the amount of time the uncrosslinked polymer is in contact with tissues, changing the polymer properties, and reducing the reactivity of functional groups. Frequently, changing the hydrophilicity or molecular weight on the polymer or grafting on a copolymer can have significant effects on toxicity, though the exact mechanisms are not always well understood [51, 52].

Network formation itself may also lead to toxicity. In photopolymerization and other radical polymerization schemes, the crosslinking is driven by radical formation and propagation. Unsurprisingly, an excess of radicals or radical-producing species will damage cells and tissues. The needs of rapid and efficient network formation must always be weighed against toxic effects. Radical polymerizing injectable systems in development use initiator types and concentrations that have been shown to exhibit minimal toxicity while

still achieving optimal crosslinking [21, 53, 54]. Similarly, any external stimuli [such as ultraviolet (UV) light, temperature changes, ionic strength changes, etc.] used to drive polymerization have to be optimized to minimize unwanted tissue damage while still achieving good network formation.

Although polymerization of these materials tends to be fairly efficient, there will invariably be some injected material that is not incorporated into the network [55]. This may include unpolymerized macromers, initiators, and buffers or solvents. These materials can leach out of the injected material and enter the surrounding tissue. In abnormal clinical conditions, the polymerization itself may also not be effective, resulting in a much higher concentration of material infiltrating into the tissue. Substances that are not fully consumed during polymerization should be carefully tested to cause minimal damage to the surrounding tissue and should be investigated as to their clearance mechanisms from the body.

17.3.4 Immunological Concerns

Although an implantable material may not exhibit significant cytotoxicity, the immune response is another material–host interaction that must be carefully considered in the design of injectable materials. Biologically derived materials and allogenic or xenogenic cells will cause a specific immune response, while entirely synthetic materials can exhibit a range of responses from a generalized foreign body reaction to a specific immunogenic reaction [56]. A severe immune response will impair tissue–material integration and largely negate the purpose of implantation and may well lead to deleterious systemic effects.

When using natural materials such as ECM molecules, proteins, and other tissue components, immune considerations become critical. Ideally, all biomolecules should be derived from bacteria using recombinant methods rather than isolated from animals or mammalian cell culture. This will reduce the presence of a variety of immunogenic impurities (such as unwanted glycosylation and protein contamination), as has been seen with early injectable biomaterials such as hyaluronan and collagen [57]. Unfortunately, even bacteriologically derived materials can contain impurities that may cause an immune reaction, so processing and purification are essential.

Cell encapsulation into injectable materials is another major source of immunogenicity. Although using the patient's own cells would eliminate this concern, the quantity and quality of autologous cells is frequently inadequate for the repair task at hand. Expansion of autologous cells in culture will increase the quantity of cells for injection, but often results in phenotypic changes from primary cells that reduce their repair potential [58]. This concern has been addressed by selecting postexpansion cells for biomarkers that are known to correlate with more robust repair, such as TiGenix's ChondroCelect system for autologous chondrocyte implantation [59]. Alternatively, many proposed materials incorporate allogenic or xenogenic cells for encapsulation. To address immunologic concerns, these cells must be effectively isolated from

the immune system. This can be accomplished by the use of material scaffolds with sufficiently small pore sizes or by injecting the material into an immunoprivileged tissue [60]. Cell-based therapies are still very new, and addressing the issues of safety and immunogenicity remains one of the main barriers to their implementation in the clinic.

It is important to note that although the major concerns for immunogenicity revolve around naturally derived materials and exogenous cells, some synthetic materials can also cause immune reactions. Synthetic peptides in particular can cause significant immune responses, though this is largely dependent on its specific bioactivity, size, and conjugation to other materials. As injectable materials continue to move toward more complex biological-synthetic composite materials, it will be necessary to carefully test the immunogenic potential of a proposed material prior to clinical use.

17.4 INJECTABLE POLYMERS IN THE CLINIC AND CLINICAL DEVELOPMENT

Injectable materials were first introduced into the clinic several decades ago, but their growth has been fairly slow. Major constraints included adequate material control, biocompatibility issues, and engineering concerns about the injection characteristics and mechanics. With the recently increased sophistication of biomaterial design and characterization, more injectable systems have been developed. This section will give an overview of the major classes of injectable polymers and their uses. It will focus on currently used materials and those under intensive development.

17.4.1 Naturally Derived Polymers

Fibrin. Fibrin glue is one of the oldest and most widely used injectable polymers in clinical use today. It utilizes mammalian clotting mechanisms to quickly form a stable, biodegradable polymer network. The glue consists of fibrinogen and thrombin in separate solutions that are mixed together during injection, often using a double-barrelled syringe/mixing system such as Duploject (Baxter). The thrombin enzyme rapidly converts fibrinogen into fibrin monomers. The fibrin is then crosslinked with Factor XIII into a network [61]. Crosslinking can occur within less than 1 min of application depending on the concentration of thrombin used [62].

Fibrin glue has been widely used in the clinic for a variety of applications. Initially intended as a hemostatic adhesive in surgery, its uses have greatly expanded in the last two decades. Most purposes still use it in an adhesive or sealant capacity, though it is no longer limited to hemostasis. Fibrin glue has been used to augment sutures in blood vessel anastomoses, improving periosteal flap seals in cartilage repair, and sealing air leaks in thoracic surgery

[63–65]. In recent years, fibrin constructs have been used for prospective tissue engineering and drug delivery scaffolds. It has been proposed for tissue engineering of cartilage, bone, vascular grafts, cornea, muscle, and more [35, 66]. Materials based on fibrin glue have been used extensively as adjuncts to tissue engineering therapies but have not yet been used as cell-supporting scaffolds in the clinic.

Major challenges to the use of fibrin include its weak mechanical strength, fairly rapid degradation, and tendency to shrink upon culture with cells. This has encouraged a variety of synthetic modifications to fibrin scaffolds. Mechanical strength has been improved through enhanced crosslinking regimes and the use of composite scaffolds with synthetic and biological polymers. Enhancing the crosslinking and the grafting of copolymers also reduces gel shrinkage and slows degradation. To enhance the bioactivity of fibrin, researchers have covalently crosslinked synthetic peptides, growth factors, and other biomolecules to the fibrin scaffolds [35]. It is unclear, though, whether tissue engineering approaches using fibrin are near to clinical applicability. Nevertheless, given its well-established track record and widespread usage, it remains a commonly used material substrate for early translational therapies.

Hyaluronic Acid. Hyaluronic acid (HA) is another commonly used base material for injectable polymers. It is a long-chain polysaccharide consisting of alternating glucuronic acid and *N*-acetyl glucosamine residues. HA is a major component of the ECM, forming the core polysaccharide for attachment to aggrecan proteoglycans through link protein. In its structural role, HA molecular weight is very high (on the order of 1–10 MDa) and exhibits unique mechanical properties. Its high hydrophilicity and water retention, further augmented by the addition of aggrecan and other GAGs, results in good resistance to compression and complex hydrodynamic properties. In articular cartilage, HA provides much of the tissue's unique mechanical properties in compression. In solution, HA is highly viscous and is a major component of the lubricants in synovial fluid. As a biomaterial, it is readily degradable by endogenous hyaluronidases and exhibits good biocompatibility [67].

HA has been used as an injectable polymer in the clinic for several purposes. The viscoelastic properties of HA have been used as an ophthalmic surgical aid including such products as Amvisc (Bausch & Lomb), Healon (Abbott), and Duovisc (Alcon). Initial products either exhibited low-viscosity "dispersive" properties to improve adherence to and protection of the corneal endothelium during phacoemulsification or high-viscosity "cohesive" properties (corresponding to much higher molecular weights) for maintaining the anterior chamber during surgery and making removal after surgery easier. Recently, second-generation "viscoadaptive" ophthalmic viscosurgical devices have claimed to contain both cohesive and dispersive properties as a function of shear rate [68]. This is not a surprising property, given the shear thinning properties of high-molecular-weight linear polysaccharides, but it is unclear how functionally different this is from similar cohesive devices. Alternatively, there exists a

well-established "soft-shell" surgical technique to use both a high-molecular-weight cohesive and a low-molecular-weight dispersive (based on either HA, chondroitin sulfate, or hydroxypropylmethylcellulose) in order to both protect the corneal endothelium and open the anterior chamber [69].

HA is also used as a longer-lasting injectable material in the dermal filler market. Dermal fillers are used primarily for aesthetic purposes of facial contouring and include Restylane (Medicis/Q-Med) and Juvederm (Allergan) [9]. They also have been used to correct human immunodeficiency virus (HIV)-associated facial lipoatrophy, and HA–dextranomer fillers have been used to treat stress incontinence (Zuidex, Q-Med) and vesicoureteral reflux (DeFlux, Q-Med) [70, 71]. A number of soft-tissue fillers have been used in the past, including autologous fat, collagen, and various synthetic materials, but HA has recently taken a large share of the market. Its advantages include very good biocompatibility, reversibility (through injection of hyaluronidase), and mechanical properties that closely approximate the "feel" of natural dermis. Early HA fillers contained fairly low concentrations and minimal degrees of crosslinking but exhibited poor long-term persistence. To increase persistence, current-day fillers are composed of a high concentration of crosslinked HA gels (using divinyl sulfone or a similar crosslinker) that are mechanically dissociated into particles approximately 100 μm in diameter. To improve the viscous properties for injection, uncrosslinked long-chain HA is also added in low concentrations [72]. Even with these improvements, patients typically return for further injections within 6 months. Furthermore, some granulomatous reactions have been noted in isolated cases, even with HA purified from bacteriological and not animal sources [73]. Nevertheless, HA is still seen as the gold standard in soft-tissue fillers, and extensive work is being done to further improve its persistence and biocompatibility.

The last major class of HA injectables in the clinic are viscosupplements used in the joint space as a symptomatic treatment of osteoarthritis. HA forms a major component of the synovial fluid and articular cartilage, providing for much of the lubrication properties of joints due to its high hydration. Since HA in the joint decreases in molecular weight and concentration with age and disease progression, these HA-containing viscosupplements aim to improve the lubrication of the joint, reducing further wear and limiting osteoarthritic pain. The HA is also hypothesized to improve endogenous HA production in the joint, probably mediated by binding to CD44, though exact mechanisms of action are currently unclear [74]. Major products include Synvisc (Genzyme), Hyalgan (Sanofi-Aventis), Supartz (Smith & Nephew), and Orthovisc (Anika/Depuy). First-generation products consisted of uncrosslinked HA of either animal or bacteriological origin. Due to very rapid clearance of injected HA, though, later products have increased the concentrations and molecular weights of injected HA and introduced crosslinked derivatives for better stability [75]. While this has improved residence times, there still exists very rapid degradation; any analgesic effects that may occur disappear within a few months. Even beyond the issue of clearance times, it is still unclear whether

injected HA has a significant clinical effect when compared to phosphate buffer saline (PBS) injection controls; this may be because injection of any fluid will flush out some of the joint debris accumulated during the progression of joint degeneration. Nevertheless, the weight of clinical evidence is trending toward HA injections having some clinical efficacy [76]. Some hypersensitivity reactions have been observed in a low percentage of patients, prompting concerns of immunogenicity, HA sourcing, and crosslinking methodologies [77].

Due to its role as a major ECM molecule, HA is also being developed for a number of other clinical applications. Photopolymerizing HA and HA–ECM composite hydrogels have been developed to regenerate cartilage, often using encapsulated adult or embryonic stem cells [6, 78, 79]. Injectable HA hydrogels are also in development for regeneration of vocal folds, skeletal muscle, and neurons [80–82]. Extensive work still needs to be done in fine-tuning scaffold mechanical properties, optimizing the non-HA components in composite materials, and directing cell-mediated tissue organization. In particular, it seems that the presentation of HA in a material—whether as a crosslinked gel, as free polysaccharides embedded in a polymer matrix, or as a spongelike solid—may have significant impact on the cellular response. Nevertheless, second-generation autologous chondrocyte implantation procedures may use HA-based materials instead of a simple cell medium. Additionally, continual improvements in the traditional uses of viscosupplementation, soft-tissue fillers, and ophthalmic surgical aids are likely, potentially utilizing more complex material designs.

Collagen. Collagen, as the most abundant protein in the ECM, is a natural candidate for use in biomaterials. It is relatively easy to isolate from a variety of connective tissues and can be injected either as a suspension or after chemical modification [83]. Collagen also exhibits some weak temperature-dependent gelation behavior, increasing its appeal as an injectable biomaterial. Collagen is composed of a triple helix of three separate helical polypeptide strands containing large amounts of proline, hydroxyproline, and glycine. These coiled coils are further organized into higher order fibrillar structures, bestowing collagen with high tensile strength [84]. Aside from its mechanical role, collagen also is an important cell- and matrix-adhesion molecule. The presence of collagen has been shown to greatly alter cell phenotype, proliferation, and differentiation. Collagen is not a homogeneous molecule in all tissues. Over 20 types of collagen have been identified, but the major molecules of interest include collagen I (largely structural roles), collagen II (hyaline cartilage and the vitreous humor), and collagen IV (basement membrane of epithelial layers). Most collagen used in the clinic today fulfills a largely structural role and is primarily composed of collagen I [85].

Like HA, one of the largest uses of collagen injections is in the facial cosmetic industry. Early collagen dermal fillers were uncrosslinked suspensions of bovine collagen (Zyderm, Allergan), which were concentrated and crosslinked by gluteraldehyde to improve persistence times (Zyplast, Allergan).

While relatively successful and still in limited use today, these fillers required allergy testing for bovine collagen prior to injection, had relatively low persistence times (6–18 months), and exhibited occasional granulomatous reactions [86]. To avoid potential immunological concerns, newer collagen fillers have used human sourcing from either cadaveric or autologous sources. Allergan has developed collagen isolated from cultured human dermal fibroblasts in both uncrosslinked (Cosmoderm) and crosslinked (Cosmoplast) varieties [87]. To avoid potential complications and toxicity from gluteraldehyde crosslinking, Ortho Dermatologics has recently developed Evolence, a porcine source collagen crosslinked by glycation with ribose [88]. Lastly, Artefill is a composite material of poly(methylmethacrylate) microspheres suspended in a collagen gel. This synthetic-biologic filler lasts far longer than collagen fillers alone (up to 5 years) but has significant issues with granulomatous reactions, nodularity, and mechanics [89].

Collagen injections have also been used for noncosmetic tissue bulking applications. A major application involves injection laryngoplasty, a procedure involving the injection of material into the vocal folds to treat paralyzed or scarred cords. Early materials were generally synthetic, including the commonly used poly(tetrafluoroethylene) (PTFE; Teflon), but concerns about the permanence and biocompatibility led to the increased use of collagen injections [80]. Aside from the purely mechanical effects, collagen injections have been postulated to increase native collagen production in scarred or atrophied vocal folds, though the evidence is not yet compelling [90]. Significant advantages to collagen injections are the better injection properties in comparison to low-viscosity Teflon microparticles, mechanics closer to the native tissue, and nonpermanent residence time. While permanent fillers have their advantages in not requiring follow-up treatment, they also leave little room for error in a very delicate and difficult procedure [80]. Collagen injections have used both bovine and human collagen sources, though generally only with products previously developed for cosmetic purposes. Due to concerns about both the short persistence and potential reactions to earlier collagen formulations, clinicians have begun using crosslinked collagens from human or autologous sources. Others have injected Cymetra (LifeCell), a micronized version of Alloderm, the widely used decellularized cadaveric dermis. This is not pure collagen but a mixture of the variety of ECM components found in the dermis [91]. Although other biomaterials are in development, including hyaluronic acid and hydroxyapetite particles, collagen-based materials are still a major material of interest for larnygoplasty.

Due to its widespread use in other areas of cell culture and biomaterials, collagen has been frequently considered as a major component for future injectable tissue engineering therapies. Significant interest has focused on collagen due to its large number of available functional groups for modification and its biological activity. Previously used crosslinked collagens (using gluteraldehyde or a similar chemical crosslinking system) have exhibited issues with toxicity. As a result, modern polymer chemistry has been used to develop new

forms of injectable collagen-based materials. Collagen has provided the cell-adhesive biological functionality of a variety of biosynthetic scaffolds, including thermoresponsive NIPAAm-grafted gelatin and spontaneously reacting PEG–collagen hydrogels for cartilage regeneration [92, 93]. Collagens and gelatins have been modified with photopolymerizable functionalities such as styrenes or cinnamates to regenerate a range of tissues including cartilage and nerve [94–96]. The potential applications of injectable collagens for tissue engineering are vast, which is reflected in the range of available materials.

Other Polysaccharides. While not as widespread in clinical use as fibrin, HA, or collagen, a variety of other ECM components have been proposed for use in injectable materials. They frequently are composed of long-chain polysaccharides. These polysaccharides are diverse and have significant variation in biological and physical properties but can all be chemically modified with ease [14]. Of the multitude of polysaccharides that have been explored, we will discuss three prominent molecules: chondroitin sulfate, alginate, and chitosan.

Chondroitin sulfate (CS) is a sulfated GAG that is present in a variety of tissues. CS proteoglycans form the majority of the sulfated GAGs in articular cartilage. It is hypothesized that the negatively charged sulfate groups contribute to the compressive mechanical properties of articular cartilage due to their hydrophilicity. CS also has a potent signaling role, probably due to its charge interaction with various proteins in the extracellular space [97]. While CS clearly has the potential to be an important component of injectable biomaterials, especially in cartilage, care must be taken in its use. CS sulfation patterns vary among different tissues and species and can have significant effects on bioactivity. Elucidating these differences and their effects is still a subject of ongoing investigation [98].

CS has been used with mixed results as an oral therapy for treating osteoarthritis. As an injectable therapy, CS has been chemically modified for photopolymerization and incorporated into a synthetic hydrogel. Bone-marrow-derived mesenchymal stem cells encapsulated in this gel exhibited enhanced chondrogenesis that mimicked the mesenchymal condensation steps in embryonic development [99]. While this and other CS-based therapies are not yet at a clinical stage, the data suggests a potent role for relevant polysaccharides in material-directed stem cell differentiation.

Alginate, as previously described in Section 17.2.3, is a long-chain polysaccharide isolated from algae that can be reversibly crosslinked by divalent cations. Its proposed clinical uses include cell encapsulation and as a drug delivery vehicle. As alginate is not a polysaccharide normally degraded by mammalian cells, its uses as a cell delivery vehicle are frequently limited to cases where only a trophic effect is desired. Classically, it has been postulated for encapsulation of pancreatic islet cells to treat diabetes. Care must be taken to ensure the alginate is sufficiently purified to ensure biocompatibility. Additionally, mechanics and porosity of the gels must be carefully tailored by appropriate selection of G : M ratios as discussed above. Despite these

limitations, preclinical studies with diabetic mice have yielded promising results [100]. Newer studies have combined alginate with cell-adhesive molecules such as gelatin to achieve scaffolds that can better simulate extracelluler matrix (ECM) and improve engraftment [101, 102].

As a drug delivery vehicle, alginate's main advantage is its mild gelation conditions, allowing for encapsulation of delicate protein therapeutics. Additionally, the gels are stable over a wide range of temperatures and can be modified or coated to change the diffusion characteristics of a given biomolecule. Alginate also possesses some mucoadhesive properties, raising the potential of targeted delivery to specific tissues [103]. Emerging uses of alginate combine it with other polymers (such as thermoresponsive NIPAAm) to yield hydrogels with complex porosity and stability dependent on combinations of temperature, ionic strength, and pH [104].

Chitosan, like alginate, is a polysaccharide purified from nonmammalian sources. It is derived through the deacetylation of chitin, normally purified from the shells of shrimp or crabs. Chitin is a simple linear polymer of *N*-acetylglucosamine, which upon deacetylation becomes a mix of glucosamine and *N*-acetylglucosamine depending on the degree of deacetylation. Chitosan is noted for its relative insolubility in water at neutral pH and is both biocompatible and readily biodegradable. The degradation and other properties of chitosan can be most easily modified by changing the degree of deacetylation, which higher deacetylation corresponding to slower biodegradation ranging from days to months [105]. The cationic nature of chitosan (due to the large number of deacetylated amines) allows unique interactions with a variety of anionic biomolecules, including deoxyribonucleic acid (DNA) and GAGs. Drawbacks to chitosan include potential immunogenicity for poorly purified derivatives as well as difficulties associated with its poor native water solubility. A wide variety of chemical modifications of chitosan are possible due to the abundant amine and hydroxyl groups of glucosamine. These modifications have allowed for the careful tailoring of the physical properties and biological activity of chitosan for two major applications: tissue engineering and delivery of drugs or DNA [106].

Photocrosslinkable chitosan has been synthesized by the addition of UV-reactive azide functionalities and has been proposed as a tissue adhesive or to repair amniotic membranes [107, 108]. Chitosan with thermogelling properties has been developed either through complex interactions with β-glycerophosphate or through covalent modification with NIPAAm [109, 110]. Furthermore, the pH-dependent solubility of chitosan has been exploited to form pH-sensitive injectable materials. When dissolved in dilute acids, it can then be injected into an environment with neutral or alkaline pH (such as the body), forming a gel-like precipitate [111]. Chitosan has also been combined with other biopolymers such as collagen or alginate to improve scaffold functionality and properties. These and similar modifications have been proposed as injectable tissue engineering scaffolds for bone, cartilage, liver, and other tissues [102, 112, 113].

Chitosan's central role as a delivery vehicle is tied to its polycationic charge, mucoadhesivity, biodegradability, and ease of processing. It forms complexes easily with anionic drugs, proteins, and DNA. Additionally, it seems to be able to perturb lipid bilayers, a significant advantage for intracellular delivery. Injectable hydrogels and suspensions of chitosan microspheres have been designed for chemotherapy, gene delivery, ocular drug delivery, and much more [114–116].

17.4.2 Synthetic Polymers

Poly(siloxane). A synthetic injectable polymer family that is well established in the clinic is poly(siloxane) and its derivatives. Poly(siloxane), also known as silicone, is a general name for polymers containing silicon and oxygen and some organic chemical group. The most commonly used silicone in clinical practice is poly(dimethylsiloxane), or PDMS. Silicone has unique viscoelastic properties, acting as a solid for low shear rates and temperature but as a viscous liquid under shear and at higher temperatures. Silicone is extremely stable at a wide range of temperatures and is resistant to chemical degradation in an aqueous environment. PDMS can be injected into the body and has been shown to be fairly bioinert, largely due to the hydrophobicity of the methyl groups that "shield" the inner silicon–oxygen backbone [117]. As a result, silicone injections are generally considered nondegradable and biocompatible. Nevertheless, concerns exist about the potential granulomatous reactions to silicone, as well as potential damage due to silicone migration in the body [118]. These injections can be composed of either uncrosslinked poly(siloxane) chains or suspensions of highly crosslinked "poly(siloxane) elastomeric particles". By varying the polymer concentration, crosslinking density, particle size, and molecular weight, the relevant viscoelastic properties can be modified.

As silicone has effectively no bioactivity, its clinical use has been directed almost exclusively by its physical properties. Unsurprisingly, silicone injections are used as bulking agents in both cosmetic applications and clinical treatments. Similar to collagen, crosslinked microparticles of silicone suspended in a carrier have been used to treat stress incontinence and vesicoureteral reflux (Macroplastique, Uroplasty) as well as provide cosmetic correction in the face (Bioplastique, Bioplasty) [119, 120]. Its main advantages are the permanence of injected material, ease of synthesis, and the good control over physical properties. Material longevity is a particular advantage, as naturally derived polymers are resorbed quickly and require frequent reinjections. Significant concerns exist over migration of the silicone particles in the body as well as the extent of foreign body reactions at the site of injection [120, 121]. Additionally, the synthesis and crosslinking of silicone uses a number of toxic reagents that must be carefully removed prior to injection; nonmedical-grade silicones have led to a number of adverse events [122].

Uncrosslinked silicone oil has also been used in a number of clinical applications. They were originally approved for intraocular injections as a "tamponade" to treat retinal detachment and tears (Silikon 1000, Richard-James Inc., Adato

Sil-ol 5000, Bausch & Lomb). The unique interfacial surface energy between the retina and silicone oil allows for the formation of relatively stable balls of silicone oil in the eye that are transparent and effectively permanent [8]. While this is far more effective than more quickly cleared tamponade materials, the permanence of the silicone oil can have its own problems; notably, long-term residence of silicone oil can cause cataracts, glaucoma, or keratopathy [123, 124]. Thus, the silicone must invariably be removed after complications arise, a complex and difficult process. These silicone oils have also been used off-label in cosmetic applications, although significant concerns about safety exist, especially given the difficulty of completely removing the material.

In sum, the permanence of injectable silicone materials is both its most attractive feature and its largest drawback. Silicone, having little biological functionality, has been used extensively for various applications that need a long-term "filler" for defective or degenerative tissue, whether in urologic, ophthalmic, or cosmetic applications. Its long-term stability means that repeat injections will rarely be necessary, easily outperforming degradable materials such as collagen or hyaluronic acid. Nevertheless, its lack of degradation by the body means that migrated silicone can potentially cause a long-term health hazard. Additionally, its physical properties and migration through tissue make it difficult to remove completely when necessary.

Polyesters. Polyesters are among the most popular hydrolytically degradable biopolymers. The three polyesters in common use are poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(caprolactone) (PCL), which differ by the number and structure of carbons between each ester linkage of the polymer backbone. These ester linkages give polyesters their degradability, with PGA degrading fastest, followed by PLA and PCL. Copolymers of polyesters, particularly poly(lactide-*co*-glycolide) (PLGA), have been produced in varying ratios to tailor the degradation rate of a polyester material [125]. Degradation of polyesters yields acidic breakdown products that may cause irritation or tissue damage in high concentrations, but in general the materials are well tolerated [15]. Polyesters are generally insoluble in aqueous conditions, which limits their utility as injected biomaterials. Nevertheless, injectable polyesters have been used for a variety of clinical applications focused on degradable tissue bulking and drug delivery systems [126].

Polyesters can easily be processed into microspheres for injection. These microspheres are frequently synthesized by various emulsion techniques between a polyester-containing organic phase and an aqueous phase. For drug delivery applications, a small-molecule therapeutic can be encapsulated into the microsphere at the time of formation and will be released as the polyester degrades. Polyester microspheres are used in the clinic for both soft-tissue fillers and drug delivery applications [127]. A suspension of PLA microspheres in carboxymethylcellulose and mannitol has been used extensively in facial plastics for treating HIV-associated facial lipoatrophy and for cosmetic correction (Sculptra, Sanofi-Aventis). While HA and collagen-based soft-tissue fillers rely

on actual tissue bulking with the ECM molecules injected, PLA microspheres are suggested to cause a greater foreign body response. The associated collagen deposition at the site of injection is theorized to provide the observed short-term correction. Unsurprisingly, the foreign body response can also be a significant detriment; development of inflammatory nodules after injection is a significant concern, though it can be mediated by appropriate injection technique [128].

Polyester microspheres have been used extensively for drug delivery. PLGA microspheres have been developed to treat prostate cancer (gonadotropin-releasing hormone (GnRH) analog; Lupron Depot, Abbott), growth hormone insufficiency (recombinant human growth hormone (hGH); Nutropin Depot, Genentech), alcoholism (opioid antagonist; Vivitrol, Alkermes), bipolar disorder (psychotropic agent; Risperdal Consta, Ortho-McNeil-Janssen), and many others [129–131]. A wide array of drugs can be encapsulated in PLGA, and any disease that requires a constant delivery of drug (as opposed to the dynamic fluctuations inherent in oral administration) may benefit from long-term release. As the microspheres degrade, drugs are released into the body at a roughly constant rate until the depot is exhausted within 1–3 months, depending on the lactide-to-glycolide ratio. Although these microspheres can migrate some in the body, there are not significant concerns associated with this as in silicone microparticles, as the PLGA will be degraded in short order. Nevertheless, there are two potential complications. First, care should be taken not to inject the microspheres into the bloodstream. Second, some granulomatous reactions at the injection site have been observed, though these tend to be relatively minor complications [132].

Although PLGA microspheres are a commonly used injectable drug delivery system, they have some practical drawbacks. In particular, the microspheres frequently clog the needle of the syringe during injection, synthesis of uniform microspheres is difficult, and particle migration in vivo is not easy to predict. To counter some of these issues, some researchers have instead developed a unique in situ forming PLGA drug reservoir. Briefly, PLGA and the drug are dissolved in an organic solvent such as *N*-methylpyrrolidone (NMP). Upon injection into an aqueous environment, the solvent diffuses into the surrounding tissue and is replaced by water. The PLGA and drug then precipitate out of solution, forming a solid implant for continuous drug release. This process has been harnessed as the Atrigel delivery system to deliver Eligard, a GnRH analog (Sanofi-Aventis). While an obvious concern is the use of an organic solvent in the injection, reported reactions have been fairly mild [133, 134]. Additionally, these in situ forming PLGA delivery systems have shown better consistency when compared to traditional microsphere systems. The rate and length of release can be tailored by the solvent-to-polymer ratio, which changes the kinetics of scaffold solidification, the polymer molecular weight, and the ratio of glycolide-to-lactide-to-caprolactone in the polymer formulation.

Poly(Ethylene Glycol). Poly(ethylene glycol) (PEG) is a widely used polymer in biomaterials. Its high hydrophilicity results in a very large hydrodynamic volume and is critical for understanding its biological and physical properties.

Its major attraction as a biomaterial is its nonadhesive and hydrophilic nature, making unmodified high-molecular-weight PEG very biocompatible [135]. Long-chain PEG forms a viscous liquid in aqueous solution and can be readily formed into a hydrogel through either chemical or physical crosslinking strategies [136]. The PEG backbone is not normally considered either hydrolytically or proteolytically degradable, but growing evidence implies a low, basal level of degradation of PEG materials in vivo, potentially catalyzed by reactive oxygen species [137–139]. PEGs have been used in almost every aspect of biomaterials science, from coatings on medical devices to drug conjugates to tissue engineering scaffolds. In injectable form in the clinic, PEG-based materials have been used for tissue adhesives, drug delivery, and are being developed for a wide range of tissue engineering applications.

Due to their biocompatibility, ease of modification, and relative strength compared to natural materials such as fibrin, it is unsurprising that PEG has been used as the core of several tissue adhesives and sealants. Some formulations, such as FocalSeal (Genzyme), are not directly injectable. Rather, the acrylate-substituted PEG and photoinitiator is brushed onto a tissue (such as a freshly sutured blood vessel or a lung) and photopolymerized by exposure to light [140, 141]. In contrast, CoSeal (Baxter) is an injectable, fast-setting PEG-based sealant that utilizes a spontaneous reaction between two differently modified tetrafunctional PEGs and the tissue surface. One PEG monomer is functionalized with amine and thiol-reactive *N*-hydroxysuccinimide (NHS) groups, and the other is end modified with thiols. The NHS–PEG can react with endogenous amines on the tissue surface and SH-PEG to form both an adhesive and cohesive gel with moderate mechanical strength [142]. CoSeal has been used to seal lungs and blood vessels against leaks as well as a coating for adhesion prevention [143–145]. Its main advantage is the lack of potentially toxic components in other sealants, such as gluteraldehyde or cyanoacrylates in Bioglue (CryoLife) and Dermabond (Ethicon), and its lack of potentially immunogenic biological components. A significant concern is its weaker mechanical integrity that poses a potential risk for sealant failure under mechanical strain [146]. On the other hand, CoSeal is less stiff than other alternatives, allowing dynamic tissues such as lungs and blood vessels more freedom of movement than stiffer sealants. Other concerns involve foreign body reactions compromising the efficacy of the sealant, and the low viscosity of the applied monomers prior to gelation, resulting in the adhesive "creeping" to surrounding structures.

Newer PEG-based adhesives in development have included biological components to enhance the biocompatibility and temper the inflammatory response to injection. Using a similar approach to CoSeal, researchers have used amine-modified PEG in conjunction with NHS-modified CS. The NHS-CS attaches to amines on both the PEGs and tissue, providing strong cohesive and adhesive properties. Besides the enhancement in biocompatibility, CS provides a large number of sites for chemical modification, making optimization of the gel

properties a simple matter of changing the degree of functionalization. Reaction kinetics can be modified by changing the pH of the mixed adhesive solution, with gelation ranging from seconds to minutes. The proteolytically degradable CS polymers also make the adhesive bioresorbable with time as the underlying tissue heals. CS-PEG adhesives have been demonstrated in preclinical studies to augment sutures in ophthalmic surgeries [36].

PEG has also been used as a crucial component in drug delivery strategies. Many such strategies have moved away from insoluble drug reservoirs such as microspheres and instead tried to incorporate the drugs into more easily injectable hydrogels. Researchers have exploited the relatively hydrophobic nature of PLGA to develop a thermogelling material composed of the block copolymer PLGA–PEG–PLGA (ReGel, BTG) [147]. Similar to Pluronics or NIPAAm, the copolymer undergoes a sol–gel phase transition as temperature is increased due to the difference in hydrophilicities between the two blocks. The water-soluble PEG allows for the easy injectability of the material, and the PLGA precipitates out of solution upon injection into body temperature, providing an in situ drug reservoir for long-term delivery. ReGel has been commercialized for sustained local delivery of the chemotherapeutic paclitaxel in esophageal cancer, marketed as OncoGel. The material has been well tolerated in clinical studies and showed promising results when compared with systemic administration of chemotherapeutics [148]. Elaborations of this idea have been postulated as potential tissue engineering scaffolds for bone [149].

Although PEG-based injectables in the clinic are still somewhat limited in scope, an enormous number of PEG-based injectables are in development, particularly in the field of tissue engineering. PEG hydrogels are being developed for applications in eye, cartilage, intervertebral disk, liver, and neural regeneration, among others [150–154]. The modifications to PEG-based materials are quite varied. PEG injectables have been modified with acrylates and other chemistries for photopolymerization, with hydrophobic polymers for thermogelation, and with various proteins and peptides for self-assembly [136, 149, 155]. ECM molecules have been grafted to PEG to add a proteolytically degradable sequence and improve the bioactivity of the material [156]. Synthetic peptides have been incorporated into PEG hydrogels in order to introduce cell or ECM binding sites as well as MMP-sensitive degradation sequences [48, 157]. Growth factors and other signals have been tethered to the PEG network to control cellular migration, proliferation, and differentiation [158]. While the proposed product space of PEG-based injectables is quite large, these regenerative therapies have a high barrier to entry into the clinic. Significant regulatory hurdles remain, especially when cells are implanted with the material. Nevertheless, the popularity of PEG materials is rooted in its long history in implanted medical devices, its excellent biocompatibility, its ease of synthesis and modification, and the similarity of PEG hydrogels to native ECM.

17.5 CASE STUDY: CHONDUX

Clearly, there exists a great diversity of candidate polymers for injectable therapies. Most injectable polymers in the clinic today are composed of simple materials that have been used in one variation or another for decades (e.g., collagen, silicones, fibrin, etc.). While the sophistication of the material design and polymer chemistry has greatly improved, the fundamental materials have not appreciably expanded to include new biosynthetic composites. Despite the promising results that have been found in innumerable cellular and animal studies, relatively few innovative injectable materials have been brought to the clinic. Nevertheless, the therapeutic potential of novel classes of injectable materials has resulted in a small but growing number of new clinical products. In this section, we will follow the clinical development of a new injectable polymer for cartilage repair.

A recent CS-based polymer that shows early promise in both preclinical and clinical models is the Chondux cartilage filler device for the repair of full-thickness cartilage lesions of the knee (Cartilix). This is a two-component injectable gel system that combines a bioadhesive based upon CS with an HA-based filler. The adhesive is composed of CS chemically modified with two separate functionalities: amine-reactive aldehydes and photoreactive acrylates. This adhesive can thus bind with amines present on a defect surface while presenting a photoreactive functionality for further attachment to a filler material, providing good tissue–material integration [159]. The photopolymerizable filler is based upon HA, a commonly used material in cartilage repair procedures. This filler device nicely demonstrates the underlying concepts of new injectable materials: It combines the bioactivity of natural polymers relevant to cartilage development and repair (HA and CS) with synthetic modifications to enable good tissue–implant integration and in situ gelation.

The adhesive simply requires painting on the base and sides of the defect and bonds in seconds. The filler material is then injected and polymerized by UV light and within 4 min forms a stable gel that is now bound in three dimensions to the cartilage lesion (Fig. 17.1). This technique achieves the stated goals of both safety and ease of use. Importantly, Chondux has been successfully utilized in cartilage repair in concert with microfracture, a somewhat efficacious long-standing orthopedic technique for cartilage repair. Preliminary clinical studies with ChonDux have shown significant improvements in cartilage repair. When compared with microfracture alone, patients experienced better function and reduced pain (Fig. 17.2). Additionally, follow-up magnetic resonance imaging (MRI) showed significant improvement and consistency in tissue fill when compared to microfracture alone [160].

It is thought that the new biomaterial guides the endogenous tissue repair and so makes an older procedure (microfracture) more robust and less capricious. The relative paucity of new injectables in the clinic may be tied to the hurdles associated with developing and validating an entirely new therapy and proving both safety and efficacy when compared to gold standards. These

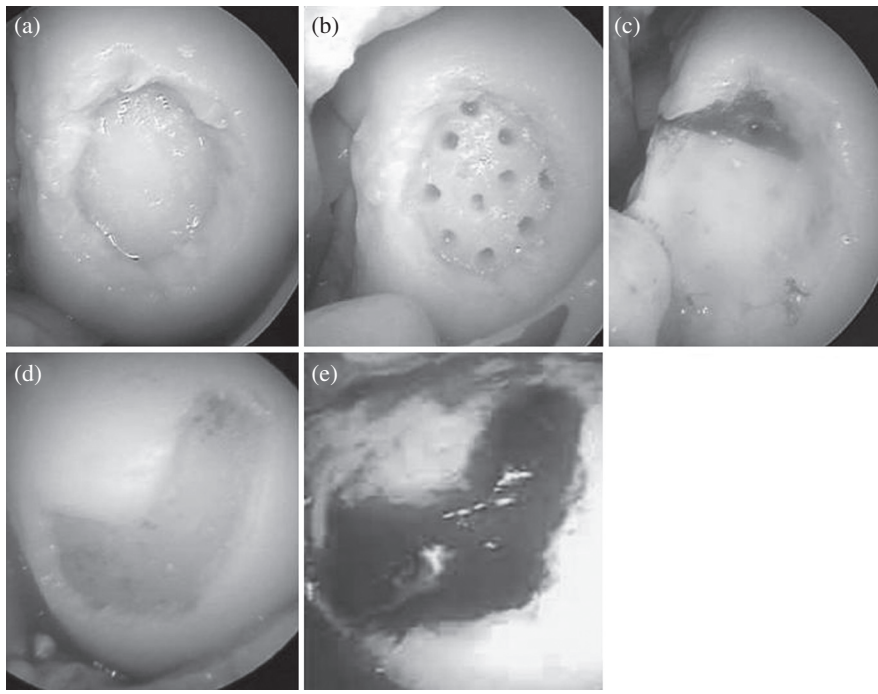

FIGURE 17.1 Cartilage lesion after (a) debridement, (b) adhesive application and microfracture, and (c) injection and photopolymerization of the hydrogel. Irregular defects were treated (d). Upon release of the tourniquet, the gel remained securely in place as blood infiltrated (e).

complex materials may indeed lead to significant improvements in patient outcomes with sufficient optimization in the clinic. The concept of combining new biomaterials with existing methods of treatment in order to produce superior, more durable and/or more consistent results is likely to be repeated in other systems.

17.6 CONCLUSION

Injectable materials are an important class of biomaterial that exhibit good integration with host tissues and minimally invasive implantation. Their primary strength, namely having liquid properties during implantation, is also their greatest limitation. Widespread use of injectable materials has been slow to develop, largely due to the technical challenges of designing biocompatible, long-lasting, in situ forming implants with appropriate physical and biological properties. Early products such as silicone or fibrin were adapted from other

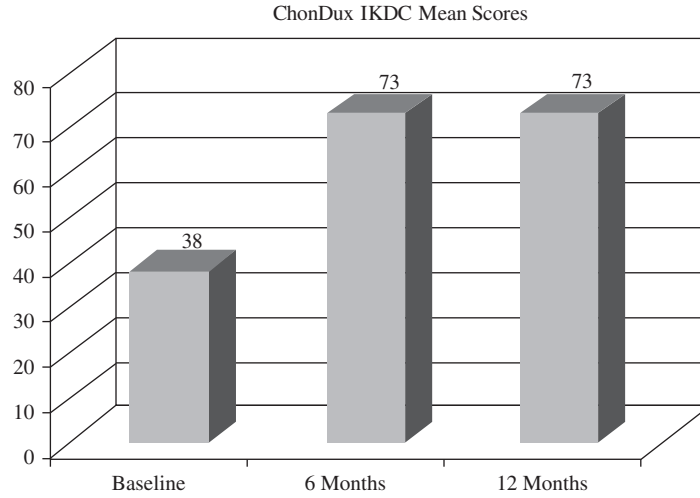

FIGURE 17.2 IKDC, a scoring system to measure patient function, improved significantly with ChonDux treatment.

purposes and were rarely ideal for their proposed usage. Naturally sourced materials were poorly controlled and rarely had adequate mechanics or longevity. Synthetic materials had significant purity concerns and rarely interacted with the surrounding tissue. In situ forming systems were primitive and often toxic.

In recent years, advances in biosynthesis and polymer chemistry have opened up a wide range of new possibilities for injectable materials. Sophisticated in situ forming chemistries have been elaborated and optimized for a range of biomaterials. Quality and control of natural materials have dramatically improved, and the range of synthetic polymers has expanded greatly to include more biocompatible materials with a range of mechanical and degradative properties. To capitalize on the strengths of both material sources, newer injectables in clinical development have blurred the lines between natural and synthetic materials. ECM molecules and other proteins are routinely functionalized with chemical reactive groups for crosslinking, and synthetic polymers are now designed with peptides or growth factors attached to interact with biological systems. These improved systems have much more tunable behavior and can be applied to a range of tissues and diseases.

A lingering question about injectable biomaterials involves regulation of these complex products. While generally classed as medical devices, regulatory agencies have not yet fully developed a methodology for evaluating in situ forming materials that are a mix of biological and synthetic components and that frequently undergo a chemical reaction at the implant site. Proposed use of injectable materials as carriers for cell transplantation adds yet another layer

of complexity to the regulatory process. Potential safety concerns range from toxicity of the monomers, chemical crosslinkers, and unpolymerized residues to immunogenicity of biological components to clearance from the body. The regulatory path is complex and requires addressing all of these concerns before approval for clinical use.

Nevertheless, injectable polymers comprise a rapidly growing class of biomaterials with a range of applications in drug delivery and tissue engineering. The rapidly expanding availability of new materials and techniques for their modification promises a dramatic improvement in the quality and quantity of clinically available injectable therapies. By focusing on the core design concepts of injectability, in situ forming chemistry, bioactivity, mechanics, degradability, and safety, injectable polymer systems will form an integral part of future regenerative medicine and drug delivery.

REFERENCES

1. Jacques, M., T. J. Marrie, and J. W. Costerton. Review: Microbial colonization of prosthetic devices. *Microb. Ecol.* 1987;**13**:173–191.
2. Campoccia, D., L. Montanaro, and C. R. Arciola. The significance of infection related to orthopedic devices and issues of antibiotic resistance. *Biomaterials* 2006;**27**(11):2331–2339.
3. Kirkpatrick, C. J., V. Krump-Konvalinkova, R. E. Unger, F. Bittinger, M. Otto, and K. Peters. Tissue response and biomaterial integration: The efficacy of in vitro methods. *Biomol. Eng.* 2002;**19**(2–6):211–217.
4. Prestwich, G. D., X. Z. Shu, Y. Liu, S. Cai, J. F. Walsh, C. W. Hughes, S. Ahmad, K. R. Kirker, B. Yu, R. R. Orlandi, A. H. Park, S. L. Thibeault, S. Duflo, and M. E. Smith. Injectable synthetic extracellular matrices for tissue engineering and repair. *Adv. Exp. Med. Biol.* 2006;**585**:125–133.
5. Kretlow, J. D., L. Klouda, and A. G. Mikos. Injectable matrices and scaffolds for drug delivery in tissue engineering. *Adv. Drug. Deliv. Rev.* 2007;**59**:(4–5), 263–273.
6. Elisseeff, J. Injectable cartilage tissue engineering. *Expert Opin. Biol. Ther.* 2004;**4**(12):1849–1859.
7. Boyd, L. M. and A J. Carter. Injectable biomaterials and vertebral endplate treatment for repair and regeneration of the intervertebral disc. *Eur. Spine. J.* 2006;**15**(Suppl 3):S414–421.
8. Soman, N. and R. Banerjee. Artificial vitreous replacements. *Biomed. Mater. Eng.* 2003;**13**(1):59–74.
9. Johl, S. S. and R. A. Burgett. Dermal filler agents: A practical review. *Curr. Opin. Ophthalmol.* 2006;**17**(5):471–479.
10. Khan, Y., M. J. Yaszemski, A. G. Mikos, and C. T. Laurencin. Tissue engineering of bone: Material and matrix considerations. *J. Bone Joint Surg. Am.* 2008;**90**(Suppl 1):36–42.
11. Leach, J. K. and D. J. Mooney. Bone engineering by controlled delivery of osteoinductive molecules and cells. *Expert Opin. Biol. Ther.* 2004;**4**(7),1015–1027.

12. Lewis, G. Injectable bone cements for use in vertebroplasty and kyphoplasty: state-of-the-art review. *J. Biomed. Mater. Res. B Appl. Biomater.* 2006;**76**(2):456–468.
13. Oliveira, S. M., C. C. Barrias, I. F. Almeida, P. C. Costa, M. R. Ferreira, M. F. Bahia, and M. A. Barbosa. Injectability of a bone filler system based on hydroxyapatite microspheres and a vehicle with in situ gel-forming ability. *J. Biomed. Mater. Res. B Appl. Biomater.* 2008;**87**(1):49–58.
14. Mano, J. F., G. A. Silva, H. S. Azevedo, P. B. Malafaya, R. A. Sousa, S. S. Silva, L. F. Boesel, J. M. Oliveira, T. C. Santos, A. P. Marques, N. M. Neves, and R. L. Reis. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. *J. Roy. Soc. Interface* 2007;**4**(17):999–1030.
15. Gunatillake, P., R. Mayadunne, and R. Adhikari. Recent developments in biodegradable synthetic polymers. *Biotechnol. Annu. Rev.* 2006;**12**:301–347.
16. Ifkovits, J. L. and J. A. Burdick. Review: Photopolymerizable and degradable biomaterials for tissue engineering applications. *Tissue Eng.* 2007;**13**(10): 2369–2385.
17. Betz, R. R. Limitations of autograft and allograft: New synthetic solutions. *Orthopedics* 2002;**25**(5 Suppl):s561–570.
18. Burkoth, A. K. and K. S. Anseth. A review of photocrosslinked polyanhydrides: In situ forming degradable networks. *Biomaterials* 2000;**21**(23):2395–2404.
19. Van Tomme, S. R., G. Storm, and W. E. Hennink. In situ gelling hydrogels for pharmaceutical and biomedical applications. *Int. J. Pharm.* 2008;**355**(1–2):1–18.
20. Nguyen, K. T. and J. L. West. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials* 2002;**23**(22):4307–4314.
21. Williams, C. G., A. N. Malik, T. K. Kim, P. N. Manson, and J. H. Elisseeff. Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. *Biomaterials* 2005;**26**(11):1211–1218.
22. Sawhney, A. S., C. P. Pathak, and J. A. Hubbell. Interfacial photopolymerization of poly(ethylene glycol)-based hydrogels upon alginate-poly(l-lysine) microcapsules for enhanced biocompatibility. *Biomaterials* 1993;**14**(13):1008–1016.
23. Elisseeff, J., K. Anseth, D. Sims, W. McIntosh, M. Randolph, and R. Langer. Transdermal photopolymerization for minimally invasive implantation. *Proc. Natl. Acad. Sci. U. S. A.* 1999;**96**(6):3104–3107.
24. Meinhardt, M., R. Krebs, A. Anders, U. Heinrich, and H. Tronnier. Wavelength-dependent penetration depths of ultraviolet radiation in human skin. *J. Biomed. Opt.* 2008;**13**(4):044030. doi:10:1117/1.2957970.
25. Ritz, J. P., A. Roggan, C. Isbert, G. Muller, H. J. Buhr, and C. T. Germer. Optical properties of native and coagulated porcine liver tissue between 400 and 2400 nm. *Lasers Surg. Med.* 2001;**29**(3):205–212.
26. Klouda, L. and A. G. Mikos. Thermoresponsive hydrogels in biomedical applications. *Eur. J. Pharm. Biopharm.* 2008;**68**: (1): 34–45.
27. Ju, X. J., R. Xie, L. Yang, and L. Y. Chu. Biodegradable "intelligent" materials in response to physical stimuli for biomedical applications. *Expert Opin. Ther. Pat.* 2009;**19**(4):493–507.
28. Desbrieres, J., M. Hirrien, and S. B. Ross-Murphy. Thermogelation of methylcellulose: Rheological considerations. *Polymer* 2000;**41**:2451–2461.

29. Smidsrod, O. and G. Skjak-Braek. Alginate as immobilization matrix for cells. *Trends Biotechnol*. 1990;**8**(3):71–78.
30. Draget, K. I., G. Skjak-Braek, and O. Smidsrod. Alginate based new materials. *Int. J. Biol. Macromol*. 1997;**21**(1–2):47–55.
31. LeRoux, M. A., F. Guilak, and L. A. Setton. Compressive and shear properties of alginate gel: Effects of sodium ions and alginate concentration. *J. Biomed. Mater. Res*. 1999;**47**(1):46–53.
32. Kong, H. J., K. Y. Lee, and D. J. Mooney. Decoupling the dependence of rheological/mechanical properties of hydrogels from solids concentration. *Polymer* 2002;**43**(23):6239–6246.
33. Kohn, R. and B. Larsen. Preparation of water-soluble polyuronic acids and their calcium salts, and the determination of calcium ion activity in relation to the degree of polymerization. *Acta Chem. Scand*. 1972;**26**(6):2455–2468.
34. Braudo, E. E., A. A. Soshkinsky, V. P. Yuryev, and V. B. Tolstoguzov. The interaction of polyuronides with calcium ions. 1: Binding isotherms of calcium ions with pectic substances. *Carbohydr. Polym*. 1992;**18**:165–169.
35. Ahmed, T. A., E. V. Dare, and M. Hincke. Fibrin: A versatile scaffold for tissue engineering applications. *Tissue Eng. Part B Rev*. 2008;**14**(2):199–215.
36. Strehin, I., W. M. Ambrose, O. Schein, A. Salahuddin, and J. Elisseeff. Synthesis and characterization of a chondroitin sulfate-polyethylene glycol corneal adhesive. *J. Cataract Refract. Surg*. 2009;**35**(3):567–576.
37. Beniash, E., J. D. Hartgerink, H. Storrie, J. C. Stendahl, and S. I. Stupp. Self-assembling peptide amphiphile nanofiber matrices for cell entrapment. *Acta Biomater*. 2005;**1**(4):387–397.
38. Lee, H. K., S. Soukasene, H. Jiang, S. Zhang, W. Feng, and S. I. Stupp. Light-induced self-assembly of nanofibers inside liposomes. *Soft Matter* 2008;**4**(5):962–964.
39. Bell, C. J., L. M. Carrick, J. Katta, Z. Jin, E. Ingham, A. Aggeli, N. Boden, T. A. Waigh, and J. Fisher. Self-assembling peptides as injectable lubricants for osteoarthritis. *J. Biomed. Mater. Res. A* 2006;**78**(2):236–246.
40. Zhang, L., E. M. Furst, and K. L. Kiick. Manipulation of hydrogel assembly and growth factor delivery via the use of peptide-polysaccharide interactions. *J. Control. Release* 2006;**114**(2):130–142.
41. Seal, B. L. and A. Panitch. Physical polymer matrices based on affinity interactions between peptides and polysaccharides. *Biomacromolecules* 2003;**4**(6):1572–1582.
42. Hart, D. S. and S. H. Gehrke. Thermally associating polypeptides designed for drug delivery produced by genetically engineered cells. *J. Pharm. Sci*. 2007;**96**(3):484–516.
43. Allahham, A., P. Stewart, J. Marriott, and D. Mainwaring. Factors affecting shear thickening behavior of a concentrated injectable suspension of levodopa. *J. Pharm. Sci*. 2005;**94**(11):2393–2402.
44. Gupta, D., C. H. Tator, and M. S. Shoichet. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. *Biomaterials* 2006;**27**(11):2370–2379.
45. Hoare, T., D. Zurakowski, R. Langer, and D. S. Kohane. Rheological blends for drug delivery. I. Characterization in vitro. *J. Biomed. Mater. Res A* 2009;**92A**(2):575–585.

46. Ambrosio, L., R. De Santis, and L. Nicolais. Composite hydrogels for implants. *Proc. Inst. Mech. Eng. H* 1998;**212**(2):93–99.
47. Zhao, H., L. Ma, C. Gao, and J. Shen. A composite scaffold of PLGA microspheres/fibrin gel for cartilage tissue engineering: Fabrication, physical properties, and cell responsiveness. *J. Biomed. Mater. Res. B Appl. Biomater.* 2009;**88** (1):240–249.
48. Raeber, G. P., M. P. Lutolf, and J. A. Hubbell. Molecularly engineered PEG hydrogels: A novel model system for proteolytically mediated cell migration. *Biophys. J.* 2005;**89**(2):1374–1388.
49. Chung, C., M. Beecham, R. L. Mauck, and J. A. Burdick.The influence of degradation characteristics of hyaluronic acid hydrogels on in vitro neocartilage formation by mesenchymal stem cells. *Biomaterials* 2009;**30**(26):4287–4296.
50. Kloxin, A. M., A. M. Kasko, C. N. Salinas, and K. S. Anseth. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* 2009;**324** (5923):59–63.
51. Jain, A., and S. K. Jain. PEGylation: An approach for drug delivery. A review. *Crit. Rev. Ther. Drug Carrier Syst.* 2008;**25**(5):403–447.
52. Vihola, H., A. Laukkanen, L. Valtola, H. Tenhu, and J. Hirvonen. Cytotoxicity of thermosensitive polymers poly(N-isopropylacrylamide), poly(N-vinylcaprolactam) and amphiphilically modified poly(N-vinylcaprolactam). *Biomaterials* 2005;**26** (16):3055–3064.
53. Cruise, G. M., O. D. Hegre, D. S. Scharp, and J. A. Hubbell. A sensitivity study of the key parameters in the interfacial photopolymerization of poly(ethylene glycol) diacrylate upon porcine islets. *Biotechnol. Bioeng.* 1998;**57**(6):655–665.
54. Atsumi, T., M. Ishihara, Y. Kadoma, K. Tonosaki, and S. Fujisawa. Comparative radical production and cytotoxicity induced by camphorquinone and 9-fluorenone against human pulp fibroblasts. *J. Oral Rehabil.* 2004;**31**(12):1155–1164.
55. Shin, H., J. S. Temenoff, and A. G. Mikos. In vitro cytotoxicity of unsaturated oligo[poly(ethylene glycol) fumarate] macromers and their cross-linked hydrogels. *Biomacromolecules* 2003;**4**(3):552–560.
56. Anderson, J. M., A. Rodriguez, and D. T. Chang. Foreign body reaction to biomaterials. *Semin. Immunol.* 2008;**20**(2):86–100.
57. Yamada, T. and T. Kawasaki. Microbial synthesis of hyaluronan and chitin: New approaches. *J. Biosci. Bioeng.* 2005;**99**(6):521–528.
58. Dell'Accio, F., C. De Bari, and F. P. Luyten. Microenvironment and phenotypic stability specify tissue formation by human articular cartilage-derived cells in vivo. *Exp. Cell Res.* 2003;**287**(1):16–27.
59. Dell'Accio, F., C. De Bari, and F. P. Luyten. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. *Arthritis Rheum.* 2001;**44**(7):1608–1619.
60. Dusseault, J., F. A. Leblond, R. Robitaille, G. Jourdan, J. Tessier, M. Menard, N. Henley, and J. P. Halle. Microencapsulation of living cells in semi-permeable membranes with covalently cross-linked layers. *Biomaterials* 2005;**26** (13):1515–1522.
61. Redl, H. and G. Schlag. Fibrin sealant (Tissucol). Application using a duploject system. *Rev. Laryngol. Otol. Rhinol. (Bord)* 1987;**108**(1):9–11.

62. Zhao, H., L. Ma, J. Zhou, Z. Mao, C. Gao, and J. Shen. Fabrication and physical and biological properties of fibrin gel derived from human plasma. *Biomed. Mater.* 2008;**3**(1):15001.

63. Cho, A. B. and R. M. Junior. Application of fibrin glue in microvascular anastomoses: Comparative analysis with the conventional suture technique using a free flap model. *Microsurgery* 2008;**28**(5):367–374.

64. Steinwachs, M. New technique for cell-seeded collagen-matrix-supported autologous chondrocyte transplantation. *Arthroscopy* 2009;**25**(2):208–211.

65. Moser, C., I. Opitz, W. Zhai, V. Rousson, E. W. Russi, W. Weder, and D. Lardinois. Autologous fibrin sealant reduces the incidence of prolonged air leak and duration of chest tube drainage after lung volume reduction surgery: A prospective randomized blinded study. *J. Thorac. Cardiovasc. Surg.* 2008;**136**(4):843–849.

66. Martin, N. E., J. W. Kim, and D. H. Abramson. Fibrin sealant for retinoblastoma: where are we? *J. Ocul. Pharmacol. Ther.* 2008;**24**(5):433–438.

67. Volpi, N., J. Schiller, R. Stern, and L. Soltes. Role, metabolism, chemical modifications and applications of hyaluronan. *Curr. Med. Chem.* 2009;**16**(14):1718–1745.

68. Sato, M., C. Sakata, M. Yabe, and T. Oshika. Soft-shell technique using Viscoat and Healon 5: A prospective, randomized comparison between a dispersive-viscoadaptive and a dispersive-cohesive soft-shell technique. *Acta Ophthalmol.* 2008;**86**(1):65–70.

69. Arshinoff, S. A. Dispersive-cohesive viscoelastic soft shell technique. *J. Cataract Refract. Surg.* 1999;**25**(2):167–173.

70. Molitierno, J. A., H. C. Scherz, and A. J. Kirsch. Endoscopic treatment of vesicoureteral reflux using dextranomer hyaluronic acid copolymer. *J. Pediatr. Urol.* 2008;**4**(3):221–228.

71. Chapple, C. R., F. Haab, M. Cervigni, C. Dannecker, A. Fianu-Jonasson, and A. H. Sultan. An open, multicentre study of NASHA/Dx Gel (Zuidex) for the treatment of stress urinary incontinence. *Eur. Urol.* 2005;**48**(3):488–494.

72. Beasley, K. L., M. A. Weiss, and R. A. Weiss. Hyaluronic acid fillers: A comprehensive review. *Facial Plast. Surg.* 2009;**25**(2):86–94.

73. Edwards, P. C. and J. E. Fantasia. Review of long-term adverse effects associated with the use of chemically-modified animal and nonanimal source hyaluronic acid dermal fillers. *Clin. Interv. Aging* 2007;**2**(4):509–519.

74. Waddell, D. D. Viscosupplementation with hyaluronans for osteoarthritis of the knee: Clinical efficacy and economic implications. *Drugs Aging* 2007;**24**(8):629–642.

75. Conrozier, T. and X. Chevalier. Long-term experience with hylan GF-20 in the treatment of knee osteoarthritis. *Expert Opin. Pharmacother.* 2008;**9**(10):1797–1804.

76. Bellamy, N., J. Campbell, V. Robinson, T. Gee, R. Bourne, and G. Wells. Viscosupplementation for the treatment of osteoarthritis of the knee. *Cochrane Database Syst. Rev.* 2006;(**2**), CD005321.

77. Magilavy, D., R. Polisson, and C. Murray. Acute local reaction to intra-articular Hylan G-F 20 (Synvisc): Part I. *J. Bone Joint Surg. Am.* 2003;**85-A**,(8):1618–1619; author reply 1619–1620.

78. Chung, C. and J. A. Burdick. Influence of three-dimensional hyaluronic acid microenvironments on mesenchymal stem cell chondrogenesis. *Tissue Eng. Part A* 2009;**15**(2):243–254.
79. Park, Y. D., N. Tirelli, and J. A. Hubbell. Photopolymerized hyaluronic acid-based hydrogels and interpenetrating networks. *Biomaterials* 2003;**24**(6):893–900.
80. O'Leary, M. A. and G. A. Grillone. Injection laryngoplasty. *Otolaryngol. Clin. North Am.* 2006;**39**(1):43–54.
81. Pan, L., Y. Ren, F. Cui, and Q. Xu. Viability and differentiation of neural precursors on hyaluronic acid hydrogel scaffold. *J. Neurosci. Res.* 2009;**87**(14) 3207–3220.
82. Wang, W., M. Fan, L. Zhang, S. H. Liu, L. Sun, and C. Y. Wang. Compatibility of hyaluronic acid hydrogel and skeletal muscle myoblasts. *Biomed. Mater.* 2009;**4** (2):25011.
83. Wallace, D. G. and J. Rosenblatt. Collagen gel systems for sustained delivery and tissue engineering. *Adv. Drug Deliv. Rev.* 2003;**55**(12):1631–1649.
84. Franchi, M., A. Trire, M. Quaranta, E. Orsini, and V. Ottani. Collagen structure of tendon relates to function. *Scienti. World. J.* 2007;**7**:404–420.
85. Cen, L., W. Liu, L. Cui, W. Zhang, and Y. Cao. Collagen tissue engineering: Development of novel biomaterials and applications. *Pediatr. Res.* 2008;**63** (5):492–496.
86. Cockerham, K. and V. J. Hsu. Collagen-based dermal fillers: past, present, future. *Facial Plast. Surg.* 2009;**25**(2):106–113.
87. Bauman, L. CosmoDerm/CosmoPlast (human bioengineered collagen) for the aging face. *Facial Plast. Surg.* 2004;**20**(2):125–128.
88. Narins, R. S., F. S. Brandt, Z. P. Lorenc, C. S. Maas, G. D. Monheit, and S. R. Smith. Twelve-month persistency of a novel ribose-cross-linked collagen dermal filler. *Dermatol. Surg.* 2008;**34**(Suppl 1):S31–39.
89. Hilinski, J. M. and S. R. Cohen. Soft tissue augmentation with ArteFill. *Facial Plast. Surg.* 2009;**25**(2):114–119.
90. Courey, M. S. Injection laryngoplasty. *Otolaryngol. Clin. North Am.* 2004;**37** (1):121–138.
91. Remacle, M. and G. Lawson. Results with collagen injection into the vocal folds for medialization. *Curr. Opin. Otolaryngol. Head Neck Surg.* 2007;**15**(3): 148–152.
92. Ohya, S. and T. Matsuda. Poly(N-isopropylacrylamide) (PNIPAM)-grafted gelatin as thermoresponsive three-dimensional artificial extracellular matrix: molecular and formulation parameters vs. cell proliferation potential. *J. Biomater. Sci. Polym. Ed.* 2005;**16**(7):809–827.
93. Funayama, A., Y. Niki, H. Matsumoto, S. Maeno, T. Yatabe, H. Morioka, S. Yanagimoto, T. Taguchi, J. Tanaka, and Y. Toyama. Repair of full-thickness articular cartilage defects using injectable type II collagen gel embedded with cultured chondrocytes in a rabbit model. *J. Orthop. Sci.* 2008; **13**(3):225–232.
94. Hoshikawa, A., Y. Nakayama, T. Matsuda, H. Oda, K. Nakamura, and K. Mabuchi. Encapsulation of chondrocytes in photopolymerizable styrenated gelatin for cartilage tissue engineering. *Tissue Eng.* 2006;**12**(8):2333–2341.

95. Dong, C. M., X. Wu, J. Caves, S. S. Rele, B. S. Thomas, and E. L. Chaikof. Photomediated crosslinking of C6-cinnamate derivatized type I collagen. *Biomaterials* 2005;**26**(18):4041–4049.

96. Gamez, E., K. Ikezaki, M. Fukui, and T. Matsuda. Photoconstructs of nerve guidance prosthesis using photoreactive gelatin as a scaffold. *Cell Transplant*. 2003;**12**(5):481–490.

97. Malavaki, C., S. Mizumoto, N. Karamanos, and K. Sugahara. Recent advances in the structural study of functional chondroitin sulfate and dermatan sulfate in health and disease. *Connect. Tissue. Res.* 2008;**49**(3):133–139.

98. Nandini, C. D., and K. Sugahara. Role of the sulfation pattern of chondroitin sulfate in its biological activities and in the binding of growth factors. *Adv. Pharmacol.* 2006;**53**:253–279.

99. Varghese, S., N. S. Hwang, A. C. Canver, P. Theprungsirikul, D. W. Lin, and J. Elisseeff. Chondroitin sulfate based niches for chondrogenic differentiation of mesenchymal stem cells. *Matrix Biol.* 2008;**27**(1):12–21.

100. Zimmermann, H., S. G. Shirley, and U. Zimmermann. Alginate-based encapsulation of cells: Past, present, and future. *Curr. Diab. Rep.* 2007;**7**(4):314–320.

101. Balakrishnan, B., and A. Jayakrishnan. Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. *Biomaterials* 2005;**26**(18):3941–3951.

102. Park, D. J., B. H. Choi, S. J. Zhu, J. Y. Huh, B. Y. Kim, and S. H. Lee. Injectable bone using chitosan-alginate gel/mesenchymal stem cells/BMP-2 composites. *J. Craniomaxillofac. Surg.* 2005;**33**(1):50–54.

103. Tonnesen, H. H., and J. Karlsen. Alginate in drug delivery systems. *Drug Dev. Ind. Pharm.* 2002;**28**(6):621–630.

104. Prabaharan, M., and J. F. Mano. Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. *Macromol. Biosci.* 2006;**6**(12):991–1008.

105. Ren, D., H. Yi, W. Wang, and X. Ma. The enzymatic degradation and swelling properties of chitosan matrices with different degrees of N-acetylation. *Carbohydr. Res.* 2005;**340**(15):2403–2410.

106. d'Ayala, G. G., M. Malinconico, and P. Laurienzo. Marine derived polysaccharides for biomedical applications: chemical modification approaches. *Molecules* 2008;**13**(9):2069–2106.

107. Suzuki, K., M. Shinya, and M. Kitagawa. Basic study of healing of injuries to the myometrium and amniotic membrane using photocrosslinkable chitosan. *J. Obstet. Gynaecol. Res.* 2006;**32**(2):140–147.

108. Ono, K., M. Ishihara, Y. Ozeki, H. Deguchi, M. Sato, Y. Saito, H. Yura, M. Sato, M. Kikuchi, A. Kurita, and T. Maehara. Experimental evaluation of photocrosslinkable chitosan as a biologic adhesive with surgical applications. *Surgery* 2001;**130**(5):844–850.

109. Cho, J., M. C. Heuzey, A. Begin, and P. J. Carreau. Physical gelation of chitosan in the presence of beta-glycerophosphate: The effect of temperature. *Biomacromolecules* 2005;**6**(6):3267–3275.

110. Lee, J. W., M. C. Jung, H. D. Park, K. D. Park, and G. H. Ryu. Synthesis and characterization of thermosensitive chitosan copolymer as a novel biomaterial. *J. Biomater. Sci. Polym. Ed.* 2004;**15**(8):1065–1079.

111. Ganguly, S. and A. K. Dash. A novel in situ gel for sustained drug delivery and targeting. *Int. J. Pharm.* 2004;**276**(1–2):83–92.
112. Kim, I. Y., S. J. Seo, H. S. Moon, M. K. Yoo, I. Y. Park, B. C. Kim, and C. S. Cho. Chitosan and its derivatives for tissue engineering applications. *Biotechnol. Adv.* 2008;**26**(1):1–21.
113. Shi, C., Y. Zhu, X. Ran, M. Wang, Y. Su, and T. Cheng. Therapeutic potential of chitosan and its derivatives in regenerative medicine. *J. Surg. Res.* 2006;**133** (2):185–192.
114. Ta, H. T., C. R. Dass, and D. E. Dunstan. Injectable chitosan hydrogels for localised cancer therapy. *J. Control. Release* 2008;**126**(3):205–216.
115. Lai, W. F., and M. C. Lin. Nucleic acid delivery with chitosan and its derivatives. *J. Control. Release* 2009;**134**(3):158–168.
116. Paolicelli, P., M. de la Fuente, A. Sanchez, B. Seijo, and M. J. Alonso. Chitosan nanoparticles for drug delivery to the eye. *Expert Opin. Drug Deliv.* 2009;**6** (3):239–253.
117. Chasan, P. E. The history of injectable silicone fluids for soft-tissue augmentation. *Plast. Reconstr. Surg.* 2007;**120**(7):2034–2040; discussion 2041–2043.
118. Narins, R. S., and K. Beer. Liquid injectable silicone: A review of its history, immunology, technical considerations, complications, and potential. *Plast. Reconstr. Surg.* 2006;**118**(3 Suppl):77S–84S.
119. ter Meulen, P. H., L. C. Berghmans, and P. E. van Kerrebroeck. Systematic review: Efficacy of silicone microimplants (Macroplastique) therapy for stress urinary incontinence in adult women. *Eur. Urol.* 2003;**44**(5):573–582.
120. Ersek, R. A., S. R. Gregory, and A. V. Salisbury. Bioplastique at 6 years: Clinical outcome studies. *Plast. Reconstr. Surg.* 1997;**100**(6):1570–1574.
121. Zimmermann, U. S., and T. J. Clerici. The histological aspects of fillers complications. *Semin. Cutan. Med. Surg.* 2004;**23**(4):241–250.
122. Pastor, J. C., J. M. Zarco, M. J. Del Nozal, A. Pampliega, and P. Marinero. Clinical consequences of the use of highly purified silicone oil. Comparative study of highly and less purified silicone oil. *Eur. J. Ophthalmol.* 1998;**8**(3):179–183.
123. Shen, Y. D. and C. M. Yang. Extended silicone oil tamponade in primary vitrectomy for complex retinal detachment in proliferative diabetic retinopathy: A long-term follow-up study. *Eur. J. Ophthalmol.* 2007;**17**(6):954–960.
124. Choi, W. C., S. K. Choi, and J. H. Lee. Silicone oil keratopathy. *Korean J. Ophthalmol.* 1993;**7**(2):65–69.
125. Sodergard, A. and M. Stolt. Properties of lactic acid based polymers and their correlation with composition. *Prog. Polym. Sci.* 2002;**27**:1123–1163.
126. Mohamed, F. and C. F. van der Walle. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. *J. Pharm. Sci.* 2008;**97**(1):71–87.
127. Wischke, C. and S. P. Schwendeman. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. *Int. J. Pharm.* 2008;**364**(2):298–327.
128. Lacombe, V. Sculptra: A stimulatory filler. *Facial Plast. Surg.* 2009;**25**(2):95–99.
129. Johnson, B. A. Naltrexone long-acting formulation in the treatment of alcohol dependence. *Ther. Clin. Risk Manag.* 2007;**3**(5):741–749.

130. Sinha, V. R. and A. Trehan. Biodegradable microspheres for parenteral delivery. *Crit. Rev. Ther. Drug Carrier Syst.* 2005;**22**(6):535–602.

131. Rabin, C., Y. Liang, R. S. Ehrlichman, A. Budhian, K. L. Metzger, C. Majewski-Tiedeken, K. I. Winey, and S. J. Siegel. In vitro and in vivo demonstration of risperidone implants in mice. *Schizophr. Res.* 2008;**98**(1–3):66–78.

132. Yasukawa, K., D. Sawamura, H. Sugawara, and N. Kato. Leuprorelin acetate granulomas: case reports and review of the literature. *Br. J. Dermatol.* 2005;**152**(5):1045–1047.

133. Sartor, O. Eligard: Leuprolide acetate in a novel sustained-release delivery system. *Urology* 2003;**61**(2 Suppl 1):25–31.

134. Perez-Marrero, R. and R. C. Tyler. A subcutaneous delivery system for the extended release of leuprolide acetate for the treatment of prostate cancer. *Expert Opin. Pharmacother.* 2004;**5**(2):447–457.

135. Gombotz, W. R., G. H. Wang, T. A. Horbett, and A. S. Hoffman. Protein adsorption to poly(ethylene oxide) surfaces. *J. Biomed. Mater. Res.* 1991;**25**(12):1547–1562.

136. Lin, C. C. and K. S. Anseth. PEG hydrogels for the controlled release of biomolecules in regenerative medicine. *Pharm. Res.* 2009;**26**(3):631–643.

137. Pol, B. J., P. B. van Wachem, L. van der Does, and A. Bantjes. In vivo testing of crosslinked polyethers. II. Weight loss, IR analysis, and swelling behavior after implantation. *J. Biomed. Mater. Res.* 1996;**32**(3):321–331.

138. Xin, A. X., C. Gaydos, and J. J. Mao. In vitro degradation behavior of photopolymerized PEG hydrogels as tissue engineering scaffold. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2006;**1**:2091–2093.

139. Christenson, E. M., J. M. Anderson, and A. Hiltner. Oxidative mechanisms of poly (carbonate urethane) and poly(ether urethane) biodegradation: In vivo and in vitro correlations. *J. Biomed. Mater. Res. A* 2004;**70**(2):245–255.

140. Torchiana, D. F. Polyethylene glycol based synthetic sealants: Potential uses in cardiac surgery. *J. Card. Surg.* 2003;**18**(6):504–506.

141. Ferguson, R. E. and B. Rinker. The use of a hydrogel sealant on flexor tendon repairs to prevent adhesion formation. *Ann. Plast. Surg.* 2006;**56**(1):54–58.

142. Wallace, D. G., G. M. Cruise, W. M. Rhee, J. A. Schroeder, J. J. Prior, J. Ju, M. Maroney, J. Duronio, M. H. Ngo, T. Estridge, and G. C. Coker. A tissue sealant based on reactive multifunctional polyethylene glycol. *J. Biomed. Mater. Res.* 2001;**58**(5):545–555.

143. Hoffmann, N. E., S. A. Siddiqui, S. Agarwal, S. H. McKellar, H. J. Kurtz, M. T. Gettman, M. H. Ereth. Choice of hemostatic agent influences adhesion formation in a rat cecal adhesion model. *J. Surg. Res.* 2009;**155**(1):77–81.

144. Napoleone, C. P., G. Oppido, E. Angeli, and G. Gargiulo. Resternotomy in pediatric cardiac surgery: CoSeal initial experience. *Interact. Cardiovasc. Thorac. Surg.* 2007;**6**(1):21–23.

145. D'Andrilli, A., C. Andreetti, M. Ibrahim, A. M. Ciccone, F. Venuta, U. Mansmann, and E. A. Rendina. A prospective randomized study to assess the efficacy of a surgical sealant to treat air leaks in lung surgery. *Eur. J. Cardiothorac. Surg.* 2009;**35**(5):817–820; discussion 820–821.

146. Saunders, M. M., Z. C. Baxter, A. Abou-Elella, A. R. Kunselman, and J. C. Trussell. BioGlue and Dermabond save time, leak less, and are not mechanically inferior to two-layer and modified one-layer vasovasostomy. *Fertil. Steril*. 2009;**91**(2):560–565.

147. Qiao, M., D. Chen, X. Ma, and Y. Liu. Injectable biodegradable temperature-responsive PLGA-PEG-PLGA copolymers: Synthesis and effect of copolymer composition on the drug release from the copolymer-based hydrogels. *Int. J. Pharm*. 2005;**294**(1–2):103–112.

148. Elstad, N. L. and K. D. Fowers. OncoGel (ReGel/paclitaxel)—Clinical applications for a novel paclitaxel delivery system. *Adv. Drug. Deliv. Rev*. 2009;**61** (10):785–794.

149. Kim, H. K., W. S. Shim, S. E. Kim, K. H. Lee, E. Kang, J. H. Kim, K. Kim, I. C. Kwon, and D. S. Lee. Injectable in situ-forming pH/thermo-sensitive hydrogel for bone tissue engineering. *Tissue Eng. Part. A* 2009;**15**(4):923–933.

150. Garagorri, N., S. Fermanian, T. Thibault, W. M. Ambrose, O. D. Schein, S. Chakravarti, and J. Elisseeff. Keratocyte behavior in three-dimensional photopolymerizable poly(ethylene glycol) hydrogels. *Acta Biomater*. 2008;**4** (5):1139–1147.

151. Hwang, N. S., S. Varghese, H. J. Lee, P. Theprungsirikul, A. Canver, B. Sharma, and J. Elisseeff. Response of zonal chondrocytes to extracellular matrix-hydrogels. *FEBS Lett*. 2007;**581**(22):4172–4178.

152. Vernengo, J., G. W. Fussell, N. G. Smith, and A. M. Lowman. Evaluation of novel injectable hydrogels for nucleus pulposus replacement. *J. Biomed. Mater. Res. B Appl. Biomater*. 2008;**84**(1):64–69.

153. Underhill, G. H., A. A. Chen, D. R. Albrecht, and S. N. Bhatia. Assessment of hepatocellular function within PEG hydrogels. *Biomaterials* 2007;**28**(2):256–270.

154. Mahoney, M. J., and K. S. Anseth. Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. *Biomaterials* 2006;**27** (10):2265–2274.

155. Lee, H. J., C. Yu, T. Chansakul, N. S. Hwang, S. Varghese, S. M. Yu, and J. H. Elisseeff. Enhanced chondrogenesis of mesenchymal stem cells in collagen mimetic peptide-mediated microenvironment. *Tissue Eng. Part A* 2008;**14** (11):1843–1851.

156. Tessmar, J. K. and A. M. Gopferich. Customized PEG-derived copolymers for tissue-engineering applications. *Macromol. Biosci*. 2007;**7**(1):23–39.

157. Hwang, N. S., S. Varghese, Z. Zhang, and J. Elisseeff. Chondrogenic differentiation of human embryonic stem cell-derived cells in arginine-glycine-aspartate-modified hydrogels. *Tissue Eng*. 2006;**12**(9):2695–2706.

158. Nie, T., R. E. Akins, Jr. and K. L. Kiick. Production of heparin-containing hydrogels for modulating cell responses. *Acta Biomater*. 2009;**5**(3):865–875.

159. Wang, D. A., S. Varghese, B. Sharma, I. Strehin, S. Fermanian, J. Gorham, D. H. Fairbrother, B. Cascio, and J. H. Elisseeff. Multifunctional chondroitin sulphate for cartilage tissue-biomaterial integration. *Nat. Mater*. 2007;**6**(5): 385–392.

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PART VIII

IPR ASPECTS OF BIODEGRADABLE POLYMERS

CHAPTER 18

GLOBAL PATENT AND TECHNOLOGICAL STATUS OF BIODEGRADABLE POLYMERS IN DRUG DELIVERY AND TISSUE ENGINEERING

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CONTENTS

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18.1 INTRODUCTION

Intellectual property rights (IPRs) are legal rights granted to innovators for *creations of the mind or intellect having commercial value*. Since creations of the mind are very different, for example, a song, a movie, a book, or an invention related to a novel drug for cancer, it is not possible to have one mechanism for protecting different creations of the mind. Accordingly, there are eight different mechanisms for protecting creations of the mind and these represent the different forms of intellectual property. These are patents (for inventions), copyrights (for literary/artistic creations), trademarks (for marks used in trade), industrial designs (for external features), registrations of geographical indications (for goods originating from a specific geographical area), layout designs of integrated circuits (for innovative electronic circuits), registration of plant varieties, (for improved/new plant varieties), and trade secrets (for undisclosed information) [1].

In this context, patents are the form of IPRs that are most important for researchers. They represent a very useful "tool" for researchers as they help to avoid duplication of work, prevent infringements, ensure access to highly technical information usually not available in journals, and also help in identification of "hot" areas of research. Another very useful aspect of patents is that they create "ownership" over research, enabling the research to be licensed out to industry, thus ensuring its practical conversion to products that benefit society.

Patents ensure rights over the invention in return for public disclosure. This ensures that the knowledge generated by an inventor is freely available for further technological developments and advancement of science. At the same time the researcher who put in original labor, time, and money is also rewarded by granting him or her exclusive rights over the work for a period of 20 years. In the absence of reward of ownership, inventors will hesitate to disclose their innovations and technical details and some will die with them, leading to loss of precious knowledge. Patents help to prevent this by bringing out the knowledge into the public domain without compromising the commercial interests of researchers who have put in the original effort.

Patents represent a vast, untapped resource of technical knowledge and innovation that can be very useful in research but about which not many researchers are aware. One of the difficulties in using patent databases in the past was the lack of digital access. One had to physically go to a patent office and check the patents. However, the Internet has radically transformed the access to patent databases. Databases comprising of millions of patents from several countries are now available in free databases that can be accessed with the click of a mouse. Some of the free patent databases are the European Patents Database representing a collection of patents of more than 80 countries and accessible at http://ep.espacenet.com and the United States of America Patent Database, which can be accessed through google at the website www.google.com/patents. Other useful databases are SciFinder and Delphion, but these are fee-based databases. It is pertinent to mention here that patent databases include both "filed patents," which disclose the technical information and inform about the latest advancements, and those that have not been granted yet. Patent applications are published 18 months after filing, whereas grant of patents takes around 3–4 years.

Thus, the present lack of use of patent databases by researchers is mainly due to lack of awareness and not difficulty in accessing information. Even today most of the researchers confine their research to the literature, with the result that a vast chunk of information documented in patents gets left out. This has serious consequences for researchers: It can lead to duplication of work since quite often an industry may just go ahead and file a patent about a cutting-edge technology without going in for publication. A second consequence of research based on the literature only is that it can lead to infringements upon patented technologies, affecting licensing of the research work.

The present book reviews the status of biodegradable polymers over a broad spectrum spanning drug delivery as well as tissue engineering. Both are applied areas in which ownership over technologies is critically important as it affects industrial linkages and investments in product development. It was felt that inclusion of patent database search would be useful to researchers in giving an overview of the technological developments and innovations that have taken place on a global level. Particularly, this review helps researchers by giving answers to a few basic questions with regard to biodegradable polymers in drug delivery and tissue engineering, namely which universities/labs contribute to the innovation process? Which industries are leaders in the area? Which countries are contributing most toward developments in this niche area? What are the problems that have been already solved? What still remain? And so on.

Global technology status regarding biodegradable polymers in drug delivery and tissue engineering was determined by searching patent databases of more than 80 countries using the official search engine of the European Patent Office (http://ep.espacenet.com). Both published patent applications as well as granted patents are reviewed. One can access the "Help Index" on the website before starting the search to enhance the reliability and completeness of the retrieved data.

18.2 METHODS

There are various modes of patent search of the European Patent Database (Fig. 18.1). In the present case, search was performed using the major keywords and terms pertaining to respective polymers as reviewed in this book. It was confined to words in the abstract and titles only to enable search of patents having greater relevance. Only the Official European Database was searched as it is the single official source of easily searchable patent database covering more than 80 countries. Latest patents from the year 2000 onward have been reviewed. The patents prior to this are mostly included in the review in the form of prior art, wherever the patents had particular importance or were "classic patents" on which technical advancement took place in subsequent patents.

Limitations of the Search. The status of patents changes every week as publication of new patents takes place on a weekly basis. Hence, the present review or status is confined to the information obtained on a particular date only. Additionally, the patents reviewed are those based on "specific keywords" confined only to the title and abstract. Accordingly, patents that may be relevant but that do not include these keywords in the title or abstract are not reviewed. This was based on practical considerations as the number of patents in a general search not confined to title or abstract yielded a very large number of patents, making their review difficult owing to sheer numbers! Additionally, it was found in some of the cases that the patents that did not include the

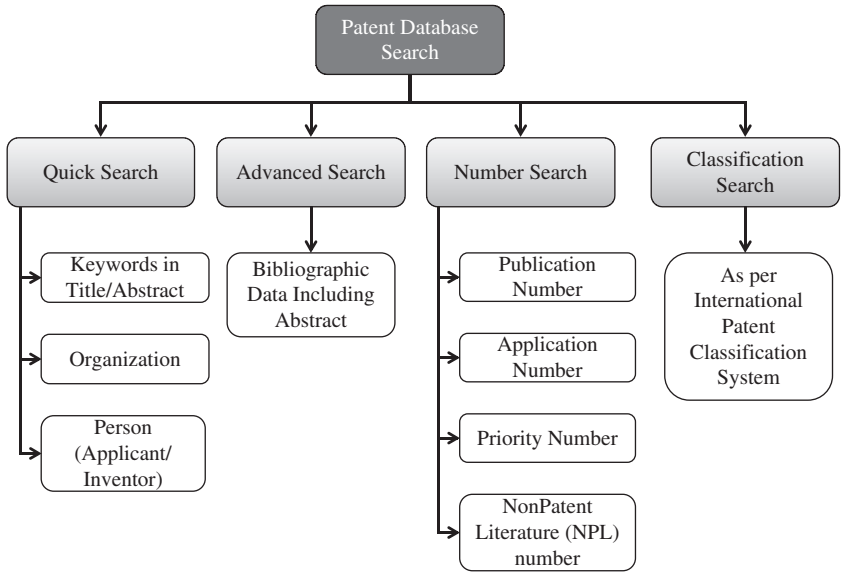


FIGURE 18.1 Search modes in European Patent Database.

respective keywords in their title or abstract were not as relevant and important as compared to those that did.

Status of patents filed in the area of natural and synthetic polymers in the field of drug delivery and tissue engineering (Date of search: 10 March 2010) is given in Table 18.1. Results obtained revealed that globally some of the major contributions in the area of polymer research were being made by industry as well as academia.

TABLE 18.1 Status of Patents Filed in the Area of Natural and Synthetic Polymers in the Field of Drug Delivery and Tissue Engineering[a]

Sr. No.	Keywords Used		No. of Patents (Filed/Granted)
Natural Polymers			
1	**Collagen**	Collagen	15,496
		Collagen and drug delivery	128
		Collagen and tissue engineering	175
2	**Gelatin**	Gelatin	21,372
		Gelatin and drug delivery	91
		Gelatin and tissue engineering	28
3	**Fibrin**	Fibrin	3,444
		Fibrin and drug delivery	16
		Fibrin and tissue engineering	30
4	**Silk fibroin**	Silk fibroin	673
		Silk fibroin and drug delivery	3
		Silk fibroin and tissue engineering	13
5	**Alginate**	Alginate	7818
		Alginate and drug delivery	60
		Alginate and tissue engineering	20
6	**Dextran**	Dextran	3950
		Dextran and drug delivery	40
		Dextran and tissue engineering	3
7	**Chitosan**	Chitosan	9,836
		Chitosan and drug delivery	160
		Chitosan and tissue engineering	80
8	**Polyhydroxyalkanoate**	Polyhydroxyalkanoate	449
		Polyhydroxyalkanoate and drug delivery	4
		Polyhydroxyalkanoate and delivery	7
		Polyhydroxyalkanoate and tissue engineering	0
9	**Arabinogalactan**	Arabinogalactan	322
		Arabinogalactan and drug	7
		Arabinogalactan and tissue	11

(*Continued*)

TABLE 18.1 (Continued)

Sr. No.	Keywords Used		No. of Patents (Filed/Granted)
Synthetic Polymers			
10	**Polyester**	Polyester	100,000
		Polyester and drug delivery	103
		Polyester and tissue engineering	57
11	**Polyorthoester**	Polyorthoester	106
		Polyorthoester and drug delivery	02
		Polyorthoester and delivery	18
		Polyorthoester and tissue engineering	02
		Polyorthoester and tissue	08
12	**Polyanhydride**	Polyanhydride	463
		Polyanhydride and drug delivery	36
		Polyanhydride and tissue engineering	05
13	**Polyurethane**	Polyurethane	100,000
		Polyurethane and drug delivery	68
		Polyurethane and tissue engineering	23
14	**PEG-based polymer**	Poly(ethylene glycol)	24,601
		PEG and drug delivery	157
		PEG and tissue engineering	10
15	**Polyaminoacid**	Polyaminoacid	84
		Polyaminoacid and delivery	6
		Polyaminoacid and tissue engineering	0
		Polyaminoacid and tissue	1
16	**Polyfumarate**	Polyfumarate	22
		Polyfumarate and delivery	0
		Polyfumarate and tissue engineering	0
		Fumarate and drug delivery	6
		Fumarate and delivery	11
		Fumarate and tissue engineering	0
		Fumarate and tissue	16

[a] Period of search- 2000 to 2010, Date of search: 10 March 2010. Database searched is the Official European Patent Database (Worldwide), which covers patent databases of nearly 80 countries including the United States Patent Database (http://ep.espacenet.com).

Patents are published by respective patent offices of each country on a weekly basis. Hence, the results (numbers) obtained are not permanent but will vary with time as new patents/patent applications get published subsequent to the date of search.

Selection of Keywords for Search. Selection of appropriate keywords for search is critical to the success of the search in helping to locate the right information. An illustrative and very interesting example is that when authors

used the keyword "formulations" in the search of title and abstract, a few results were obtained. However, when the keyword "formulation" was used in the search of title and abstract, several patents were obtained. Thus, the choice of keywords drastically affects the outcome of data obtained. Recognizing the sensitivity of the results to the keywords, the authors devised a simple and practical strategy in which a "search map" was prepared (Table 18.1). Different permutations and combinations of words were selected and agreed upon by various team members of the group, based on their expertise. Once the list of words that were relevant technically and also their possible synonyms, plurals, and the like were decided, the search was performed. Accordingly, for the readers it is advised that they should make various permutations and combinations of keywords, inventors, and so forth before embarking on a search to make sure that no relevant patent is missed.

18.3 REVIEW OF PATENTS RELATING TO POLYMERS IN DRUG DELIVERY AND TISSUE ENGINEERING

Biodegradable polymers can be broadly classified on the basis of their origin into two categories, namely natural and synthetic, both of which have been extensively explored in the field of drug delivery and tissue engineering. Natural polymers were the first biodegradable biomaterials used clinically. They possess several inherent advantages such as bioactivity, the ability to present receptor-binding ligands to cells, susceptibility to cell-triggered proteolytic degradation, and natural remodeling. However, they have their drawbacks, which include a strong immunogenic response associated with most of the polymers, the complexities associated with their purification, and the possibility of disease transmission. Synthetic biomaterials on the other hand are generally biologically inert, have batch-to-batch uniformity, and an advantage having tailored property profiles for specific applications, devoid of many of the disadvantages of natural polymers. Extensive research has thus gone into custom designing biodegradable polymer systems with predictable erosion kinetics as drug/gene delivery vehicles or as scaffolds for tissue engineering.

Table 18.2 and 18.3 show the contribution of industry as well as academia in the development of polymers for drug delivery and tissue engineering pertaining to natural and synthetic polymers, respectively. It was found that both industry and academia are responsible for the growth of this field with the maximum chunk of intellectual property coming from the People's Republic of China and the United States. The patent review for both natural and synthetic polymers is discussed in Sections 18.4 and 18.5, respectively. Table 18.4 gives the global landscape of the patents related to the use of biodegradable polymers in drug delivery and tissue engineering, discussed in the present review.

TABLE 18.2 Academic Institutes and Industries Involved in Filing Patents in the Area of Natural Polymers[a]

Sr. No.	Industry	Sr. No.	Academia/ Hospital
1	Baio Berude K. K., Japan	**1**	American National Red Cross, USA
2	Baxter International Inc., USA	**2**	Beijing University of Chemical Technology, People's Republic of China
3	Beijing Saier Taihe Biological Medical Technology Co., Ltd., People's Republic of China	**3**	California Institute of Technology, USA
4	Beijing Yiling Bioengineering Co., Ltd., People's Republic of China	**4**	Children's Medical Center Corporation, USA
5	Chondros Inc., USA	**5**	Clemson University, USA
6	E.I. du Pont de Nemours and Company, USA	**6**	Council of Scientific and Industrial Research, India
7	Enzrel, Inc., USA	**7**	Dalian University of Technology, People's Republic of China
8	Fuji Photo Film B.V., Netherland	**8**	Fourth Military Medical University, People's Republic of China
9	Hadasit Medical Research Services & Development Limited, Israel	**9**	Fujian Normal University, People's Republic of China
10	Innocoll Technologies Limited, Ireland	**10**	Guangzhou Institute of Traumatic Surgery, People's Republic of China
11	ISP Investments Inc., USA	**11**	Hadasit Medical Research & Development Ltd.
12	Medgel Corporation, Japan	**12**	Industry Foundation of Chonnam National University, S. Korea
13	Metabolix, Inc., USA	**13**	Inst Science & Tech Kwangju, Korea
14	Nuviance, Inc., USA	**14**	Jinan University People's Republic of China
15	NVR Labs, Ltd., Israel	**15**	Loyola University of Chicago
16	Orquest, Inc., USA	**16**	Nankai University, People's Republic of China
17	Pharmagenesis, Inc., USA	**17**	Northwest University, People's Republic of China
18	Polichem S.A., Luxembourg	**18**	Purdue Research Foundation, USA
19	Procter & Gamble, USA	**19**	Renomedix Institute Inc. Japan
20	SK Chemicals Co. Ltd., S. Korea	**20**	Sun Yat-Sen University, People's Republic of China

Sr. No.	Industry	Sr. No.	Academia/ Hospital
21	Sofradim Production, France	**21**	State University of New York, USA
22	Tepha, Inc., USA	**22**	The Children's Mercy Hospital, USA
23	V.I. Technologies, Inc., USA	**23**	The University of Texas System, USA
24	Xiros PLC, UK	**24**	Tianjin Medical University, People's Republic of China
25	Yissum Research Development Company, Israel	**25**	Tianjin University, People's Republic of China
		26	Tufts College, USA
		27	University of California, USA
		28	University of Michigan, USA
		29	Zhejiang University, People's Republic of China
		30	Zhongshan Ophthalmic Center, People's Republic of China

[a] Date of patent database search: 10 March 2010.

TABLE 18.3 Academic Institutes and Industries Involved in Filing Patents in the Area of Synthetic Polymers[a]

Sr. No.	Industry	Sr. No.	Academia/Hospital
1	Advanced Cardiovascular Systems, Inc., USA	**1**	Aston University
2	Advanced Polymer Systems Inc.	**2**	Changchun Applied Chemistry, People's Republic of China
3	Aesculap AG & Co KG, DE	**3**	Chinese Academy of Sciences, People's Republic of China
		4	Commonwealth Scientific and Industrial Research Organisation, Australia
4	Alcon Manufacturing Ltd., USA	**5**	Council of Scientific and Industrial Research, India
5	Alza Corp., USA	**6**	Fudan University, People's Republic of China
6	Amorepacific Corp., Korea	**7**	Guangdong Provincial People's Hospital, People's Republic of China
7	AP Pharma, Inc., USA	**8**	Industrial Technology Research Institute, Taiwan

(*Continued*)

TABLE 18.3 (Continued)

Sr. No.	Industry	Sr. No.	Academia/Hospital
8	Atrix Laboratories, USA	**9**	Korea Advanced Institute of Science and Technology, Korea
9	Bausch & Lomb Incorporated, USA	**10**	Korea Institute of Science and Technology, S. Korea
10	Biomedlab Corporation), Japan	**11**	Korea Research Institute of Chemical Technology, S. Korea
11	Bonwrx Inc, USA	**12**	Liverpool John Moores University, UK
12	Canada Natural Resources, CA	**13**	Massachusetts Institute of Technology, USA
13	Cellology Ltd, IE	**14**	Mayo Foundation for Medical Education and Research, USA
14	Cheil Jedang Corp, Korea	**15**	Mogam Biotechnology Research Institute, S. Korea
15	Chemical & Medical Res Co Ltd, Korea	**16**	Nat Science Council, Taiwan
16	Chiron Corporation, USA	**17**	National Cardiovascular Centre, Japan
17	Curexo USA, Inc., USA	**18**	National Institute of Pharmaceutical Education and Research (NIPER), India
18	Cygnus Therapeutic Systems, USA	**19**	National Science Council, Taiwan
19	Dong Sung Chemical Ind Co Ltd, Korea	**20**	Commonwealth Scientific and Industrial Research Organisation, Australia
20	Dow Chemical Co, USA	**21**	Penn State Res Found, USA
21	Edwards Life Sciences Corp, USA	**22**	Philadelphia Children Hospital, USA
22	ELLA-CS, Czech Republic	**23**	Polynovo Biomaterials Limited, Australia
23	Ethicon Inc., USA	**24**	Qingdao University of Science and Technology, Peop. Rep. China
24	Expression Genetics Inc., USA	**25**	Regents of the University of California, USA
25	Flamel Technologies, France	**26**	Rice University, USA
26	Gensia Pharma USA	**27**	Shanghai Institute of Ceramics, China
27	Guilford Pharm Inc USA	**28**	Sichuan University, People's Republic of China
28	Hande Biolog Tech Co Ltd Yunan, People's Republic of China	**29**	Sungkyunkwan University, Foundation for Corporate Collaboration, S. Korea
29	Hydrophilix Corp, USA	**30**	The Governors of the University of Alberta, Canada

Sr. No.	Industry	Sr. No.	Academia/Hospital
30	Indevus Pharmaceuticals, Inc., USA	**31**	The Penn State Research Foundation, USA
31	Insite Vision Inc., USA	**32**	The State University of New Jersey, USA
32	Integra Lifesciences I Ltd, USA	**33**	The University of Nottingham, UK
33	Isis Biopolymer LLC, USA	**34**	Università Degli Studi di Palermo, Italy
34	Isotis N.V., Neth.	**35**	University of British Columbia
35	Macromed, Inc., USA	**36**	University of California, USA
36	Mastcell Pharmaceuticals Inc., USA	**37**	University of Fudan, People's Republic of China
37	Medtronic Inc., USA	**38**	University of Gent, BE
38	Meta Dental Co., S. Korea	**39**	University of Huazhong Science Tech, People's Republic of China
39	Nipro and Co., Ltd., Japan	**40**	University of Johns Hopkins, USA
40	Nova Pharmaceutical Corp., USA	**41**	University of Minas Gerais, BR
41	Ono Pharmaceutical Co, Japan	**42**	University of Ningbo, People's Republic of China
42	Oreal, France	**43**	University of Qingdao Science & Tech, People's Republic of China
43	Organogenesis, Inc., USA	**44**	University of Sichuan, People's Republic of China
44	Pacific Corp., S. Korea	**45**	University of South Carolina, USA
45	Polymaterials AG, Germany	**46**	University of Southern California, USA
46	Poly-Med, Inc., USA	**47**	University of Sungkyunkwan Foundation, Korea
47	Polynovo Biomaterials Limited, Australia		
48	Procter & Gamble, USA	**48**	University of Texas, USA
49	Salviac Ltd, USA	**49**	University of Tongji, People's Republic of China
50	Samyang Corporation, S. Korea	**50**	University of Vanderbilt
51	Shearwater Corporation, USA	**51**	University of Virginia, USA
52	Solco Biomedical Co., Ltd.	**52**	University of Wuhan Tech, People's Republic of China
53	Spherics, Inc., USA	**53**	University of Xi An Jiaotong, People's Republic of China
54	Squibb Bristol Myers Co USA	**54**	University of Yonsei Seoul, Korea

(*Continued*)

TABLE 18.3 (Continued)

Sr. No.	Industry	Sr. No.	Academia/Hospital
55	Telomolecular Corporation, USA	**55**	Universidade Federal de Minas Gerais, Brazil
56	The Dow Chemical Company, USA	**56**	Università degli Studi di Palermo, Italy
57	Tyndale Plains-Hunter Ltd., USA	**57**	Universiteit Gent, Belgium
58	Umd Inc.	**58**	University of Michigan, USA
59	Valera Pharmaceuticals, Inc., USA	**59**	University of South Carolina, USA
60	Vitaphore Corp USA	**60**	William Marsh Rice University, USA
61	Zhejiang Jiayuan Pharmaceutical Industry Co., Ltd., People's Republic of China;	**61**	Wuhan University of Technology, People's Republic of China
62	Zhejiang Puluo Pharmaceutical, People's Republic of China		

Date of patent database search: 10 March 2010.

TABLE 18.4 Global Patent Landscape of Biodegradable Polymers for Use in Drug Delivery and Tissue Engineering Applications

Polymer	Patent Number	Innovation/Application	Reference
Natural Polymers			
Collagen	US2008241245	Prolong delivery of analgesics and anesthetics	182
	WO2006068232	Drug delivered specifically to astrocytes	4
	WO2007103555	Transdermal delivery compositions to promote collagen biosynthesis	5
	JP2008125916	Crosslinked material as an anchor for tissue regeneration or a carrier for a drug delivery	6
	WO2002017713	Porous bilayer matrices for tissue regeneration and drug delivery	7
	WO2009152384	Solutions and methods of preparing a decellularized tissue	8
	WO2009140573	Conjugates of collagen peptides and metal binding agents	9
	CN101549171	Collagen sponge bracket for regenerating cartilages	10

Polymer	Patent Number	Innovation/Application	Reference
	CN101584884	Preparation of artificial bone material	11
	CN101554490	Method for preparing new blood vessel middle layer scaffold	12
	WO2009016519	Bioresorbable wall reinforcement implants	13
	WO2009007531	Materials and methods for improving the biological properties of collagen scaffolds	14
	CN101066471	Cell-removing cornea substrate for corneal transplantation	15
	CN1887359	Skin wound repairing agar/collagen dressing	16
	US2005020506	Chemically crosslinked collagen and demineralized bone matrix as an implant for tissue engineering	17
Gelatin	WO2006085653	Water-based medical hydrogel as a drug delivery vehicle	18
	WO2004089291	Nanoparticles or microparticles of gelatin for targeting drugs to tumors	183
	CN1868553	Dermal tissue-engineered stent	19
	WO2004085473	Scaffold comprising RGD-enriched gelatin	20
	WO2008107126	Novel biodegradable fiber fabric	21
	CN1799647	Novel nanocomposite	22
	CN1720876	Novel uterine neck tissue engineering scaffold	23
	CN1546182	Novel nerve damage renovation material	24
	CN1608684	Preparation process and applications of the epidermal transplant	25
Fibrin	WO2002089868	Novel bioerodible fibrin material	26
	WO9420133	Methods of production and use of a novel tissue sealant	27
	WO9915637	Novel microbeads of a fibrin	28
	CN101002964	Novel composite skin material	29
	CN1569259	Novel tissue-engineered auto corneal epithelium	30
	WO9843686	Novel biocompatible material	31
Silk fibroin	WO2005123114	Novel sustained-release delivery system	32

(*Continued*)

TABLE 18.4 (Continued)

Polymer	Patent Number	Innovation/Application	Reference
	WO2002081793	Novel process for preparing extruded fibers	33
Alginates	WO9812228	Novel alginate chains modified by covalent bonding to a drug molecule	34
	CN101549158	Liver-targeted nanometer drug delivery system	35
	WO2008033497	Novel thermoreversible gel	36
	WO2002076429	Novel sustained-release drug delivery composition	37
	WO2004082594	Novel alginate sponge	38
	WO9618424	Methods and compositions for reconstruction of breast tissue	39
Dextran	WO2009064977	Novel tissue adhesive	40
	KR2009058420	Preparation method for dextran acetate nanoparticles	41
	US6303585	New dextran-based carrier	42
	WO2000069473	Novel macromolecular carriers	43
	WO2004029095	Novel cohesive biopolymer gels	44
	WO2001079315	Copolymer of polylactic acid with dextran	45
Chitosan	EP1958613	Novel liquid compositions	46
	WO2009091992	Novel polymer-surfaced microcolloid	47
	WO2009036022	Novel compositions	48
	WO2007083984	Novel conjugate for transmucosal delivery of active agents	49
	CN101209241	Preparation method for controlled-release nanoparticle	50
	CA2537724	Novel emulsions and creams	51
	WO2006062506	Novel pharmaceutical compositions	52
	CN101280467	Preparation method and application of nanofibers	53
	CN101225123	Preparation method and application of chitosan derivative	54
	CN101148520	Novel temperature-sensitive chitosan hydrogel	55
	CN101491702	Method for preparing a porous tissue engineering frame material	56
	CN101502673	Method for preparing injectable hydrogel	57

Polymer	Patent Number	Innovation/Application	Reference
Polyhydroxyalkanoate	WO2001019422	Novel liquid compositions or microdispersions	58
	WO2001015671	Methods for fabricating flushable drug delivery systems	59
	WO2000056376	New devices of with controlled degradation rates	60
	WO9851812	Novel chemically modified polymer	61
Arabinogalactan	WO2002002607	Novel acid-modified composition	62
	WO2007034495	Novel modified polymer conjugate	63
	US2002012705	Novel applications of polymer	64
Syntheic Polymers			
Polyesters	US2008113027	Novel pharmaceutical composition consisting of a water insoluble polymer matrix, for controlled release of an active moiety to a target site	66
	WO9959548	A sustained controlled-release system where drug is covalently conjugated to polymer	67
	WO2008025111	Production of a monolithic intraocular pharmaceutical formulation	68
	WO2004096178	A nano- or microparticle drug delivery system comprising a linear aliphatic polyester, with at least one aliphatic polyol residue	69
	US6365173	Stereocomplexes for drug delivery	70
	US7628977	Method for sustained pulmonary delivery	71
	US2007190103	An implantable medical device with surface eroding characteristics	72
	US6565874	Flowable controlled-release implant for leuprolide acetate delivery	73

(*Continued*)

TABLE 18.4 (Continued)

Polymer	Patent Number	Innovation/Application	Reference
	WO2009036083	An interpenetrating polymer network for controlled drug delivery	74
	US2009157158	Self-expanding biodegradable stent	75
	WO2004096190	Novel composition containing magnetic nanoparticles for targeted drug delivery	76
	US2009149800	Novel iontophoretic drug delivery system	77
	US2003009004	Self-assembling amphiphilic block copolymers	78
	US6410057	Micellar delivery system as particulate gene carrier	79
	US2004161464	Novel formulations for drug delivery	80
	WO2002060508	Method for the preparation of a porous scaffold/implant	81
	US2007275034	Swellable fiber- and microfiber-forming polyether-esters	82
	WO2010025176	Electroprocessed drug delivery devices	83
	CN101530631	An inventive liver tissue engineering scaffold and its preparation method	84
	CN101474428	Preparation method of a composite stent material	85
	US6207749	Novel comb polymer for modulating cell surface interactions	86
	US2002025340	Tissue volume replacement compositions and their preparation methods	87
	WO2009044403	Injectable compositions for tissue augmentation	88
	JP2007268239	Fabrication of an artificial, polymer-based blood vessel	89
	KR2001011083	Scaffold for treatment of periodontitis	90
	JP2000245450	Skin replacement graft for acute and chronic wounds	91
	JP2003126236	Porous scaffold for ocular tissue regeneration	92

Polymer	Patent Number	Innovation/Application	Reference
	US2009035349	Composite scaffold for complex tissue grafts	93, 94
Polyorthoesters	US2007264338	A stabilized, semisolid as a topical or injectable delivery system	98
	US2009142408	Nanoparticulate delivery system for human telomerase	99
	WO9414416	Novel controlled drug delivery system	100
	WO2002026209	Microparticles with adsorbent surfaces for polypeptide/ nucleic acid delivery	101
	US2004043135	Surface modification of porous scaffolds by graft polymerization and low-temperature plasma discharge technique	102
	KR2004101787	Method of preparation of porous scaffolds without organic solvent	103
	US7329413	Novel method for coating implantable medical device	104
Polyanhydride	US6486214	Novel polyanhydride linkers	109
	WO2002009767	Polyanhydride linkers for drug delivery	110
	US4916204	Novel method for synthesizing polyanhydrides in solution	111
	WO 0244232	Polyanhydrides with aliphatic hydrocarbon terminals	112
	EP260415	Novel synthesis procedure	113
	US4888176	Controlled drug release devices	114
	WO9009783	Controlled delivery of water-soluble compounds	115
	WO, 9603984	Localized delivery to solid tumors	116
	WO9213567	Novel polymeric blends for drug delivery	117
	US5179189	Novel polymers with fatty-acid modified end groups	118
	US2003105067	Localized drug delivery systems	119
	US4891225	Novel polymers for controlled drug delivery	120
	US2003199449	New therapeutic method for cancer treatment	121

(*Continued*)

TABLE 18.4 (Continued)

Polymer	Patent Number	Innovation/Application	Reference
	IN589/DEL/2007	Novel injectable drug delivery system	122
	WO2005056708	Novel bioadhesive polymers	123
	WO9912990	Novel polymers	124
	WO8901006	Novel polymers	125
	CN101357239	Novel application	126
	CN101402737	Novel electricity-conducting biomedical polymer	127
	JP2002020523	Porous scaffold	128
	KR2003097156	Porous scaffold	129
Polyurethanes	CN101503501	Novel synthesis procedure	133
	WO2005013936	Fabrication procedure for a long-term drug delivery device	134
	US2009208540	Drug delivery device	135
	US4743673	Synthesis procedure for hydrophilic carboxy polyurethanes	136
	US5387419	Controlled, site-specific delivery system for antiarrhythmic agents	137
	WO9421237	Controlled, site-specific delivery system for antiarrhythmic agents	138
	WO2006068950	Ocular drug delivery device	139
	WO2000059483	Drug reservoir for transdermal drug delivery systems	140
	WO9709970	High-capacity drug reservoir for transdermal drug delivery systems	141
	US5567488	Impermeable multilayer barrier film	142
	EP272918	Diffusion matrix for transdermal drug delivery	143
	WO2006032501	Fabrication of open-pore foam-based scaffold	144
	US6579322	CBD-RGD peptide modified biomaterial for vascular tissue engineering	145
	CN101157748	Preparation and use of a degradable mesh	146
	US2003149406	Medicated multilayered foam dressing	147
	WO2009043099	High modulus compositions for biomedical applications	148

Polymer	Patent Number	Innovation/Application	Reference
	WO2004009227	Preparation and use of flowable, injectable compositions for in situ generation of tissue engineering scaffolds	149
PEG-based polymers	US6214966	Novel derivatives for controlled delivery of bound molecules	150
	CN1995099	Stable, chemical crosslinking gel for drug delivery and tissue engineering	151
	US2010076209	Novel PEG-lipid conjugates	152
	US5939453	Novel polymers forming micellar systems	153
	WO2000018821	Novel thermosensitive polymers	154
	KR838809	Thermo/pH-sensitive block copolymers	155
	US2005019303	Novel micellar systems for drug/cosmetic delivery applications	156
	WO2008071009	Novel micellar systems for targeted drug delivery	157
	IN751/DEL/2009	Novel polymer for polymersome fabrication	158
	IN922/DEL/2010	Novel vesicular delivery system (polymersome) for amphotericin B	159
	IN913/DEL/2010	Novel vesicular delivery system (polymersome) for doxorubicin	160
	US2009247666	Thermosensitive block copolymer	161
	WO2002016557	Scaffolds modified with matrix-enhancing molecules	162
Polyaminoacids	WO2008152669	Novel protein/peptide delivery systems	164
	EP734720	Particulate drug delivery systems	165
	WO2002028521	Particulate drug delivery systems	166
	WO9958151	Novel nucleic acid delivery system	167
	WO9823226	Fabrication and use of a tissue transplant	168
Polyfumarates	WO2005099667	Ocular drug delivery composition	174
	WO2007008927	Composition for skeletal tissue reconstruction	175
	US2004023028	Polymeric microspheres for controlled drug delivery	176

(*Continued*)

TABLE 18.4 (Continued)

Polymer	Patent Number	Innovation/Application	Reference
	EP325866	Compositions for biomedical applications	177
	WO2006102530	Hydrogels for cell/drug delivery	178
	WO2006055940	Novel photocrosslinkable and self-crosslinkable block copolymers	179
	WO2008008288	Hydrogels for nerve growth	180
	WO2008063878	Novel device for nerve regeneration	181

18.4 NATURAL POLYMERS

Natural polymers have the advantage of having an intrinsic property of environmental responsiveness via degradation, bioactivity, ability to present receptor-binding ligand to cells, and remodeling by cell-secreted enzymes. They are also generally nontoxic, even at high concentrations and, therefore, can be readily fabricated into a desired form as tissue engineering scaffold or used as growth factor delivery systems. However, they suffer from some drawbacks such as immunogenic response, batch-to-batch variation, restrictions with the versatility of designing devices with specific biomechanical properties, variable rate of in vivo degradation, and the possibility of disease transmission [2]. These limitations may be overcome in the case of synthetic polymers that are readily synthesized in large quantities and can also be "tailor-made" to suit specific requirements and also by making suitable modifications in the natural polymers as per one's needs.

18.4.1 Collagen

Collagen is the most abundant protein in the animal kingdom and is the major component of the extracellular matrix and connective tissues rendering strength and flexibility to connective tissues such as tendons, bones, cartilage, blood vessels, skin, and other musculoskeletal tissues. Collagen has been extensively explored for an array of tissue engineering and biomedical applications owing to its mechanical, hemostatic, and cell-binding properties. Integra Dermal Regeneration Template, Orcel and Apligraf are a few of the products of collagen approved by the U.S. Food and Drug Administration (FDA)

Use of Collagen in Drug Delivery. Collagen has a vast array of applications in drug delivery, for example, prolonged delivery of various agents such as analgesics and anesthetics and for transdermal and ophthalmic drug delivery of

various other agents. A new method for fibrillar collagen matrix and drug substance amino amide anesthetics, amino ester anesthetics, and mixtures has been reported [3]. In this matrix, drug substance is present in an amount sufficient to provide a sufficient duration of local analgesia, local anesthesia, or nerve blockade, which lasts for one day after administration. Another application describes astrocyte-specific drug carrier containing a retinoid derivative and/or a vitamin A analog as a constituent and a therapeutic method with the use of the drug. By binding a drug carrier to a retinoid derivative such as vitamin A or a vitamin A analog or encapsulating the same in the drug carrier, a drug for therapeutic use can be delivered specifically to astrocytes. As a result, an astrocyte-related disease can be efficiently and effectively inhibited or prevented while minimizing side effects. Thus, the secretion of type I to type IV collagens can be inhibited and fibrosis can be effectively avoided [4]. Another application describes transdermal delivery compositions and topical compositions that promote collagen biosynthesis and delivery of chemical agents such as antifungal agents, anesthetics, and nonsteroidal anti-inflammatory drugs [5]. Another application describes a crosslinked protein material (collagen, gelatin, and albumin) as an anchor for tissue regeneration or a carrier for a drug delivery system, which has low toxicity and in vivo stability for a relatively long period of time, can control solubility and decomposability, and holds cell proliferation ability [6]. For tissue regeneration on orthopedic implants and drug delivery, another patent describes porous bilayer matrices of collagen and polysaccharides such as hyaluronic acid [7].

Use of Collagen in Tissue Engineering. For tissue engineering applications, solutions and methods of preparing a decellularized tissue for recellularization has been provided [8]. The solutions provide collagen conditioning to restore the collagen triple helix structure, strengthening of the collagen structure of the tissue, and biologically preparing the decellularized tissue by placing it in an environment that promotes recellularization. Another application describes conjugates of collagen peptides and metal binding agents. Resulting compositions are useful in various tissue engineering and regeneration applications such as cell culture, cell adhesion, cosmetic surgery, construction of artificial skin substitutes, management of severe burns and burn surgery, reconstruction of bone, and a wide variety of dental, orthopedic, and surgical purposes, and as a drug delivery vehicle [9]. Another use belongs to biomedicine field, relating to a type II collagen sponge bracket for regenerating cartilages in tissue engineering. This possesses uniform communication aperture suitable for cell growth and preferable mechanical intensity and three-dimensional stability of apertures structure. This can be used as tissue engineering implant, bracket materials of cell culture, or drug delivery for the application of preventing and treating of renovating cartilage densification [10]. Another patent discloses a method for preparing biomimetic artificial bone materials containing collagen, chitosan, and hydroxyapatite for biodegradable tissue engineering. This artificial bone materials prepared by this method are excellent in bone inducing activity and immune

compatibility, thereby thoroughly putting an end to the inevitable viral hidden trouble of animal collagen scaffold materials and greatly improving safety [11].

A new method for preparing blood vessel middle-layer scaffold material used for tissue engineering has been described [12]. The prepared scaffold material greatly improves the safety and efficacy owing to its excellent mechanical capacity, biocompatibility, blood and immunity compatibility, as well as lower immunity rejection reaction and virus transmission. Another application discloses bioresorbable wall reinforcement implants consisting of a bioresorbable porous matrix based on a collagen sponge that defines first pores, a bioresorbable porous three-dimensional knit that defines second pores, with the matrix filling the knit, and all the first and second pores being partially interconnected with one another [13]. Another application relates the materials and methods for improving the biological properties and electromechanical performance of collagen scaffolds used for cell transplantation, by fixation of biocompatible reagents, and adhesion molecules that control cell adhesion, apoptosis, survival, and/or differentiation simultaneously. The grafting of adhesion molecules to collagen matrices renders them suitable for use in vascular and cardiothoracic surgery/medicine, as well as in cell therapy for the heart and in artificial heart muscle engineering [14]. Another application in corneal transplantation describes cell-removing cornea substrate that is constructed of hundreds of mutually parallel collagen fiberboards, between which there are interspaces formed due to cell removing. The cell-removing cornea substrate is used for repairing surface tissue trauma of the eyes, rebuilding and shaping the surface tissue of the eyes, curing ocular surface tissue disease, and constructing the carrier of tissue engineering biology cornea. The cell-removing cornea substrate can retain for a long time or decompose gradually, showing low immunogenicity and good tissue compatibility after transplantation [15]. Further, the preparation process and applications of an agar-collagen dressing, is described for skin wound repair [16]. Agar and collagen or gelatin are mixed, and the mixture is subjected to freeze-drying to obtain porous compound rack or compound film for promoting the repair of skin wound, and the rack may be also used in other soft tissue engineering applications. Another application of collagen in the form of a porous matrix or scaffold describes a composition comprising of a collagen and demineralized bone matrix chemically crosslinked with a carbodiimide in the presence of *N*- hydroxysuccinimide. The composition can be used as an implant for tissue (e.g., soft tissue or bone) engineering [17].

18.4.2 Gelatin

Gelatin is derived by denaturing collagen and is hence free of any antigenicity property associated with collagen. Due to its promising properties such as biodegradability and biocompatibity, gelatin has been used in drug delivery, mainly in the form of microspheres. It has also been used for tissue engineering applications. Gelfoam is an absorbable gelatin sponge available in powder form.

Use of Gelatin in Drug Delivery. For application in drug delivery, water-based medical hydrogel obtained by crosslinking of gelatin has been reported [18]. This medical hydrogel posseses appropriate properties for continuous drug delivery. Another application describes a composition including fast-release and slow-release formulation for tumor targeting (nanoparticles or microparticles). This invention also uses drug-loaded gelatin and poly(lactide-*co*-glycolide) nanoparticles and microparticles for targeting drugs to tumors in the peritoneal cavity, bladder tissues, and kidneys [19]. Another application describes a dermal tissue-engineered stent for cervix uteri composed of a circular disk-type base, a semispherical or projected cylindrical head, and a neck with a central through hole. It is a single-layer or double-layer structure. It is prepared by mixing gelatin, chitosan, chitosan-derivative iodine polymer, glycerine, and hyaluronic acid, stirring, laying aside for at least 6 h , loading in mold, and then freeze-drying [19].

Use of Gelatin in Tissue Engineering. A new application concerns a cell support comprising (Arg-Gly-Asp) RGD-enriched gelatin that has a more even distribution of the minimum level of RGD sequences than naturally occurring gelatin. The invention also relates to RGD-enriched gelatins that are used for attachment to integrins. In particular the RGD-enriched gelatins of the invention can be used for coating a cell culture support for growing anchor-dependant cell types. Furthermore, the RGD-enriched gelatins of the invention may find use in medical applications, in particular, as a coating on implant or transplant material or as a component of drug delivery systems [20]. Another application provides a nonwoven fiber fabric in the form of a flat material that can be used as a biodegradable material in medicine, in particular, as an implant or carrier material for living cells (tissue engineering) but can also be used in food technology in a variety of applications [21]. Another patent discloses a nanohydroxyapatite/chitosan/gelatin composite composed of a porous bone tissue engineering cradle material. The porous cradle material comprises chitosan and gelatin with their mass ratio being 3 : 7−7 : 3 and the nanohydroxyapatite of 40−80 nm deposited on it. The material prepared in this invention possesses good mechanical property and biocompatibility [22]. Another application provides a uterine neck skin tissue engineering scaffold and its preparation method, wherein the scaffold mainly comprises a disk-shaped bottom, a hemispherical head, and a choked neck connected at the center with a central axial through hole; the scaffold is combined by an inner and an outer layer [23]. Another patent describes a complex type of tissue engineering nerve damage renovation material, which comprises type I collagen, heparitin sulfate, type IV collagen, and gelatin. The material has simple adjusting inner diameter and uniform single orientation axial bore. It is easy to be made into different external forms, for example, cylinder or rectangle [24]. Another application describes epiderm transplant based on chitosan and gelatin. Present invention also provides the preparation process and applications of the epiderm transplant with excellent mechanical performance, simple operation, short preparation period, and fast wound healing [25].

18.4.3 Fibrin

Fibrin is a biopolymer that is derived from fibrinogen and is one of the earliest biopolymers used as a biomaterial due to the excellent biocompatibility, biodegradability, and injectability. Tisseel, Evicel, Crosseal, and Bioseed are a few of the fibrin-based products available that are duly patented.

Use of Fibrin in Drug Delivery. The mechanical behavior and high retention properties for therapeutic agents causes the fibrin material to be ideally suited for use as a drug delivery device, capable of delivering biomolecules (proteins, hormones, enzymes) and chemotherapeutic agents (antibiotics, antineoplastic agents). A new drug delivery application based on bioerodible fibrin material obtained by mixing fibrinogen and thrombin has been reported. It has a tight structure with thin fibers and small pores suitable for use as an antiadhesion barrier [26]. Another application provides methods of production and use of supplemented and unsupplemented tissue sealants such as fibrin glue. This sealant does not interfere with skin wound healing and can be used for localized delivery of a growth factors and/or drugs such as 5-fluorouracil or free-base tetracycline [27].

Use of Fibrin in Tissue Engineering. A new application describes microbeads of a fibrin (including a fibrinogen) that are biologically active and extensively crosslinked and consist of cells bonded to these microbeads. A method for culturing, separating, and transplanting a cell and methods for engineering a tissue using the fibrin microbeads have also been provided [28]. Another application describes a composite skin material composed of the hypodermal cell layer and epithelial cell layer attached on fibrin scaffold. Preparation process includes steps such as isolating fibroblasts and epithelial stem cells from patient itself/foreign person, dispersing them in fibrinogen liquid, preparing serozyme solution, mixing them together, and coating the mixture on the surface of the wound [29]. Another application describes tissue-engineered autocorneal epithelium that consists of fibrin biostent and the patient's autocorneal epithelium stem cells and corneal epithelium cells anchored on it. The preparation process involves amplifying and differentiating a patient's autocorneal epithelium stem cells, inoculating to the fibrin biostent, and culturing, constructing tissue-engineered autocorneal epithelium in vitro. The tissue-engineered corneal epithelium can be applied as biomaterial for corneal transplantation [30]. Another application of fibrin includes a biocompatible material suitable for promoting cell growth, wound healing, and tissue regeneration. Applications of this technology include use on implantable devices and tissue and cell scaffolding. Other applications include use in surgical adhesive or sealant, as well as in peripheral nerve regeneration and angiogenesis [31].

18.4.4 Silk Fibroin

Silk represents protein polymer spun into fibers by silkworms, spiders, scorpions, mites, and flies. Degradable silk is a mechanically robust biomaterial

that offers a wide range of mechanical and functional properties for biomedical applications including drug delivery.

A novel sustained release silk-based delivery system and its preparation method has been reported. In this, silk fibroin solution is combined with a therapeutic agent to form a silk fibroin article, which was thereafter used [32]. Another application relates to a novel process for preparing a solution of silk or silk-like protein from various sources, and spinning it to produce a reformed silk fiber for biomedical and other applications. The process comprises dissolving silk, waste silk or silklike material in an organic solvent to form a silk fibroin solution, filtering the solution, and spinning the filtered solution into a coagulation bath to form extruded fibers. The silk fibers are useful for implantable devices such as absorbable artificial ligaments and engineering matrices, drug delivery implants, and for many other uses [33].

18.4.5 Alginates

Alginates are naturally derived polysaccharides. Alginates undergo reversible gelation in aqueous solution under mild conditions through interaction with divalent cations such as Ca^{2+}. This has led to their wide use as cell transplantation vehicles to grow new tissues, as wound dressings, and also in three-dimensional culture of chondrocytes. Several alginate-based commercially available products include Nu-Derm, Curasorb, and AlgiSite.

Use of Alginates in Drug Delivery. A new application discusses materials that contain alginate or modified alginate chains that are advantageously modified by covalent bonding to a drug molecule for cell adhesion or other cellular interaction, particularly for cell transplantation and tissue engineering applications [34]. Another application describes a sodium alginate liver-targeted nanometer drug delivery system as carrier material and uses glycyrrhetic acid as liver-targeted compound. The sodium alginate liver-targeted nanometer drug delivery system of the invention has the advantages of sustained release, reduced drug dosage, reduced drug taking times, reduced toxic side effects, increased drug effect, simple and practical preparing method, and excellent application prospect [35]. Another application describes a thermoreversible gel containing submicron-size particles containing a therapeutic molecule and alginate polyelectrolyte complex and a chitosan polyelectrolyte complex or mixtures. The therapeutic molecule used in the thermoreversible gel is lidocaine [36]. Another application describes a pH-dependent sustained-release drug delivery composition based on alginate, capable of being formed into tablets or pellets. This composition was comprised of sodium alginate, propylene glycol, and a pharmaceutical medicament [37].

Use of alginates in Tissue Engineering. A novel polymer based on alginates for cellular transplantation and its application for tissue engineering has been reported. These polymers are advantageously modified by covalent

bonding of a biologically active molecule for cell adhesion or other cellular interactions. Processes for preparation of these alginate materials and methods for using them, particularly as polymeric matrices, for example, for cell transplantation and tissue engineering applications have been described [34]. Another application describes an alginate sponge and its preparation method. This alginate sponge possesses significantly improved flexibility, structural integrity, water absorptivity, and processability to be used for medical and tissue engineering purposes with a simple preparation method [38]. Another application relates to methods and compositions for reconstruction or augmentation of breast tissue. Dissociated cells, preferably muscle cells, are implanted in combination with a suitable biodegradable polymeric matrix to generate new tissue. There are two forms of matrices that can be used: a polymeric hydrogel formed of a material such as alginate having cells suspended therein and a fibrous matrix having an interstitial spacing between about 100 and 300 μm. Other materials, such as bioactive molecules that enhance vascularization of the implanted tissue and/or that inhibit growth of fibrotic tissue, can be implanted with the matrix to enhance development of normal tissues [39].

18.4.6 Dextran

Dextrans are polysaccharides available in a wide range of molecular weights. They contain a high density of hydroxyl groups that make the polymers highly hydrophilic and capable of being further functionalized chemically. They are also used as the hydrophilic part of amphiphilic block copolymers.

Use of Dextran in Drug Delivery. A novel application discusses tissue adhesive formed by reacting primary amino groups of aminodextran with aldehyde groups of an oxidized dextran. The dextran-based polymer tissue adhesive is particularly useful in medical applications where low swell and slow degradation are needed, for example, sealing the dura, ophthalmic procedures, tissue repair, antiadhesive applications, drug delivery, and as a plug to seal a fistula or the punctum [40]. Another application describes a preparation method for dextran acetate nanoparticles. These nanoparticles have excellent delivery property for hydrophobic drug and radio-labeled material, with excellent biodegradation property. A preparation method of dextran acetate drug delivery media comprises mixing of dextran acetate nanoparticles with organic solvent and dialyzing the mixed solution followed by freeze-drying [41]. Another drug delivery application describes a dextran-based carrier and its preparation method. The preparation method involves reaction of polysaccharide with an oxidizing agent to open sugar rings on the polysaccharide and to form reactive aldehyde groups. The formed aldehyde groups are reacted with a second polysaccharide to form dimer via formation of oxime linkage [42]. Macromolecular carriers based on dextran for drugs and diagnostic agents make use of the chemical attachment of new leashes to oligomeric backbone structures. The synthesis of these leashes and their facile creation, reaction, and

conjugation with chelators and ligands makes them ideal candidates for use in medicine and in diagnostics [43].

Use of Dextran in Tissue Engineering. A novel application concerns cohesive biopolymer gels comprising coprecipitates of sulfated polysaccharides (e.g., dextran sulfate) and fibrillar proteins (e.g., gelatin) useful for clinical applications. The cohesive biopolymer gels may be used clinically as a scaffold for a cell-bearing implant, as a depot for sustained release of bioactive agents, or for research and biotechnology [44]. Another application describes preparation of biocompatible, biodegradable, copolymer by crosslinking a polylactic acid with a polysaccharide such as dextran. The resulting copolymer is a biodegradable hydrogel or solid having both hydrophobic and hydrophilic properties. The biologically active agents may be covalently bonded to the dextran prior to incorporation of the dextran into a copolymer, and release occurs as the copolymer degrades [45].

18.4.7 Chitosan

Chitin is a linear polymer found in the shells of crabs, lobsters, shrimp, and insects. Chitosan is the fully or partially deacetylated form of chitin and has attracted much attention in the tissue engineering with a wide variety of applications ranging from skin, bone, cartilage, and vascular grafts. It has been found to enhance blood coagulation accelerating wound healing, thus it can act as an ideal wound dressing. It also exhibits a positive charge, film-forming capacity, mild gelation characteristics, and a strong tissue adhesive property. HemCon is a commercially available chitosan-based bandage.

Use of Chitosan in Drug Delivery. A novel application describes liquid compositions containing chitosan or a chitosan derivative. The composition forms a dermal film after application onto the skin, useful for delivery of active agents onto the skin surface and in the stratum corneum [46]. Another application describes chitosan or silica-based nontoxic polymer-surfaced microcolloid that reduces acrolein-mediated cell death/cytotoxicity following neural insult. The cytoprotective mechanism may be (a) chitosan acts as a membrane fusogen to restore cell function, and (b) a polymer-surfaced microcolloid preferential target that damaged nerve tissues to restore conduction of nerve impulses, to seal/restore nerve fiber membranes, and to reduce baseline efflux of a large intracellular enzyme. These polymer-surfaced microcolloids are further used as a drug delivery vehicle for acrolein scavengers including hydralazine [47]. Another application describes compositions for enhanced delivery of therapeutic agents such as nucleic acids and anionic-modified polysaccharides (e.g., chitosan). The compositions may be useful in transfection of a therapeutic nucleic acid [48]. Another application describes a conjugate including an active agent covalently bound to chitosan or its derivative and a method for transmucosal delivery of active agents. The conjugate exhibits excellent absorption rate and biocompatibility, biodegradability, and

superior bioavailability even with oral administration [49]. Another patent discloses a preparation method for drug targeting controlled-release nanoparticles via eye drops. These can significantly prolong the eye surface-acting time of the drug and release the drug continuously and constantly up to 6 h [50]. Another application relates to novel chitosan–sorbitan ester emulsions and creams, particularly, chitosan sorbitan monolaurate, monooleate, and triolate emulsions, of use in the food, cosmetic, and drug delivery fields [51]. Another application comprises chitosan–based pharmaceutical compositions for administering nutraceuticals. Particularly provided are proliposomal compositions that are advantageously used to deliver nutraceuticals, including polycyclic, aromatic antioxidant, or anti-inflammatory compounds to the gastrointestinal tract after oral administration [52]. Another application describes the preparation method and application of chitosan-based nanofibers. These prepared nanofibers are biodegradable and have the imitated extracellular matrix structure, by which the controlled release of bioactivator can be achieved. By adding collagen or gelatin, the cellular compatibility of nanofibers or the control of the release speed of the bioactivator can be improved [53]. Another application describes water-soluble chitosan derivative and the preparation method and application of the same. The preparation method enjoys the advantages of mild conditions, simple operation, natural and nontoxic prepared chitosan derivatives, biodegradation and biocompatibility, and the ability to be used as injectable hydrogel support and cartilage repair materials for tissue engineering scaffold material after further polymerization [54].

Use of Chitosan in Tissue Engineering. A novel application discusses one kind of temperature-sensitive chitosan hydrogel that is liquid at lower temperatures and becomes a gel at body temperature and may be applied as the injection-implanted material. The preparation process is ecofriendly and requires mild conditions. The prepared hydrogel has high temperature sensitivity, high mechanical strength, and wide applications in tissue engineering [55]. Another application described in Section 18.3.1., chitosan has been used for the preparation of biomimetic artificial bone materials for tissue engineering [11]. Another application relates to a method for preparing a porous (80–88% porosity, pore size 100–800 μm) carbapatite/chitosan-poly (lactic acid) bony tissue engineering frame material [56]. Another application describes a method for preparing an injectable chitosan/sodium glycero-phosphate/collagen hydrogel, as a technology for preparing temperature-sensitive injectable material. The invention has the beneficial effects that the chitosan/sodium glycero-phosphate/collagen hydrogel can be maintained in the liquid state at low temperature when the pH value is within physiological range (pH value ranges from 7.0 to 7.2), whereas the mixture turns into gel when the temperature is raised to body temperature (37°C) [57].

18.4.8 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are biological polyesters produced by microorganisms under unbalanced growth conditions. For tissue engineering applications,

mainly PHA including poly-3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly-4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx), and poly-3-hydroxyoctanoate (PHO) are available.

Use of Polyhydroxyalkanoate in Drug Delivery. A novel application describes PHA materials in the form of liquid PHA polymer compositions or PHA microdispersions suitable for repair of soft tissue and for augmentation and viscosupplements in animals and humans [58]. Another application describes materials suitable for preparing components of flushable drug delivery systems. Methods for fabricating these devices, including transdermal drug delivery patches, are described. These components include drug-impermeable and drug-permeable materials, including materials that can be used to control the rate of release of drugs from the device and pressure-sensitive adhesive compositions that are drug compatible [59].

Use of Polyhydroxyalkanoates in Tissue Engineering. A novel application describes devices of biocompatible PHA with controlled degradation rates. These devices include sutures, suture fasteners, meniscus repair devices, rivets, tacks, staples, screws, bone plates and bone plating systems, surgical mesh, repair patches, slings, cardiovascular patches, orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, atrial septal defect repair devices, pericardial patches, bulking and filling agents, vein valves, bone marrow scaffolds, meniscus regeneration devices, ligament and tendon grafts, ocular cell implants, spinal fusion cages, skin substitutes, dural substitutes, bone graft substitutes, bone dowels, wound dressings, and hemostats. The PHA compositions provide favorable mechanical properties, biocompatibility, and degradation times within desirable time frames under physiological conditions [60]. Another application describes PHA from which pyrogen has been removed that are provided for use in numerous biomedical applications. PHA have been chemically modified to enhance physical and/or chemical properties, for targeting, or to modify biodegradability or clearance by the reticuloendothelial system. PHA provided are suitable for in vivo applications such as in tissue coatings, stents, sutures, tubing, bone and other prostheses, bone or tissue cements, tissue regeneration devices, wound dressings, drug delivery, and for diagnostic and prophylactic uses [61].

18.4.9 Arabinogalactan

Arabinogalactan is a highly branched water-soluble natural polysaccharide. It is extracted from the Larix tree and is available in a 99.9% pure form with reproducible molecular weight and physicochemical properties. The high water solubility, biocompatibility, biodegradability, and ease of drug conjugation in

an aqueous medium make arabinogalactan attractive and a potential drug carrier.

Use of Arabinogalactan in Drug Delivery. A novel application describes an acid-modified arabinogalactan protein composition, having an arabinose–galactose ratio of less than 3.5 : 1 or less than 80% of the arabinose : galactose ratio of the arabinogalactan protein component of the composition prior to acid modification. It is capable of reconstitution into an aqueous intravenously injectable formulation and is useful for stimulating hematopoiesis, inducing the proliferation or maturation of megakaryocytes, stimulating the production of interleukin-1β, interleukin-6, tumor necrosis factor-α, interferon-γ, granulocyte macrophage colony stimulating factor or granulocyte colony stimulating fator, stimulating the production or action of neutrophils, treating neutropenia, anemia, or thrombocytopenia, accelerating recovery from exposure (e.g., accidental or nontherapeutic exposure, as well as therapeutic exposure) to cytotoxic agents or radiation, treating cachexia, emesis, or drug withdrawal symptoms, or modifying biological responses or protecting hepatic cells in hepatitis B [62]. Another application discloses modified polymer conjugates of a polymer and a drug having reduced toxicity relative to the unmodified parent compound while retaining substantially the same degree of therapeutic activity as of the unmodified parent compound [63].

Use of Arabinogalactan in Tissue Engineering. A novel application discusses polysaccharide polymers employed in various medical applications. For example, chitosan-arabinogalactan and polysaccharide amine polymers are disclosed. The polymers can be used to prevent wound adhesion and to provide scaffolds for tissue transplantation and carriers for cell culture [64].

18.5 SYNTHETIC POLYMERS

Synthetic polymers provide the flexibility to tailor drug release, mechanical properties, and degradation kinetics to suit various applications, as discussed earlier. Furthermore, synthetic polymers can be designed on a need basis and are available in a wide variety of compositions with readily adjustable properties [65]. Similar to natural polymers, synthetic polymers are also being utilized and developed by industry and academia alike. Table 18.3 summarises the technological capacity overview of synthetic biodegradable polymers.

18.5.1 Polyesters

Poly(α-ester)s are thermoplastic polymers with hydrolytically labile aliphatic ester linkages in their backbone. This class is the most highly explored polymer class for drug delivery as well as tissue engineering applications owing to its immense diversity and synthetic versatility. Polyesters have hydrolytically labile

aliphatic ester linkages in their backbone and hence are biodegradable. Among the class of poly(α-ester)s, the most extensively investigated polymers are the poly(α-hydroxy acid)s, which include poly(glycolic acid) (PGA) and the stereoisomeric forms of poly(lactic acid) (PLA) and their copolymers poly (lactic-co-glycolic acid) (PLGA) due to their good biocompatibility and controllable degradation rates tailored to specific demands of each tissue type.

Use of Polyesters in Drug Delivery. The major application of polyesters in drug delivery is for development of medical devices (e.g., drug-eluting stents) and delivery systems, which may range from particulate delivery systems to various kinds of responsive and targeted drug delivery systems. For instance, a polyester polymer with a melting point of less than 60°C has been used for the development of a bioerodible water-insoluble polymer matrix, for controlled release of an active moiety to a target site [66]. Conjugation of molecules to be released with biodegradable polyester polymers via covalent bond has been used for the development of molecular sustained controlled-release systems [67]. Furthermore, an intraocular pharmaceutical formulation, composed of a monolithic system of drug dispersed in polyester has been described for treatment of retinal degenerations and diseases such as diabetic retinopathy, age-related macular degeneration, retinitis by cytomegalovirus, endophthalmitis, and uveitis [68]. Linear aliphatic polyester consisting of a polymer backbone containing aliphatic dicarboxylate residues and residues of an aliphatic polyol have been used for development of nano- or microparticle drug delivery systems for pharmaceutically active agents. The polymer backbone includes at least one aliphatic polyol residue, containing a moiety capable of interacting with the pharmaceutically active agent [69]. Polyesters have also been used in the form of a sterocomplex with the molecules to be delivered incorporated on or within the complex [70]. Improved porous particles made up of functionalized polyester graft copolymers consisting of a linear α-hydroxy-acid polyester backbone having at least one amino acid group incorporated therein and at least one poly(amino acid) side chain extending from an amino acid group in the polyester backbone have been used for administration to the respiratory tract to permit systemic or local delivery of a wide variety of therapeutic agents [71]. These particles are greater than 5 μm for drug delivery to the pulmonary system (alveolar region of the lung) and have a mass density less than 0.4 g/cm^3.

Various implantable systems have also been prepared using polyesters. An implantable medical device comprising therapeutic agents coated on the device using polyesters for the drug reservoir layer that exhibits surface-eroding characteristics has been developed for treating vascular diseases [72]. The molecular release rate from the above system can be regulated to be proportional to the chemical degradation rate of the biodegradable polyester polymers, resulting in near zero-order kinetics profile of release without showing a burst effect. Moreover, the high loading efficiency of hydrophilic drugs can be achieved in this system. Furthermore, a flowable controlled-release implant consisting of a biodegradable thermoplastic polyester that is at least

substantially insoluble in aqueous medium or body fluid has been developed for delivery of leuprolide acetate [73]. Also, a polymer composition comprising of an interpenetrating polymer network (IPN) of a branched polyether and biodegradable aliphatic polyester has been synthesized for use in drug delivery systems and medical devices such as drug-eluting stents [74]. A self-expanding, biodegradable compressible, resilient mesh stent was fabricated using polyesters that could be compressed during delivery to a biological vessel or channel but could later be expanded to the contours of the vessel or channel upon delivery. The stent includes a substantially cylindrical main body portion composed of polydioxanone monofilament fiber, which is slightly flared, and has longitudinally opposed first and second open ends [75]. A targeting drug delivery system comprising of magnetic nanoparticles (500-nm size) has been prepared by encapsulating magnetic Fe-based magnetic material and drug with biodegradable synthetic polyester [76]. Polyester-based films have been used for fabrication of a manually activated iontophoretic drug delivery system that includes electrodes controlled by a microprocessor controller to drive charged molecules contained in a drug reservoir through the skin into the tissues of a patient [77].

Polyesters have also been utilized in the form of block copolymers with other classes of polymers. For instance, a biodegradable amphiphilic block copolymer having a polyethylenimine hydrophilic block and a polyester-based polymer hydrophobic block, which self-assembles in aqueous solution and hence can be applied for the solubilization of a hardly soluble drug and the drug delivery system of proteins, genes, and charged drugs, has been described. The polymer assembly is prepared by dispersing the block copolymer into a solvent and has an average particle size of 10–1000 nm [78]. An amphiphilic polyester–polycation copolymer and an amphiphilic polyester–sugar copolymer have been used as biodegradable carriers for delivery of a selected negatively charged bioactive molecule such as a nucleic acid into a targeted host cell. The polymer helps to improve delivery efficiency by providing a particulate gene carrier for which the particle size and charge density are easily controlled by various means. Various kinds of ligands and other functional compounds may also be introduced to the carrier [79]. Poly(ester-anhydrides) or polyesters formed from ricinoleic acid and natural fatty diacids have been used for delivering bioactive agents including small drug molecules, peptides and proteins, and DNA (deoxyribonucleic acid) and DNA complexes with cationic lipids or polymers or nano- and microparticles loaded with bioactive agents [80].

Use of Polyesters in Tissue Engineering. Along with the vast array of applications in drug delivery, polyesters have also been used for a variety of applications in the field of tissue engineering, mostly for growth factor/gene delivery or as a scaffold. A number of patents deal with fabrication of polyester-based porous scaffolds for tissue engineering applications and various polymers used for the same applications [81, 82]. A polydioxanone-based electroprocessed drug delivery device, which incorporates monocyte chemotactic protein-1

(MCP-1), has been used to influence macrophage infiltration and adherence and to allow extended chemokine release [83].

Polyesters along with galactosylated chitosan, galactosylated, or galactose-modified degradable macromolecular materials have been used for developing a degradable, liver tissue engineering scaffold, having physiological blood vessel network structure. The preparation method comprises the following steps: selecting degradable aliphatic polyester materials to obtain a physiological blood vessel tree model by molding blood vessel; then preparing degradable liver tissue engineering scaffold having physiological blood vessel network structure by a combined method of infiltration forming and lyophilization/filtering technology. The scaffold can cause the vascular endothelial cell and liver cell to grow well in pores of tubes in the blood vessel network and porous sponge body, which is good for forming new blood vessels and the demand of the cell number in vivo implanted by the scaffold. The liver tissue engineering scaffold has a good biocompatibility, mechanical strength, and toughness [84].

Recently, polyester-enhanced degradable bioactive porous silicate calcium composite stent material has been developed. It comprises the following steps: (1) calcium silicate powder, pore former, and bonding agent are mixed uniformly to be molded and calcined to obtain a porous calcium silicate ceramic skeleton; (2) the porous calcium silicate ceramic skeleton is immersed in a polyester solution or polyester/calcium silicate ceramic mixed suspension to be infused into the porous calcium silicate ceramic skeleton; and (3) the immersed and infused porous calcium silicate ceramic skeleton is taken out to be dried. Pores of the polyester-enhanced degradable bioactive porous silicate calcium composite stent material prepared by the invention are highly communicated. Compared with a pure porous silicate calcium stent, the mechanical strength is improved by 3–30 times. The polyester-enhanced degradable bioactive porous silicate calcium composite stent material can be used for the hard tissue defect repairing or as a bone tissue engineering cell stent [85]. Synthetic polyester-based comb copolymers, which elicit controlled cellular response, have been used for modifying biomaterial surfaces, in tissue engineering applications, and as drug delivery devices. The comb copolymers are comprised of hydrophobic polymer backbones and hydrophilic, non-cell-binding side chains, which can be end capped with cell-signaling ligands that guide cellular response. By mixing non-cell-binding combs with ligand-bearing combs, the surface concentration and spatial distribution of one or more types of ligands, including adhesion peptides and growth factors, can be tuned on a surface to achieve desired cellular response [86].

Polyesters have also been used as tissue volume replacements, comprising preferably a solid polymer particle phase and a gel phase and may also comprise single-phase compositions [87]. Injectable polymers consisting of liquid or pasty hydroxy fatty-acid-based copolyesters, polyester–anhydrides, or combinations thereof, which show an increase in viscosity upon contact with bodily fluids to form a solid or semisolid implant, have been used for soft tissue repair and/or augmentation. These polymer compositions can also incorporate

various active agents or additives and are typically administered by injection [88]. Improved long-term patency of an artificial blood vessel, by having a coating layer mainly composed of a biodegradable synthetic material, preferably PLA, on the outside of a tubular body containing collagen. This effect is observed even when used in an artery in the vicinity of the heart [89].

Another application is to form a regenerated layer of periodontal tissue, which is biodegraded and does not need to be removed by operation for treatment of periodontitis. This regenerated layer comprises heat-pressed two layers consisting of (a) biodegradable polyester-based nonwoven scaffold with micropores with the mean diameter below 10 μm and (b) a biodegradable scaffold with around 100–400 μm pore size [90].

Polyesters have been applied in skin tissue engineering applications for obtaining a subject sheet having an epidermal cell culture layer made up of collagen, fibrin, hyaluronic acid or gelatin, and a biological absorbent polyester-based mesh, with a corium supplementing layer such as a human fibroblast between them. This composition is useful for repair of an acute skin defect wound such as a heat wound, an external wound, or a surgical wound and a chronic skin defect wound such as a bed sore or a diabetic or arteriosclerotic ulcer [91]. One or more kind of polyesters have been used for the preparation of a porous support body (pore diameter: 10–800 μm, porosity: 50–99%) for regenerating damaged ocular tissues [92].

Polyesters have also been used for the fabrication of a composite scaffold for engineering a heterogeneous tissue. The composite scaffold includes: (a) a first scaffold being capable of supporting the formation of a first tissue type thereupon and (b) a second scaffold being capable of supporting the formation of a second tissue type thereupon, where the distance between any cell of the second tissue type and the first tissue type does not exceed 200 μm [93, 94].

18.5.2 Poly(Ortho ester)s

Poly(ortho ester)s are amorphous hydrophobic polymers containing hydrolytically labile, acid-sensitive, backbone linkages. These were developed by ALZA Corporation (Alzamer) as hydrophobic surface-eroding polymers particularly for drug delivery applications. By using diols with varying levels of chain flexibility, the rate of degradation for these polymers, pH sensitivity, and glass transition temperatures can be controlled. Up to now, four different classes of poly(ortho ester)s have been developed (POE I, II, III, and IV). Few orthopedic applications of this class of polymers have been explored, but the major use of POEs has been limited to drug delivery systems [95].

Use of Poly(ortho ester)s in Drug Delivery. Poly(ortho ester)s are particularly useful for controlled-release drug delivery. Devices made of poly(ortho ester)s can be formulated in such a way that the device undergoes "surface erosion" and hence tends to release drugs embedded within the polymer at a constant rate [96, 97]. Due to their ability to provide well-controlled release

profiles, poly(ortho ester)s have been investigated for controlled drug delivery of a wide range of pharmaceutical agents, including proteins. A poly(ortho ester)-based semisolid delivery vehicle in the form of topical, syringable, or injectable formulation has been developed for local delivery of active agents [98].

Poly(ortho ester)s along with various other synthetic polymers have been proposed for use as nanoparticulate carriers for the delivery of therapeutic compounds such as catalytic subunit hTert of human telomerase to cells in a cell culture or in a living animal. The nanoparticle may incorporate a plasticizer to facilitate sustained release of telomerase such as L-tartaric acid dimethyl ester, triethyl citrate, or glyceryl triacetate. A polymer that affects the charge or lipophilicity or hydrophilicity of the particle, for example, a hydrophilic polymer such as poly(vinyl alcohol) may also be included [99].

Once-a-day eye drop formulation comprising of a plasticized or unplasticized bioerodible polymer, such as a poly(ortho ester), suspended as a discontinuous phase in a hydrophobic liquid such as silicone oil or mineral oil, as a continuous phase was developed as a controlled-release medicament delivery system. Medicament is entrapped by the discontinuous phase. For preparation, the polymer is heated to an elevated temperature for a sufficient time to substantially reduce its molecular weight, and medicament is added at a temperature above which there is complete phase separation [100].

Other applications include formulation of microparticles with adsorbent surfaces using cationic, anionic, or nonionic detergents, for stimulating an immune response, for immunizing a host animal against a viral, bacterial, or parasitic infection, and for vaccination. Polymers, such as poly(α-hydroxy acid), polyhydroxy butyric acid, polycaprolactone (PCL), poly(ortho ester), polyanhydride, and the like, have been used for the same applications. The surface of the microparticles efficiently adsorbs polypeptides, such as antigens, and nucleic acids, such as ELVIS vectors and other vector constructs, containing heterologous nucleotide sequences encoding biologically active macromolecules, such as polypeptides, antigens, and adjuvants [101].

Use of Poly(ortho ester)s in Tissue Engineering. The major application of poly(ortho ester)s in tissue engineering is as a biocompatible support or scaffold for use in various biomedical applications. Poly(ortho ester)s have been described for the development of biodegradable porous polymer supporters for tissue engineering, to improve the cell tropism and the growth rate of tissue by modifying the surface of a supporter [102, 103]. Additionally, poly(ortho ester)s have been used for fabricating a coating for an implantable medical device [104].

18.5.3 Polyanhydrides

Polyanhydrides are a class of hydrolytically unstable surface-eroding polymers that are either aliphatic, aromatic, or a combination of the two. They find application in both tissue engineering and drug/growth factor delivery. A wide

variety of drugs and proteins including insulin, bovine growth factors, angiogenesis inhibitors, enzymes, and anesthetics have been incorporated into polyanhydride matrices, and their in vitro and in vivo release characteristics have been evaluated. Polyanhydrides derived from bis-*p*-(carboxyphenoxy propane) and sebacic acid are currently being marketed under the name Gliadel for the delivery of chemotherapeutic agent and BCNU (bis-chloroethylnitrosourea) to the brain for the treatment of glioblastoma multiformae, a universally fatal brain cancer.

To obtain polyanhydrides with high mechanical strength for load-bearing applications, osteocompatible poly(anhydride-*co*-imides) have also been designed and shown to support endosteal bone growth [105]. A copolymer of 1 : 1 sebacic acid and erucic acid dimer has been found to be useful as a potential delivery vehicle for gentamicin (Septacin) in the treatment of osteomyelitis. Readers may refer to literature reviews for details of hydroxy-fatty-acid-based polyanhydride and poly(ester-anhydride) drug delivery systems [106–108].

Use of Polyanhydrides in Drug Delivery. Patents are an excellent source of technical knowhow for the selection, synthesis, and use of any polymer for a specific application and also a means to successfully protect the same while ensuring its availability. For example, the method of preparation and administration of polymeric drug delivery systems prepared via linking low-molecular-weight drugs containing a carboxylic acid group and an amine, thiol, alcohol, or phenol group to polyanhydride linkers has been protected by a number of patents [109, 110]. Others describe a method for synthesizing polyanhydrides in solution using coupling agents such as phosgene, diphosgene, and acid chlorides and a removable acid acceptor to effect a one-step polymerization of dicarboxylic acids, for drug delivery applications. The method is also useful in the polymerization of dicarboxylic acids including heat liable dipeptides of glutamic or aspartic acid. Insoluble acid acceptors include insoluble polyamines and crosslinked polyamines such as polyethyleneimine and polyvinylpyridine and inorganic bases such as K_2CO_3, Na_2CO_3, $NaHCO_3$, and $CaCO_3$. The only by-product formed is a removable hydrochloric acid–acid acceptor [111]. Polyanhydrides with aliphatic hydrocarbon terminals having ester or amide bonds have also been described [112].

Another patent describes synthesis of high-molecular-weight ($>$20,000) polyanhydrides or an intrinsic viscosity of greater than 0.3 dL/g, by melt polycondensation of highly pure isolated prepolymers in an organic solvent at room temperature, with the help of catalysts used for transterification, ring-opening polymerization, and related polymerizations for controlled drug delivery [113]. A bioerodible controlled drug release device prepared by solvent casting from the aforementioned polymer exhibits zero-order release, with improved correlation between the rate of release and polymer degradation, and an induction period between the introduction to the eroding environment and the initial release of the biologically active substance. It is stable for extended periods of time, flexible, and durable and is not subject to

fracture and disintegration [114]. Polyanhydrides have also been reported for controlled release of water-soluble proteins due to their surface-eroding characteristics [115] and for localized delivery of chemotherapeutic agents to solid tumors [116].

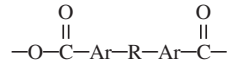
Blending polymers may result in the emergence of distinct properties making them suitable for use as carriers of pharmaceutically active agents. It has been discovered that the rate of release of the agent from the blends is different than the rate of release from the individual polyanhydride and polyester polymer components, being a function of the blend composition. Two or more polyanhydrides, polyesters, or mixtures of polyanhydrides and polyesters have been used together so as to provide a means for altering the characteristics of a polymeric matrix without altering the chemical structure of the component polymers using solvent mixing or melt mixing procedures [117].

Fatty-acid-terminated polyanhydrides are suitable for use as controlled-release matrices in biodegradable sustained-release drug delivery systems [118]. These polymers are more hydrophobic, more soluble in organic solvents, and have a lower melting point than the corresponding non-fatty-acid-terminated polyanhydrides and combine the properties of thermodynamic and hydrolytic stability and easy storage. The polymers can be produced with a controlled and low molecular weight. Biodegradable polymeric wafers composed of a polyanhydride copolymer of 1,3-bis(*p*-carboxyphenoxy)propane and sebacic acid in a 20 : 80 molar ratio for localized delivery of 1,25 D3, directly to a target area, thus maximizing the efficacy of this drug while minimizing systemic exposure and toxicity, for the treatment of intracranial tumors as well as neurodegenerative disorders such as Alzheimer's disease [119]. The polyanhydride-based hydrophobic polymeric matrix, which is suitable for use after implantation in vivo, has been investigated for controlled release and delivery of biologically active substances such as drugs, antibiotics, and steroids and may also be used outside the body for release of fragrances, pesticides and the like [120]. A new therapeutic method to treat cancer combines radiofrequency (RF) ablation and local controlled drug delivery of 5-fluorouricil (5-FU) from polyanhydride implants [121]. Our lab has developed a novel polyanhydride-based (low-molecular-weight random copolymer of sebacic acid and recinoleic acid maleate) drug delivery system that is injectable at around 40°C and can carry a variety of active pharmaceutical agents and deliver them at a controlled rate for a period of not more than 15 days [122].

Bioadhesive materials composed of a compound containing an aromatic group (catechol derivative, dihydroxyphenylalanine, DOPA), which contains one or more hydroxyl groups grafted onto a polymer (polyanhydride) or coupled to individual monomers, have been reported for fabricating new drug delivery or diagnostic systems with increased residence time at tissue surfaces, and consequently increase the bioavailability of a drug or a diagnostic agent [123].

Use of Polyanhydrides in Tissue Engineering. Aromatic polyanhydrides composed of a repeating unit with structure **1** (wherein Ar is a substituted or

unsubstituted aromatic ring, and R is a difunctional organic moiety substituted on each Ar ortho to the anhydride group) have been reported for use as implantable medical devices such as scaffolding implants for tissue reconstruction as well as drug delivery systems [124]:



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Polyanhydrides have also been used as a bioerodible bone cement where the polymer is first cast as a solution onto a bone fracture and then crosslinked by radiation or radical polymerization to yield a strong, adhesive material [125]. Another application is where calcium metaphosphate (CMP) compounded with polymeric materials such as PLA, PGA, polyhydroxybutyric acid, PCL, polyurethane, polyanhydride, collagen, chitosan, gelatin, hyaluronic acid, and fibrin protein gel is used as bone cement. Compared with the prior art, the invention has the advantages that in the CMP–polymer composite materials, the polymer materials provide a stable mechanical environment and are easy to shape; and the CMP promotes cell adhesion and affinity. Before the adsorption of the CMP composite support materials, cells may be amplified to a sufficient number to generate sufficient extracellular matrix, thereby enhancing the adhesion capacity of cells and materials, and enhancing the anticoagulation and antithrombotic capacity of the tissue engineering supports [126]. Polyanhydrides have also been reported for the preparation of degradable electricity-conducting biomedical polymer material, which can be prepared into guide pipes, suture threads, membranes, sheet bodies, block bodies, and materials for frames of tissue engineering [127].

Polyanhydrides have been utilized for the preparation of biodegradable scaffolds for supporting cellular growth. A biodegradable porous polymer supporter for tissue engineering may be prepared to obtain the open cell-structured porous polymer supporter, with an improved degree of pore and to allow the size of pore to be controlled. The biodegradable porous polymer supporter is prepared by making a polymer species by using a polymer solution containing a biodegradable polymer and an anisotropic mixture. boiling (foaming) the polymer species in a boiling medium; and drying it. Preferably, the biodegradable polymer is selected from the group consisting of PGA, PLA, PLGA, PCL, poly(glutamic acid), polyanhydride, poly(orthoester), their derivatives, and their mixtures; and has a molecular weight of 5000–2,000,000. The anisotropic mixture comprises a carbonate and an organic acid. Preferably the carbonate is selected from the group consisting of sodium hydrogen carbonate, sodium carbonate, ammonium hydrogen carbonate, ammonium carbonate, potassium hydrogen carbonate, potassium carbonate, calcium carbonate, and their mixtures; and the organic acid is selected from the group consisting of citric acid, succinic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, malonic acid, malic acid, gluconic acid, mucic acid, some amino acids, and their mixtures [102, 128].

Further, biodegradable polymers such as polyanhydride, chitin, chitosan, alginic acid, PLA, PGA, PLGA, polyhydroxybutyric acid, PCL, and polyalkylcyano acrylate. may be combined with tissues under the mucous membrane of small intestine or a bone powder of mammals for the preparation of a porous/biodegradable carrier so as to incorporate the advantages of biodegradable synthetic polymer carrier and natural material into the carrier [129].

18.5.4 Polyurethanes

Polyurethanes (PURs) are prepared by the polycondensation reaction of diisocyanates with alcohols and amines. Conventional polyols are polyethers or polyesters. The resulting polymers are segmented block copolymers, with the polyol segment providing a low-glass-transition temperature (i.e., $< 25^{\circ}C$), soft segment, and the diisocyanate component, often combined with a hydrocarbon chain extender, providing the hard segment. A wide range of physical and mechanical properties have been realized with commercial polyurethanes. Biostable polyurethanes have been extensively investigated as materials for the preparation of long-term medical implants, especially cardiac pacemakers and vascular grafts due to their excellent biocompatibility, mechanical properties, and their synthetic versatility [130]. Attempts have also been made to develop biodegradable polyurethanes. Biodegradable polyurethanes have recently been investigated as candidate materials for bone regenerative medicine. Poly(α-hydroxy acids), including PLA, PGA, and PCL, have been used as soft segments for biodegradable polyurethanes [131]. Polyurethanes for biomedical engineering have been reviewed by Gunatillake and Meijs [132].

Polyurethanes in Drug Delivery. Polyurethanes have been widely explored for various drug delivery applications. The synthesis of biodegradable, nontoxic, amphiphilic multiblock polyurethane (MW 5000−100,000), by alternate copolymerization of soft segments composed of polymer diol and hard segments composed of diisocyanate and a chain extender, has been described [133]. The polyurethane material has a unique multiblock structure and amphiphilicity, can form a stable self-assembly micellar structure in water, and easily introduces active sites at a main chain, a side chain, and chain ends of polyurethane for molecular modification. Further, the structure and performance of material is controllable, thereby making it suitable for drug delivery.

Polyurethanes have been used for controlled drug delivery in the form of reservoir devices/systems [134]. Polyurethane-based drug delivery device has been used to deliver naltrexone and formulations thereof at a constant rate for an extended period of time [135]. Hydrophilic polyurethane polymers having carboxy groups in the polymer backbone have been used as drug delivery systems, as burn and wound dressings, in cosmetic applications, in body implants, as coatings on cannulae, and a host of other applications [136]. A biocompatible polyurethane-based patch system has been developed for

controlled release and site-specific delivery of myocardial agents such as antiarrhythmic agents [137, 138]. Similarly, a drug delivery device based on polyurethane–siloxane-containing copolymers crosslinked with hydrophilic monomers for placement in the eye has been developed [139].

Furthermore, polyurethanes find various applications in transdermal drug delivery. Polyurethane-based drug reservoirs, which can be processed at temperatures below those that cause degradation of temperature-sensitive drugs and/or excipients, have been described as transdermal drug delivery devices [140]. Hydrogels formulated from polyurethanes crosslinked with diisocyanate crosslinking agents or cured with radiation in the presence of a photoinitiator have been used for the preparation of high-capacity reservoirs (as high as 65–70 wt% or higher) for incorporation into transdermal drug delivery systems [141]. A few patents also describe the use of polyurethane films in transdermal delivery devices [142, 143].

Polyurethanes in Tissue Engineering. Polyurethanes have been utilized as scaffolds for cell growth as well as for specific applications such as bone, cartilage, and skin graft fabrication. Injectable polyurethane foam scaffolds >70 vol% porosity with at least 50% interconnected pores and scaffold density from about 50 to about 250 kg/m^3, are useful in the field of tissue engineering. Preparation of a biocompatible, optionally biodegradable open-cell polyurethane foam having open pores is also protected [144]. Polyurethane, coated with (cellulose-binding domain) CBD-RGD peptide made by the genetic engineering method, has been reported for improving the adhesive and proliferating ability of tissue cell such as endothelium, fibroblast and cuticular cell [145]. A novel polyurethane/urea with the degradable mesh structure was prepared by the reaction of polyether polyol containing a secondary amino group on a main chain and the fatty diisocyanate, for the preparation of the stents and for repair of the damaged bone or the cartilage [146].

A medicated multilayered polyurethane foam dressing and drug delivery device with cooling properties is described for use in body cavities and on damaged tissues, particularly burns. The dressing has at least one therapeutic agent dispersed into the polyurethane layers; an optional outer layer of either a hydrogel formulated from a polyurethane or an adhesive elastomeric material; an optional drug-free fluid-retaining layer and/or a drug-reservoir layer, each comprised of hydrophilic polyurethane foam; a nonadherent, drug-loaded surface-contacting layer of a polyurethane hydrogel; and an optional cover sheet for surface dressings [147].

Polyurethane or polyurethane/urea compositions have been used in biomedical vascular stents, an orthopedic implant, a drug delivery coating, or in tissue engineering. The composition has a tensile strength greater than 10 MPa, a modulus of elasticity greater than 400 MPa, an elongation break greater than 30% at a temperature of between 0 and 60°C, and a relative humidity of between 0 and 100% [148]. Aforementioned compositions that are capable of in vivo curing with low heat generation to form materials suitable for use in

scaffolds in tissue engineering applications such as bone and cartilage repair by noninvasive surgery. These polymers are desirably flowable and injectable and can support living biological components to aid in the healing process. They may be cured ex vivo for invasive surgical repair methods or alternatively utilized for relatively noninvasive surgical repair methods such as by arthroscopy [149].

18.5.5 PEG-Based Polymers

Polyethylene glycol (PEG) is a biocompatible and nontoxic polymer that has been mainly used to modify other polymers since it is known to reduce protein adsorption and modify polymer conformation. PEG-based hydrogels have been widely studied for drug delivery as well as for other biomedical applications in tissue engineering and delivery of growth factors.

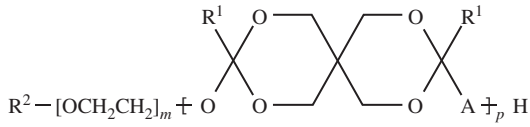
Use of PEG-Based Polymers for Drug Delivery. PEG and related polymer derivatives having weak, hydrolytically unstable linkages near the reactive end of the polymer have been reported for conjugation to drugs, including proteins, enzymes, small molecules, and others. These derivatives provide a sufficient circulation period for a drug–PEG conjugate, followed by hydrolytic breakdown of the conjugate and release of the bound molecule. In some cases, drugs that demonstrate reduced activity when permanently coupled to PEG maintain a therapeutically suitable activity when coupled to degradable PEG in accordance with the invention. The PEG derivatives of the invention can be used to impart improved water solubility, increased size, a slower rate of kidney clearance, and reduced immunogenicity to a conjugate formed by attachment thereto. Controlled hydrolytic release of the bound molecule into an aqueous environment can then enhance the drug's delivery profile by providing a delivery system that employs such polymers [150].

A decomposable temperature-sensitive chemical crosslinking gel composed of PEG as the hydrophilic block and decomposable polyester as the hydrophobic block to form the host of temperature-sensitive amphipathy block copolymers, which forms a gel under human body temperature (or normal temperature) in water, using a macromolecular monomer technique to obtain stable chemical crosslinking gel as drug slow-release carrier or culture rack of tissue engineering cell [151]. PEG has also been reported for liposomes and drug delivery in the form of diacylglycerol–polyethylene glycol (DAG–PEG) conjugates [152].

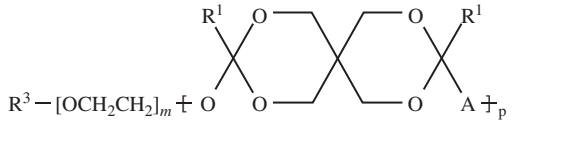
Block copolymers, namely PEG–POE, PEG–POE–PEG, and POE–PEG–POE, which contain both hydrophilic and hydrophobic blocks, form micelles in aqueous solution rendering them suitable for encapsulation or solubilization of hydrophobic and water-insoluble materials for sustained release of active agents. Structure **2** describes a block copolymer of formula (I), formula (II), or formula (III) in which m and n are independently an integer from 5 to 500; p and q are independently an integer from 5 to 500; R1 and R2 are independently C1–C4 alkyl; R3 is H or C1–C4 alkyl; and A is –O–R4–, –O–R5–, or a mixture thereof; R4 is selected from the structures depicted by

(IV), where t is an integer from 1 to 12, R6 is C1–C4 alkyl, and R7 is H or C1–C4 alkyl; R5 is depicted by (V) in which x is an integer from 1 to 10, and R8 is H or C1–C6 alkyl. [153]:

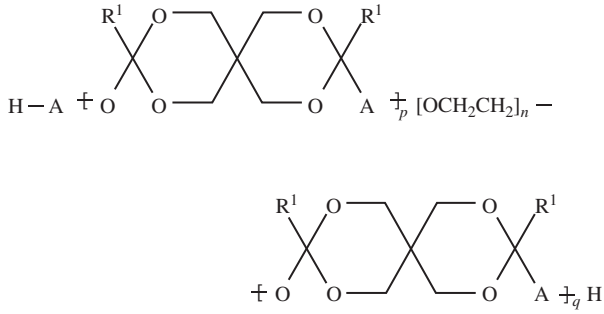
(I)



(II)



(III)



2

Furthermore, PEG has been widely explored as a hydrophilic block in the preparation of thermosensitive triblock polymers. A water-soluble, biodegradable ABA- or BAB-type triblock polymer made up of a major amount of a hydrophobic A polymer block made of a biodegradable polyester and a minor amount of a hydrophilic PEG B polymer block, having an overall average molecular weight of between about 2000 and 4990, possesses reverse thermal gelation properties. Effective concentrations of the triblock polymer and a drug may be uniformly contained in an aqueous phase to form a drug delivery composition. At temperatures below the gelation temperature of the triblock polymer the composition is a liquid, and, at temperatures at or above the gelation temperature, the composition is a gel or semisolid. The composition may be administered to a warm-blooded animal as a liquid by parenteral, ocular, topical, inhalation, transdermal, vaginal, transurethral, rectal, nasal, oral, pulmonary, or aural delivery means and is a gel at body temperature. The

composition may also be administered as a gel. The drug is released at a controlled rate from the gel, which biodegrades into nontoxic products. The release rate of the drug may be adjusted by changing various parameters such as hydrophobic/hydrophilic component content, polymer concentration, molecular weight, and polydispersity of the triblock polymer. Because the triblock polymer is amphiphilic, it functions to increase the solubility and/or stability of drugs in the composition [154]. A temperature- and pH-sensitive block copolymer having excellent gel strength, including: (a) a PEG-based compound (A) or a copolymer (B) of the PEG-based compound (A) and a biodegradable polymer and (b) a polyurethane-based oligomer (C), has also been developed [155].

Another use of PEG involves a biodegradable copolymer with a core block comprising a bioresorbable hydrophobic polyester segment and from 3 to 10 shell blocks comprising a hydrophilic PEG segment linked by a urethane linkage. This copolymer forms a micellar system when the concentration of the copolymer in a dispersing medium reaches or exceeds the critical micelle concentration. The micelles possess good drug and bioactive agent delivery characteristics and are suitable for use in drug delivery or cosmetic applications [156].

Poly(ethylene oxide)–block-poly(ester) block copolymers having reactive groups on both the poly(ethylene oxide) block and the poly(ester) block therein are another group of micelle-forming polymers, whose biodegradability and biocompatibility with a large number of bioactive agents make them suitable as carriers for various bioactive agents. The bioactive agent, such as DNA, RNA (ribonucleic acid), oligonucleotide, protein, peptide, drug, and the like, can be coupled to the reactive groups on the polyester block of the copolymer. A variety of targeting moieties can be coupled to the reactive group on the poly(ethylene oxide) block for targeting the bioactive agent to a particular tissue [157].

Our lab has developed a branched copolymer having multiple, low-molecular-weight PEG chains and a hydrophobic biodegradable polymer (PLA) chain attached via linker for polymersomes formation. Because of multiple chains of low-molecular-weight PEG, it improves the surface density of PEG on the polymersomes and hence can extend the plasma circulation time. The resulting polymersomes have the capability of encapsulating/entrapping and controlling the release of hydrophilic, hydrophobic, as well as amphoteric molecules [158]. The same polymer was utilized for development of a novel vesicular amphotericin B [159] and doxorubicin-loaded formulation [160].

Use of PEG-Based Polymers in Tissue Engineering. PEG-based polymers have been also used in various tissue engineering applications, especially for growth factor delivery by encapsulation or conjugation. An amphiphilic diblock copolymer, composed of a hydrophilic segment made up of an endcapped PEG or derivatives thereof and a hydrophobic segment made up of random polymer polymerized of lactone or the cyclic C3–C6 molecule and lactic acid/glycolic acid, forms a thermosensitive material having a phase transfer temperature of 25–50°C. The said biodegradable copolymer is formed by one-pot ring-opening

polymerization and can be applied to biological factor delivery, tissue engineering, cell culture, and as biological glue [161].

Conjugation of matrix-enhancing molecules, such as (transforming growth factor-β) TGF-β to a tether, such as PEG monoacrylate, on scaffolds is another approach to increase extracellular matrix production by cells for tissue engineering, tissue regeneration, and wound-healing applications. The matrix-enhancing molecule retains activity after attachment to the scaffold and causes cells growing in or on the scaffold to increase extracellular matrix production, without substantially increasing proliferation of the cells, even when the scaffold additionally contains cell adhesion ligands. The increased extracellular matrix produced by the cells aids in maintaining the integrity of the scaffold, particularly when the scaffold is degradable, either by hydrolysis or by enzymatic degradation [162].

18.5.6 Poly(Amino Acids)

Since proteins are composed of amino acids, many researchers have tried to develop synthetic polymers derived from amino acids that is, poly(amino acids) to serve as models for structural, biological, and immunological studies. Poly (amino acids) are usually prepared by the ring-opening polymerization of the corresponding *N*-carboxy anhydrides that are obtained by reaction of the amino acid with phosgene.

Poly(amino acids) are advantageous as biomaterials due to their diversity and the availability of side chains, which offer sites for the attachment of small peptides, drugs, crosslinking agents, or pendent groups that can be used to modify the physicomechanical properties of the polymer. Since these polymers release naturally occurring amino acids as the primary products of polymer backbone cleavage, their degradation products may be expected to show a low level of systemic toxicity. Poly (aspartic acid), poly(L-lysine), and poly(L-glutamic acid) are a few examples of this class. Bourke and Kohn have replaced the peptide bonds in the backbone of synthetic poly(amino acids) by a variety of such "nonamide" linkages as ester, iminocarbonate, urethane, and carbonate bonds [163]. The term pseudo-poly(amino acid) is used to denote this new family of polymers in which naturally occurring amino acids are linked together by nonamide bonds. Hydroxyproline-derived polyesters, serine-derived polyesters, and tyrosine-derived polyiminocarbonates and polycarbonates represent specific embodiments of these synthetic concepts.

Poly(amino Acids) in Drug Delivery. Copolymers with a polyaspartamide (specifically, α, β-poly-hydroxyethyl-aspartamide, or PHEA) backbone derivatized with hydrophobic functionalities, ionizable functionalities, and thiol functionalities in side chains have been used as delivery systems for the release of active ingredients, specifically peptides and proteins, by means of their incorporation in nanoparticles, nanoaggregates, or complexes. Such polymer systems are proposed to release peptide drugs or proteins, such as insulin, from oral dosage forms in an effective manner, and in order to increase the

physicochemicai stability of proteins in liquid or solid pharmaceutical dosage forms [164]. Low-cost poly(amino acid)-based nano- or microparticles have been developed as vectors useful for the particularly oral or parenteral delivery of medicinal or nutritional active principles [165]. Additionally, a suspension of biocompatible particles based on a double-block, hydrophilic neutral poly(amino acid)/hydrophobic neutral poly(amino acid) copolymer for sustained/delayed delivery of active principles. These carrier particles spontaneously form stable aqueous suspensions even in the absence of surfactants or organic solvents. The application describes the carrier particles in dry form, the method for preparing them, and pharmaceutical compositions (in dry form or suspension) comprising said carrier particles associated with an active principle [166].

Poly(amino acids) have been also explored in nucleic acid delivery. Self-assembly of a polyelectrolyte complex can be achieved by use of a cationic polyelectrolyte polymer material including (a) a derivative of an amino acid or a synthetic amino acid, or a vinyl-type polymer (e.g., polyglutamine or polyasparagine derivative) and (b) bringing the nucleic acid material into association with said cationic polyelectrolyte polymer material for nucleic acid delivery [167].

Poly(Amino acid) in Tissue Engineering. Poly(amino Acids) have been used for fabrication of transplants, suitable for tissue or cell transplantation, in subjects suffering from cell or tissue malfunction or deficiency, for example, diabetes. Here, a biological tissue transplant coated with a stabilized multilayer alginate coating is used. Coating has three primary layers of the alginate with a poly(amino acid) barrier emplaced between a primary and a secondary layer. The secondary "halo" layer of soft gel is formed by a gel gradient created between weakly bound crosslinking gelling divalent cations of an alginate used for the primary layer of the coating and nongelling counter ions of a nonionic alginate of the secondary layer of the coating. It has been used for growth of pancreatic islet cells and hepatocytes [168].

18.5.7 Polyfumarates

Polyfumarates are a class of novel, injectable, biodegradable, and biocompatible materials that find applications for the delivery of bioactive molecules/drugs in various pharmaceutical and biomedical fields. Oligo[poly(ethylene glycol) fumarate] (OPF) is one such water-soluble, synthetic polyfumarate that can be injected into a defect site and crosslinked in situ at physiological conditions, thereby eliminating the need for invasive implantation and retrieval surgeries [169]. Poly(propylene)fumarate (PPF), another polymer from this class, is a linear polyester whose repeating units contain two ester bonds and one unsaturated carbon−carbon double bond. It undergoes bulk erosion via hydrolysis of its ester bonds and the degradation products formed are primarily fumaric acid and propylene glycol. The double bonds in PPF allow the polymer to be crosslinked thermally or by photoinitiator into a solid, polymeric network. These networks are mechanically strong, biocompatible,

and biodegradable [170, 171]. Polyfumarates suffer a limitation particularly with respect to bone tissue engineering, that is, lack of mechanical strength due to flexible C−O−C region in its backbone. Hence, several strategies have been devised that include incorporation of ceramics or nanoparticles in them [170, 172]. Other polyfumarate-based polymers, namely poly(caprolactone fumarate) and poly(ethyleneglycol fumarate) have also been developed into injectable systems and termed as "self-crosslink," as no crosslinking agent is required, but a photoinitiator and accelerator are required [173].

Polyfumarates in Drug Delivery. Compositions based on PPF polymer deliver a pharmaceutically active agent to the eye, and/or treat an ophthalmic disorder [174]. Bioresorbable compositions made from a PLA, PGA, or PLGA polymer having a relatively low molecular weight (less than 10,000, such as from about 500 to about 5000) have been incorporated with fumarate groups to provide crosslinking sites. Ethylene oxide groups and ceramic particles may further be incorporated to vary the properties of the final compositions. These may contain various therapeutic, beneficial, and pharmaceutical agents that may be released during degradation of the polymer [175]. PPF, a polymeric material other than PPF (e.g., PLGA), and a bioactive agent have been used for fabrication of microspheres as a delivery system. The bioactive agent is selected depending on the physiological effect desired. For example, in bone regeneration applications, the bioactive agent may be selected from osteoinductive agents, peptides, growth hormones, osteoconductive agents, cytokines and mixtures thereof. The bioactive agent is dispersed in the microsphere, the microsphere has a diameter in the range of 1−300 μm, the PPF and PLGA are distributed in the microsphere, and the microsphere releases the bioactive agent in a sustained manner after an initial burst release. The microspheres may be covalently attached to a PPF scaffold for tissue regeneration applications in which the bioactive agent is released from the scaffold [176].

Polyfumarates in Tissue Engineering. PPF synthesis with controlled end-group ratio and a range of molecular weights (average molecular weight between 500 and 3000) with minimal low-molecular-weight and excessively high-molecular-weight fractions has been described for use in biomedical applications such as in bone cements and tissue implants or glues. One of three methods may be used: melt polymerization using nonvolatile starting materials; step polymerization where in each step the polymer is increased by the addition of two groups to the polymer ends; or under reaction conditions maintained so that only the condensation by-product, water, is removed during the reaction, thereby keeping the ratio between propylene glycol and fumaric acid constant. Variations of the PPF polymers include polymers with increased sensitivity to hydrolysis through incorporation of lactic acid groups into the polymer and polymers formed from maleic acid, maleic anhydride, citraconic acid, or citraconic anhydride [177].

Furthermore, photocrosslinkable, injectable, biodegradable OPF hydrogels may be prepared by photopolymerization of an OPF macromer with ultraviolet

(UV) light and a photoinitiator. The biodegradable OPF hydrogels can be injected as a fluid into a bodily defect of any shape, may incorporate various therapeutic agents, for example, cells and/or growth factors, and may be implanted via minimally invasive arthroscopic techniques. Hydrogels with varying mechanical properties and water content can be made with changes in macromer and crosslinking agent concentration in a precursor solution [178]. Biocompatible and bioresorbable block copolymer of PPF and PCL have been reported for injectable and in situ hardening scaffolds for tissue and/or skeletal reconstruction, which are both self-crosslinkable and photocrosslinkable [179].

OPF hydrogel has also been used as a scaffold for nerve tissue regeneration, which can be injected as a fluid into a patient's body via minimally invasive arthroscopic techniques. The photocrosslinkable material includes a copolymer formed by reacting OPF selected from monomers, oligomers, and polymers and a second charged reactant (e.g., unsaturated quaternary ammonium compounds) selected from charged monomers, charged oligomers, and charged polymers. Nerve cells are contained within or attracted to the copolymer. It may further include a bioactive agent such as a nerve growth factor [180]. OPF hydrogels have also been used as tissue guidance conduits. Devices for use in regeneration or repair of body tissue (such as nerves) comprise a multilumen scaffold and, optionally, an outer sheath. The outer sheath is formed of a stronger material than the scaffold and preferably comprises a region at each end for suturing the device in place [181].

18.6 CONCLUSION

Natural and synthetic polymers represent a niche area of research in which both academia and industry are actively involved. Both industries as well as academia are involved in filing patents in the said field. However, it is the industrial patents that in most cases represent products that have been actually commercialized and are available to the end users, that is, the patients. In case of patents filed by universities and institutes, technology transfer and licensing are major challenges that affect the commercialization of promising technologies. In a few cases, joint patents involving industry–academia collaboration or with medical institutes were also observed. The major countries involved in research in the area of polymer research pertaining to tissue engineering and drug delivery are the United States, the People's Republic of China, Korea, and Japan, with the United States topping the list of innovations as reflected by the high number of patents filed. India also represents an emerging country in the area of polymer research relating to biomedical applications. The present review can serve as a useful guide and reference chapter for researches as it gives an overview of the innovations, technological challenges, and also the global map of the players involved, that is, industry and academia in the field of drug delivery and tissue engineering.

REFERENCES

1. Bansal, P. *IPR Handbook for Pharma Students and Researchers*. Pharma Book Syndicate: Hyderabad, India, 2008, p.214.
2. Dang, J. M. and K. W. Leong. *Adv. Drug Deliv. Rev*. 2006;**58**:487–499.
3. Myers, M. and P. W. Reginald. Collagen-based drug delivery device for providing local analgesia, local anesthesia or nerve blockage (Innocoll Technologies Limited, Ire.). U.S. Patent 2008241245, 2008. Cont -in-part of U.S. Ser No US 692,337.
4. Niitsu, Y., J. Kato, and Y. Sato. Drug carrier and drug carrier kit for inhibiting fibrosis. Hokkaido, Japan, Renomedix Institute Inc., WO, 2006068232, 2006, p. 57.
5. Sand, B. J., M. Babich, A. Z. Haghighi. Transdermal drug delivery and topical compositions comprising at least two permeation enhancers, such as benzyl alcohol and lecithin for application on the skin. Nuviance, Inc., USA, WO, 2007103555, 2007, p. 94.
6. Sugai, H., J. Kanamune, K. Matsumura, and C. X. Xuan. Scaffold materials for tissue regeneration, and carriers for drug delivery systems, and production thereof. K. K. Baio Berude, Japan, JP, 2008125916, 2008, p. 10.
7. Spiro, R. C., and L. S. Liu. Collagen/polysaccharide bilayer matrix for tissue regeneration. Orquest, Inc., USA, WO, 2002017713, 2002, p. 20.
8. Hopkins, R. Solutions for tissue engineering and methods of use. The Children's Mercy Hospital, USA, WO, 2009152384, 2009, p. 49.
9. Chmielewski, J. A., M. M. Pires, and D. E. Przybyla. Collagen peptide conjugates and uses thereof as biomaterials. Purdue Research Foundation, USA, WO, 2009140573, 2009, p. 95.
10. Chen, H., X. Yang, and S. Qin. Method for preparing type-II collagen sponge scaffold and its application to cartilage repair. Guangzhou Institute of Traumatic Surgery, Peop. Rep. China, CN, 101549171, 2009, p. 17.
11. Fan, D., C. Zhu, X. Ma, Y. Mi, P. Ma, X. Zhu, W. Xue, J. Hui, Y. Luo, and C. Gao. Method for manufacturing biodegradable biomimetic artificial bone material for tissue engineering. Northwest University, Peop. Rep. China, CN, 101584884, 2009, p. 13.
12. Fan, D., C. Zhu, X. Ma, Y. Mi, Y. Luo, P. Ma, J. Hui, X. Zhu, W. Li, L. Liang, and L. Chen. Method for preparing biodegradable vascular inner layer scaffold with high biocompatibility, blood compatibility, and immune compatibility used in tissue engineering. Northwest University, Peop. Rep. China, CN, 101554490, 2009, p. 9.
13. Bayon, Y., P. Gravagna, A. Meneghin, M. Therin, and O. Lefranc. Bioresorbable implant for wall reinforcement treatment and tissue engineering. Sofradim Production, Fr., WO, 2009016519, 2009, p. 46.
14. Schussler, O., and R. Michelot. Collagen scaffold modified by covalent grafting of adhesion molecules, associated methods and use thereof for cardiovascular and thoracic cell therapy and contractile tissue engineering. Fr., WO, 2009007531, 2009, p. 79.
15. Wang, Z., Corneal acellular matrix containing collagen fiber. Zhongshan Ophthalmic Center, Sun Yat-Sen University, Peop. Rep. China, CN, 101066471, 2007, p. 8.

16. Tang, S., and X. Mao. Wound dressings and tissue engineering scaffolds containing crosslinked agar and collagen and gelatins. Jinan University, Peop. Rep. China, CN, 1887359, 2007, p. 8.
17. Drapeau, S. J., M. Torrianni, F. Everaerts, and W. F. McKay. Crosslinked compositions comprising collagen and demineralized bone matrix, methods of making and methods of use. U.S. Patent 2005020506, 2005, p. 14.
18. Tabata, Y., and T. Asahara. Hydrogels containing crosslinked gelatin derivatives for medical use. Medgel Corporation, Japan, WO, 2006085653, 2006, p. 16.
19. Yang, Y. A cervical epidermal tissue engineering stent. Peop. Rep. China, CN, 1868553, 2006, p. 8.
20. Bouwstra, J. B., A. J. J. Van Es, and Y. Toda. RGD-enriched gelatin-like proteins with enhanced cell binding and therapeutic use. Fuji Photo Film B.V., Neth., WO, 2004085473, 2004, p. 31.
21. Ahlers, M., and D. Reibel. Fiber matting composed of gelatin for medical and food technology applications. Gelita AG, Germany; Carl Freudenberg KG, WO, 2008107126, 2008, p. 45.
22. Yao, K., J. Li, Y. Chen, H. Yao, and L. Pu. Method for preparing hydroxyapatite nanoparticle/chitosan/gelatin composite porous stent material. Tianjin University, Peop. Rep. China, CN, 1799647, 2006, p. 7.
23. Yang, Y. Manufacture of cervical skin tissue engineering scaffold. Peop. Rep. China, CN, 1720876, 2006, p. 9.
24. Luo, Z. Tissue engineering compound material for repairing nerve injury and its preparation. Fourth Military Medical University, PLA, Peop. Rep. China, CN, 1546182, 2004.
25. Cao, Y., L. Cui, W. Liu, G. Yang, and J. Yang. Manufacture of epidermal prosthetic materials by tissue-engineering. Cao Yilin, Peop. Rep. China, CN, 1608684, 2005, p. 12.
26. Delmotte, Y. and J. Diorio. Fibrin material and method for producing and using the same. Baxter International Inc., USA, WO, 2002089868, 2002, p. 48.
27. Nunez, H. A., W. N. Drohan, W. H. Burgess, H. P. Greisler, J. O. Hollinger, C. I. Lasa, Jr., T. Maciag, and M. J. MacPhee. Supplemented and unsupplemented tissue sealants, methods of their production and use. American National Red Cross, USA; Loyola University of Chicago; United States Dept. of the Army, WO, 9420133, 1994, p. 140.
28. Marx, G. and R. Gorodetsky. Preparation of fibrin microbeads for transplantation of cells. V.I. Technologies, Inc., USA; Hadasit Medical Research & Development Ltd., WO, 9915637, 1999, p. 59.
29. Han, B. Composite material for tissue-engineered skin graft. Beijing Saier Taihe Biological Medical Technology Co., Ltd., Peop. Rep. China, CN, 101002964, 2007, p. 13.
30. Han, B., S. Tian, Y. Du, and X. Zhang. Tissue-engineered auto-corneal epithelium grafts and its preparation method. Beijing Yiling Bioengineering Co., Ltd., Peop. Rep. China, CN, 1569259, 2005, p. 13.
31. Hubbell, J. and J. C. Schense. Enzyme-mediated modification of fibrin for tissue engineering. California Institute of Technology, USA, WO, 9843686, 1998, p. 42.
32. Kaplan, D. L. and L. Meinel. Silk-based drug delivery systems for sustained release of drugs. Trustees of Tufts College, USA, WO, 2005123114, 2005, p. 39.

33. Crighton, J. S., Process for forming silk fibers use in biodegradable implants. Xiros PLC, UK, WO, 2002081793, 2002, p. 32.
34. Mooney, D. J. K. H. Bouhadir, W. H. Wong, and J. A. Rowley. Materials containing polysaccharide chains, particularly alginates or modified alginate chains. Regents of the University of Michigan, USA, WO, 9812228, 1998, p. 97.
35. Yuan, Z., W. Wang, C. Zhang, W. Huang, and Q. Tian. Sodium alginate hepatic-targeted nanodrug delivery system and the preparation method thereof. Nankai University, Peop. Rep. China, CN, 101549158, 2009, p. 7.
36. Chu, B. and B. S. Hsiao. Nanostructured thermo-reversible smart gel comprising alginate or chitosan polyelectrolyte complex, for time release delivery of drugs such as lidocaine. The Research Foundation of State University of New York, USA, WO, 2008033497, 2008, p. 41.
37. Moroni, A. and W. Drefko. pH-dependent sustained-release pharmaceutical compositions. ISP Investments Inc., USA, WO, 2002076429, 2002, p. 12.
38. Han, S.-M., I.-S. Kim, and N.-K. Han. Preparation of alginate sponges. SK Chemicals Co. Ltd., S. Korea, WO, 2004082594, 2004, p. 19.
39. Vacanti, J. P., A. Atala, D. J. Mooney, and R. S. Langer. Breast tissue engineering. Children's Medical Center Corporation, USA, WO, 9618424, 1996.
40. Lu, H. S. M., Dextran-based polymer tissue adhesive for medical use formed by reacting aminodextran containing primary amine groups with oxidized dextran containing aldehyde groups. E.I. du Pont de Nemours and Company, USA, WO, 2009064977, 2009, p. 46.
41. Lee, G. Y., G. H. Jung, and J. Kim. Method for manufacturing dextran acetate nanoparticles with good biodegradability and biocompatibility for drug delivery. Industry Foundation of Chonnam National University, S. Korea, KR, 2009058420, 2009, p. 10.
42. Spiro, R. C., A. Y. Thompson, and L. Liu. Crosslinked polysaccharide drug carrier. Orquest, Inc., USA, US, 6303585, 2001, p. 7.
43. Vera, D. R. Macromolecular carrier for drug and diagnostic agent delivery. The Regents of the University of California, USA, WO, 2000069473, 2000, p. 46.
44. Nevo, Z., L. Astachov, S. Rochkind, and A. Shahar. Cohesive coprecipitates of sulfated polysaccharide and fibrillar proteins. NVR Labs, Ltd., Israel, WO, 2004029095, 2004, p. 49.
45. Gevaert, M. R. and S. Massia. Polylactide-dextran graft copolymers for biomaterial and tissue engineering applications. Clemson University, USA, WO, 2001079315, 2001, p. 19.
46. Mailland, F. and M. Legora. Dermal film-forming liquid formulations for drug release to skin. Polichem S.A., Luxembourg, EP, 1958613, 2008, p. 16.
47. Cho, Y., R. Shi, A. Ivanisevic, and R. Borgens. Repairing damaged nervous system tissue with nanoparticles. Purdue Research Foundation, USA, WO, 2009091992, 2009, p. 43.
48. Roy, K., B. Ghosn, and S. Kasturi. Enhancement of polysaccharide-mediated nucleic acid delivery through functionalization with secondary and tertiary amines. Board of Regents, The University of Texas System, USA, WO, 2009036022, 2009, p. 32.
49. Sangyong, J., L. Eunhye, L. J. Ju, and L. In-Hyun. Transmucosal delivery of pharmaceutical active substances. Inst Science & Tech Kwangju, Korea, WO, 2007083984, 2007.

50. Sun, H., X. Yuan, E. Tian, X. Li, and W. Jiang. Nanoparticles drug-targeting controlled release eye drops. Eye Center, Tianjin Medical University, Peop. Rep. China, CN, 101209241, 2008, p. 6.
51. Grant, J., J. Cho, and C. Allen. Chitosan-sorbitan ester emulsions. Can., CA, 2537724, 2007, p. 41.
52. Yatvin, M. B., Chitosan-coated liposome drug delivery of antioxidant or anti-inflammatory compounds. Enzrel, Inc., USA, WO, 2006062506, 2006, p. 60.
53. Zeng, R., M. Tu, Z. Zha, and H. Liu. Preparation method and application of chitosan-based nanofibers. Jinan University, Peop. Rep. China, CN, 101280467, 2008, p. 15.
54. Nie, J., Y. Zhou, D. Yang, X. Gao, and T. Wang. Preparation of water-soluble derivatives of chitosan. Beijing University of Chemical Technology, Peop. Rep. China, CN, 101225123, 2008, p. 15.
55. Liang, W., M. Chen, and Y. Wang. Temperature-sensitive chitosan hydrogel. Zhejiang University, Peop. Rep. China, CN, 101148520, 2008, p. 9.
56. Liu, R., X. Xiao, and Q. Huang. Method for preparing nanoscale dahllite/chitosan-poly(lactic acid) scaffold material for bone tissue engineering. Fujian Normal University, Peop. Rep. China, CN, 101491702, 2009, p. 11.
57. Liu, T., B. Jiang, S. Guan, W. Zhang, K. Song, D. Ge, X. Li, X. Ma, and R. Lu. Preparation method of injectable chitosan/sodium glycerophosphate/collagen hydrogel with good biocompatibility. Dalian University of Technology, Peop. Rep. China, CN, 101502673, 2009, p. 7.
58. Williams, S. F. and D. P. Martin. Polyhydroxyalkanoate compositions for soft tissue repair, augmentation, and viscosupplementation. Tepha, Inc., USA, WO, 2001019422, 2001, p. 24.
59. Williams, S. F. Flushable disposable polymeric products. Tepha, Inc., USA, WO, 2001015671, 2001, p. 15.
60. Williams, S. F., D. P. Martin, and F. Skraly. Medical devices and applications of polyhydroxyalkanoate polymers. Metabolix, Inc., USA, WO, 2000056376, 2000, p. 71.
61. Williams, S. F., D. P. Martin, and T. Gerngross, and D. M. Horowitz. Polyhydroxyalkanoates for in vivo applications. Metabolix, Inc., USA, WO, 9851812, 1998, p. 51.
62. An, J., K. S. Leu, E. S. Lennox, and J. H. Musser. Acid-modified arabinogalactan protein from Astragalus membranaceus. Pharmagenesis, Inc., USA, WO, 2002002607, 2002, p. 32.
63. Domb, A. J., I. Polacheck, M. Soskolni, and J. Golenser. Conjugates of therapeutically active compounds. Hadasit Medical Research Services & Development Limited, Israel; Yissum Research Development Company, WO, 2007034495, 2007, p. 37.
64. Domb, A. J. Polymer constructs. Chondros Inc, USA, US, 2002012705, 2002.
65. Gunatillake, P. A. and R. Adhikari. *Eur. Cell Mater.* 2003;**5**:1–16; discussion 16.
66. Asgharian, B., M. A. Chowhan, and M. B. Wax. Water-insoluble polymer matrix for drug delivery. Alcon Manufacturing Ltd., USA, US, 2008113027, 2008, p. 17.
67. Oh, J. E., K. H. Lee, T. G. Park, and Y. S. Nam. Controlled drug delivery system using the conjugation of drug to biodegradable polyester. Mogam Biotechnology Research Institute, S. Korea; Korea Advanced Institute of Science and Technology, WO, 9959548, 1999, p. 72.

68. Da Silva Cunha, Jr., R. Camargo Siqueira, and S. Ligorio Fialho. Biodegradable device comprising polyester matrix for intraocular drug delivery, for the treatment of retinal degenerations and posterior segment eye diseases, Universidade Federal De Minas Gerais, Brazil, WO, 2008025111, 2008, p. 29.
69. Garnett, M. C., G. Hutcheon, S. Higgins, P. Kallinteri, and C. St. Pourcain. Nano and microparticle drug delivery systems comprising polyesters containing aliphatic dicarboxylate residues and residues of aliphatic polyols. The University of Nottingham, UK; Liverpool John Moores University; Aston University, WO, 2004096178, 2004, p. 33.
70. Domb, A. J. and Z. Zeev. Stereocomplex polymeric carriers for drug delivery. Efrat Biopolymers Ltd., US, 6365173, 2002, p. 10.
71. Edwards, D. A., R. S. Langer, R. Vanbever, J. Mintzes, J. Wang, and D. Chen. Particles for inhalation having sustained release properties. Massachusetts Institute of Technology, USA; The Penn State Research Foundation, US, 7628977, 2009, p. 24.
72. Hossainy, S. F. A., S. D. Pacetti, and M. V. Simhambhatla. Implantable medical device with surface-eroding polyester drug delivery coating for treating vascular diseases. Advanced Cardiovascular Systems, Inc., USA, US, 2007190103, 2007, p. 10.
73. Dunn, R. L., J. S. Garrett, H. Ravivarapu, and B. L. Chandrashekar. Polymeric delivery formulations of leuprolide with improved efficacy. Atrix Laboratories, USA, US, 6565874, 2003, p. 15.
74. Zhang, W., T. T. Tran, S. Hahn, J. Y. Yang, Y. Yuk, and K. D. Brown. Interpenetrating polymer network compositions for controllable drug delivery. Curexo USA, Inc., USA, WO, 2009036083, 2009, p. 43.
75. Ondracek, V. and J. Kufrova. Self-expanding biodegradable stent comprising polydioxanone fibers. ELLA-CS, Czech Rep., US, 2009157158, 2009, p. 8.
76. Si-Young, S., H. Seung-Joo, P. Seong-Bae, G. Jin-Gu, K. Han-Soo, and S. Yong-Gun. Composition comprising magnetic nanoparticle encapsulating magnetic material and drug with biodegradable synthetic polymer. Univ Yonsei Seoul, Korea, WO, 2004096190, 2004.
77. Durand, E. A., Iontophoretic drug delivery device and software application. Isis Biopolymer LLC, USA, US, 2009149800, 2009, p. 36.
78. Nam, Y. S., H. S. Kang, S. H. Han, and I. S. Chang. Amphiphilic biodegradable block copolymers and self-assembled polymer aggregates formed from the same in aqueous milieu. Pacific Corp., S. Korea, US, 2003009004, 2003, p. 16.
79. Kweon-Choi, Y. and J. S. Kim. Biodegradable mixed polymeric micelles for drug delivery. Samyang Corporation, S. Korea, US, 6410057, 2002, p. 9.
80. Domb, A. J. Polymeric formulations for drug delivery. Efrat Biopolymers Ltd, US, 2004161464, 2004, p. 16.
81. Bezemer, J. M., J. R. De Wijn, R. E. Haan, and M. Blom, Biodegradable porous scaffold material. Isotis N.V., Neth., WO, 2002060508, 2002, p. 20.
82. Shalaby, S. W., J. M. Lindsey III, M. S. Taylor, D. E. Linden, and D. R. Ingram. Swellable fiber- and microfiber-forming polyether-esters and applications thereof in biomedical and tissue engineering devices. Poly-Med, Inc., USA, US, 2007275034, 2007, p. 8.

83. Bowlin, G. L., M. J. Smith, and K. L. White, Jr. MCP-1 electroprocessed delivery system comprising electrospun scaffold such as polydioxanone. Organogenesis, Inc., USA, WO, 2010025176, 2009, p. 13.
84. Wan, T., S. Li, Y. Yan, H. Wen, B. Cao, and F. Zhao. In-vivo degradable liver tissue-engineered porous sponge scaffold with physiological vascular network structure. Wuhan University of Technology, Peop. Rep. China, CN, 101530631, 2009, p. 6.
85. Chang, J., K. Lin, X. Pang, W. Gu, Y. Zhang, and Y. Bao. Polyester-enhanced degradable bioactive porous calcium silicate composite scaffold material, and preparation method and application thereof. Zhejiang Jiayuan Pharmaceutical Industry Co., Ltd., Peop. Rep. China; Shanghai Institute of Ceramics, Chinese Academy of Sciences, CN, 101474428, 2009, p. 20.
86. Mayes, A. M., L. G. Griffith, D. J. Irvine, P. Banerjee, and T. D. Jonson. Comb copolymers for regulating cell-surface interactions. Massachusetts Inst Technology, USA, US, 6207749, 2001.
87. Dyer, W. K. Methods and compositions for tissue augmentation. USA, US, 2002025340, 2002, p. 9.
88. Lior, Y. and A. J. Domb. Injectable biodegradable polymer compositions for soft tissue repair and augmentation. Juvenis Ltd; Yankelson Lior; Domb Abraham J, WO, 2009044403, 2009, p. 4.
89. Kuroiwa, T., K. Minatotani, S. Funamoto, N. Nagatani, T. Fujisato, T. Nakaya, N. Sasayama, A. Shirakazu, H. Hattori, H. Takano, T. Narumi, and T. Moritan. Collagen-based artificial blood vessel having biodegradable or biocompatible coatings. National Cardiovascular Center, Japan; Nipro and Co., Ltd., JP, 2007268239, 2007, p. 14.
90. Choi, S. U., H. Y. Kim, D. R. Lee, S. C. Lee, J. S. Oh, and S. S. Oh. Regenerated layer of periodontal tissue and preparation method thereof. Meta Dental Co., S. Korea, KR, 2001011083, 2001.
91. Yanaga, H. The sheet for the culture epidermis which improves raw wearing factor. Terumo Corp., Japan, JP, 2000245450, 2000, p. 9.
92. Kim, Y.-H., S.-H. Kim, S.-Y. Lee, J.-C. Kim, and J.-H. Oh. Porous biodegradable polymer supports for regeneration of damaged eyeball tissues. Korea Institute of Science and Technology, S. Korea, JP, 2003126236, 2003, p. 10.
93. Dan, G., D. Abraham, T. Gadi, P. Gadi, and A. Tony. Composite scaffolds and methods using same for generating complex tissue grafts. Yissum Res Dev Co, US, 2009035349, 2009, p. 35.
94. Dan, G., A. J. Domb, T. Gadi, P. Gadi, and A. Tony. Composite scaffolds and methods using same for generating complex tissue grafts. Yissum Res Dev Co; Gazit Dan; Domb Avraham J; Turgeman Gadi; Pelled Gadi; Azzam Tony, WO, 02087411, 2002, p. 87.
95. Sokolsky-Papkov, M., K. Agashi, A. Olaye, K. Shakesheff, and A. J. Domb. *Adv. Drug Deliv. Rev.* 2007;**59**:187–206.
96. Pachence, J. M., M. P. Bohrer, and J. Kohn. Biodegradable polymers. In R. Lanza, R. Langer, J. Vacanti (Eds.), *Principles of Tissue Engineering*. Academic Press: Burlington. 2007, pp. 1095–1109.

97. Heller, J., R. V. Sparer, and G. M. Zentner. *J. Bioact. Comper. Polym.* 1990;**3**:97–105.
98. Shah, D. T., J. Barr, B. Baxter, and J. Heller. Base-stabilized polyorthoester formulations. AP Pharma, Inc., USA, US, 2007264338, 2007, p. 44.
99. Lin, X., G. Zhai, M. Sarad, and P. N. Lohstroh. Telomerase delivery by biodegradable nanoparticles. Telomolecular Corporation, USA, US, 2009142408, 2009, p. 17.
100. Bowman, L. M., S. K. Chandrasekaran, R. Patel, and H. V. Vo. Bioerodible controlled delivery system. Insite Vision Inc., USA, WO, 9414416, 1994, p. 60.
101. O'Hagan, D., G. Otten, J. J. Donnelly, J. M. Polo, S. Barnett, M. Singh, J. Ulmer, and T. W. Dubensky, Jr. Microparticles for delivery of the heterologous nucleic acids. Chiron Corporation, USA, WO, 2002026209, 2002, p. 100.
102. Han, D. K., K. D. Ahn, Y. M. Ju, and S. Ahn. Preparation method of biodegradable porous polymer scaffolds having an improved cell compatibility for tissue engineering. Korea Institute of Science and Technology, S. Korea; Solco Biomedical Co., Ltd., US, 2004043135, 2004, p. 7.
103. Jung, S. I., S. H. Kim, Y. H. Kim, and S. H. Lee. Method for preparing biodegradable porous support for tissue engineering without organic solvent. Korea Institute of Science and Technology, S. Korea, KR, 2004101787, 2004.
104. Pacetti, S. D., S. F. A. Hossainy, Y. Tang, A. C. Tung, and T. Glauser. Polymer coatings for drug delivery implantable devices having gradient of hydration. Advanced Cardiovascular Systems, Inc., USA, US, 7329413, 2008, p. 13.
105. Ibim, S. E., K.E. Uhrich, M. Attawia, V. R. Shastri, S. F. El-Amin, and R. Bronson. *J. Biomed. Mater. Res.* 1998;**43**:374–379.
106. Jain, J. P., D. Chitkara, and N. Kumar. *Expert Opin. Drug Deliv.* 2008;**5**:889–907.
107. Jain, J. P., S. Modi, A. J. Domb, and N. Kumar. *J. Control. Release* 2005;**105**: 541–563.
108. Jain, J. P., S. Modi, and N. Kumar. *J. Biomed. Mater. Res. A* 2008;**84**:740–752.
109. Uhrich, K. E. Polyanhydride linkers for production of drug polymers and drug polymer compositions using them, Rutgers, The State University of New Jersey, USA, US, 6486214, 2002, p. 7.
110. Uhrich, K. E. Therapeutic polyanhydride compounds for drug delivery, Rutgers, The State University of New Jersey, USA, WO, 2002009767, 2002, p. 38.
111. Domb, A. J., R. S. Langer, E. Ron, S. Giannos, R. Kothari, and E. Mathiowitz. Pure polyanhydride from dicarboxylic acid and coupling agent. Massachusetts Inst. Technology, USA, US, 4916204, 1990.
112. Domb, A. J. Polyanhydrides. Efrat Biopolymers Ltd., WO, 0244232, 2009, p. 29.
113. Langer, R. S., and A. J. Domb. Process for manufacture of high-molecular-weight controlled-release polyanhydride drug delivery devices. Massachusetts Institute of Technology, USA, EP, 260415, 1988, p. 22.
114. Langer, R. S., A. J. Domb, and C. T. Laurencin. Controlled drug delivery using high molecular weight polyanhydrides. Massachusetts Institute of Technology, USA, US, 4888176, 1989, p. 27.
115. Laurencin, C. T., P. A. Lucas, G. T. Syftestad, A. Domb, J. Glowacki, and R. S. Langer. Delivery system for controlled release of bioactive factors. Massachusetts Institute of Technology, WO, 9009783, 1990, p. 28.

116. Brem, H., R. J. Langer, and A. J. Domb. Controlled local delivery of chemotherapeutic agents for treating solid tumors. Massachusetts Institute of Technology; The Johns Hopkins University, WO, 9603984, 1996, p. 75.

117. Domb, A. J., M. Maniar, and A. T. S. Haffer. Biodegradable polymer blends for drug delivery. Nova Pharmaceutical Corp., USA, WO, 9213567, 1992, p. 34.

118. Domb, A. J. and M. Maniar. Fatty acid-terminated polyanhydrides as controlled-release pharmaceutical matrixes. Nova Pharmaceutical Corp., USA, US, 5179189, 1993, p. 9.

119. Burke, M., M.-C. White, M. C. Watts, J. KyooLee, B. M. Tuler, G. H. Posner, and H. Brem. Vitamin D_3 analog loaded polymer formulations for cancer and neurodegenerative disorders. Univ. Johns Hopkins (USA), US, 2003105067, 2003.

120. Langer, R. S. and H. Rosen. Bioerodible polyanhydrides for controlled drug delivery, Massachusetts Institute of Technology, USA, US, 4891225, 1990, p. 13.

121. Tarcha, P. J., J. R. Haaga, and N. T. Stowe. Combination of ablation and controlled drug delivery. for the treatment of cancer. USA, US, 2003199449, 2003, p. 12.

122. Kumar, N. and J. P. Jain. Injectable depot forming drug delivery system. National Institute of Pharmaceutical Educationa and Research (NIPER), SAS Nagar, India, IN, 589/DEL/2007, 2008, p. 32.

123. Schestopol, M. A., J. S. Jacob, R. Donnely, T. L. Ricketts, A. Nangia, E. Mathiowitz, and Z. E. Shaked. Bioadhesive polymers with catechol functionality. Spherics, Inc., USA, WO, 2005056708, 2005, p. 53.

124. Uhrich, K. Aromatic polyanhydrides with therapeutically useful degradation products. Rutgers, The State University, USA, WO, 9912990, 1999, p. 29.

125. Domb, A. J. and R. S. Langer. Unsaturated polyanhydrides useful for biomedical products. Massachusetts Institute of Technology, USA, WO, 8901006, 1989, p. 31.

126. Wu, Y., X. Yu, and X. Xiao. Calcium metaphosphate biomaterial for non-hard tissue engineering. Guangdong Provincial People's Hospital, Peop. Rep. China, CN, 101357239, 2009, p. 9.

127. Li, S., Y. Yan, Q. Zhang, X. Chen, T. Wan, Y. Wang, X. Wang, and T. Feng. Degradable conducting biomedical polymer. Wuhan University of Technology, Peop. Rep. China, CN, 101402737, 2009, p. 14.

128. Hahn, D. G., K. D. Ahn, J. M. Kim, and Y. M. Joo. Biodegradable polymer supports for tissue engineering and their manufacture. Korea Institute of Science and Technology, S. Korea; Biomedlab Corporation, JP, 2002020523, 2002, p. 7.

129. Choi, J. S., G. S. Kang, J. A. Kang, I. Y. Kim, H. B. Lee, I. U. Lee, S. J. Lee, and P. G. Shin. Tissue engineering natural/synthetic hybrid carrier and method for producing the same. Korea Research Institute of Chemical Technology, S. Korea, KR, 2003097156, 2003.

130. Nair, L. S. and C. T. Laurencin. *Prog. Polym. Sci.* 2007;**32**:762–798.

131. Gorna, K. and S. Gogolewski. *J. Biomed. Mater. Res.* 2002;**60**:592–606.

132. Gunatillake, P. A. and G. F. Meijs. Polyurethanes in biomedical engineering. In K. H. J. Buschow, R. W. Cahn, M. C. Flemings, B. I. Ilschner, E. J. Kramer, S. Mahajan, and P. Veyssière (Eds.). *Encyclopedia of Materials: Science and Technology*. Elsevier: Oxford. 2008, pp. 7746–7752.

133. Tan, H., M. Ding, Q. Fu, J. Li, Z. Wang, X. Zhang, X. Fu, and Q. Zhang. Preparation of amphiphilic multi-block polyurethane material with no toxicity and high biodegradability. Sichuan University, Peop. Rep. China, CN, 101503501, 2009, p. 20.

134. Kuo, S.-H., and P. Kuzma. Manufacture of long term drug delivery devices with polyurethane based polymers. Valera Pharmaceuticals, Inc., USA, WO, 2005013936, 2005, p. 32.

135. Kuzma, P., H. Quandt, and S.-H. Kuo. Polyurethane implantable device for the delivery of naltrexone. Indevus Pharmaceuticals, Inc., USA, US, 2009208540, 2009, p. 15.

136. Johnston, C. W. and J. M. Teffenhart. Hydrophilic carboxylated polyurethanes. Tyndale Plains-Hunter Ltd., USA, US, 4743673, 1988, p. 7.

137. Levy, R. J. and A. Sintov. Biocompatible polymeric matrix system for controlled release of antiarrhythmic agents. University of Michigan, USA, US, 5387419, 1995, p. 15.

138. Levy, R. J. and A. Sintov. System for controlled release of antiarrhythmic agents. Board of Regents of the University of Michigan, USA, WO, 9421237, 1994, p. 83.

139. Lai, Y.-C., R. Shi, W. O. Lever, Jr., D. V. Ruscio, and Y. Huang. Drug delivery device comprising crosslinked polyurethane-siloxane-containing copolymers. Bausch & Lomb Incorporated, USA, WO, 2006068950, 2006, p. 20.

140. Venkatraman, S. S., T. M. Stein, J. Snider, and R. D. Hamlin. Transdermal drug delivery devices comprising a polyurethane drug reservoir. Alza Corp., USA, WO, 2000059483, 2000, p. 31.

141. Chen, T.-F., C.-M. Chiang, J. Jona, P. Joshi, and A. Ramdas, Polyurethane hydrogel drug reservoirs for use in transdermal drug delivery systems. Cygnus, Inc., USA; Chen, Tung-Fen; Chiang, Chia-Ming; Jona, Janan; Joshi, Priti; Ramdas, Asha, WO, 9709970, 1997, p. 39.

142. Allen, S. I., M. Ferguson, and H. Tung. Multilayer barrier film for transdermal drug delivery system and ostomy applications. The Dow Chemical Company, USA, US, 5567488, 1996, p. 8.

143. Cleary, G. W. A diffusion matrix for transdermal drug administration and removable and repositionable transdermal drug delivery devices containing it. Cygnus Research Corp., USA, EP, 272918, 1988, p. 8.

144. Wiese, H. and G. Maier. Open-pored polyurethane foam without skin formation, formulation for the production thereof and use thereof as a carrier material for cell and tissue cultures or medicaments. Polymaterials AG, Germany, WO, 2006032501, 2006, p. 37.

145. Hsu, S.-H. and D. C. Chen. Biomedical material for improving the adhesion and proliferation of cells and a modified artificial vessel. National Science Council, Taiwan, US, 6579322, 2003, p. 9.

146. Zhang, S., M. Wang, and B. Yuan. Method for preparing biodegradable network-structured polyurethane/polyurea, Qingdao University of Science and Technology, Peop. Rep. China, CN, 101157748, 2008, p. 9.

147. Martineau, L. and P. N. Shek. Multi-layer dressings as drug delivery system. Can., US, 2003149406, 2003, p. 19.

148. Moore, T. G., P. A. Gunatillake, R. Adhikari, and S. Houshyar. High modulus polyurethane and polyurethane/urea compositions for medical applications. Polynovo Biomaterials Limited, Australia, WO, 2009043099, 2009, p. 38.

149. Adhikari, R., and P. A. Gunatillake. Biodegradable polyurethane/urea compositions as scaffolds in tissue engineering. Commonwealth Scientific and Industrial Research Organisation, Australia, WO, 2004009227, 2004, p. 79.

150. Harris, J. M. Soluble, degradable poly(ethylene glycol) derivatives for controllable release of bound molecules into solution. Shearwater Corporation, USA, S, 6214966, 2001, p. 13.

151. Ding, J. and L. Yu. Method for preparing degradable temperature-sensitive chemically crosslinked hydrogel. Fudan University, Peop. Rep. China, CN, 1995099, 2007, p. 12.

152. Wu, N. and B. C. Keller. PEG-lipid conjugates for liposomes and drug delivery. USA, US, 2010076209, 2009, p. 25.

153. Heller, J. and S. Y. Ng. PEG-poly(ortho ester), PEG-poly(ortho ester)-PEG, and poly(ortho ester)-PEG-poly(ortho ester) block copolymers. Advanced Polymer Systems, Inc., USA, US, 5939453, 1999, p. 12.

154. Rathi, R. C., G. M. Zentner, and B. Jeong. Biodegradable low molecular weight triblock polyester-polyethylene glycol copolymers having reverse thermal gelation properties. Macromed, Inc., USA, WO, 2000018821, 2000, p. 41.

155. Lee, D. S., D. Kasala, and B. S. Kim. Manufacture temperature- and pH-sensitive block copolymer with good gel strength, and drug delivery system using this block copolymer. Sungkyunkwan University, Foundation for Corporate Collaboration, S. Korea, KR, 838809, 2008, p. 22.

156. Tsai, B.-H., J.-H. Chen, M.-L. Chen, Y.-H. Chen, and M.-J. Liu. Biodegradable copolymer, and polymeric micelle composition containing the same. Taiwan, US, 2005019303, 2005, p. 11.

157. Lavasanifar, A. and X.-B. Xiong. Ligand guided micelle-forming poly(ethylene oxide)-block-poly(ester) copolymers for targeted drug delivery. The Governors of the University of Alberta, Can., WO, 2008071009, 2008, p. 105.

158. Kumar, N. and J. P. Jain. Polymersomes for controlled delivery of drugs. National Institute of Pharmaceutical Education and Research (NIPER), S. A. S. Nagar, India, IN, 751/DEL/2009, 2009.

159. Kumar, N. and J. P. Jain. Amphotericin B-loaded nanoformulation. National Institute of Pharmaceutical Education and Research (NIPER), S. A. S. Nagar, India, IN, 922/DEL/2010, 2010.

160. Kumar, N. and W. Y. Ayen. Doxorubicin nanopolymersomes. National Institute of Pharmaceutical Educationa and Research (NIPER), SAS Nagar, India, IN, 913/DEL/2010, 2010.

161. Yu, Y.-J., C. F. Chen, T.-Y. Lin, S.-J. Yeh, S.-Y. Wang, and P.-L. Lai. Biodegradable copolymer and thermosensitive material. Industrial Technology Research Institute, Taiwan, US, 2009247666, 2009, p. 37.

162. West, J. L. and B. K. Mann. Tissue engineering scaffolds promoting matrix protein production. Rice University, USA, WO, 2002016557, 2002, p. 25.

163. Bourke, S. L. and J. Kohn. *Adv. Drug Deliv. Rev.* 2003;**55**:447–466.

164. Licciardi, M., G. Giammona, G. Cavallaro, and G. Pitarresi. Colloidal vectors with polyaminoacid structure for oral release of peptides and proteins and their production. Università degli Studi di Palermo, Italy, WO, 2008152669, 2008, p. 35.

165. Huille, S., A. Lemercier, and G. Soula. Particles based on polyamino-acid(s) for use as carriers for pharmaceutical and nutritional active agents. Flamel Technologies, Fr., EP, 734720, 1996, p. 19.

166. Bryson, N. and G. Soula. Colloidal suspension of submicron particles for carrying active principles. Flamel Technologies, Fr., WO, 2002028521, 2002, p. 41.

167. Schacht, E. H. and L. Dekie. Polymeric system for delivery of nucleic acid material to target cells in biological systems. Universiteit Gent, Belg., WO, 9958151, 1999, p. 63.

168. Cochrum, K. C. and S. A. Jemtrud. Biological tissue transplant coated with stabilized multilayer alginate coating suitable for transplantation and method for preparation thereof. Regents of the University of California, USA, WO, 9823226, 1998, p. 57.

169. Holland, T. A., Y. Tabata, and A. G. Mikos. *J. Control. Release* 2005;**101**:111–125.

170. Peter, S. J., L. Lu, D. J. Kim, and A. G. Mikos. *Biomaterials* 2000;**21**:1207–1213.

171. Yaszemski, M. J., R. G. Payne, W. C. Hayes, R. Langer, and A. G. Mikos. *Biomaterials* 1996;**17**:2120–2130.

172. Horch, R. A., N. Shahid, A. S. Mistry, M. D. Timmer, A. G. Mikos, and A. R. Barron. *Biomacromolecules* 2004;**5**:1990–1998.

173. Jabbari, E., S. F. Wang, L. C. Lu, J. A. Gruetzmacher, S. Ameenuddin, T. E. Hefferan, B. L. Currier, A. J. Windebank, and M. J. Yaszemski. *Biomaterials* 2005;**6**:2503–2511.

174. Ammon, D. M., J. C. Salamone, H. Ueda, S. Jo, and A. G. Mikos. Sustained release drug delivery compositions comprising a matrix of fumarate polymers for eye diseases. Bausch & Lomb Incorporated, USA; William Marsh Rice University, WO, 2005099667, 2005, p. 31.

175. Jabbari, E. Bioresorbable composition comprising polymer for repairing skeletal tissue, University of South Carolina, USA, WO, 2007008927, 2007, p. 41.

176. Yaszemski, M. J., B. L. Currier, L. Lu, X. Zhu, and E. Jabbari. Blend, crosslinkable poly(propylene fumarate) for immobilization and controlled drug delivery. Mayo Foundation for Medical Education and Research, USA, US, 2004023028, 2004, p. 23.

177. Domb, A. J. Poly(propylene glycol fumarate) compositions for biomedical applications. USA, EP, 325866, 1989, p. 28.

178. Dadsetan, M., M. Yaszemski, and L. Lu. Photocrosslinkable oligo(poly(ethylene glycol) fumarate) hydrogels for cell and drug delivery. Mayo Foundation for Medical Education and Research, USA, WO, 2006102530, 2006, p. 43.

179. Wang, S., M. J. Yaszemski, L. Lu, and B. L. Currier. Block copolymers of polycaprolactone and poly (propylene fumarate). Mayo Foundation for Medical Education and Research, USA, WO, 2006055940, 2006, p. 30.

180. Dadsentan, M., M. J. Yaszemski, and L. Lu. Charged oligo(poly(ethylene glycol) fumarate) hydrogels for nerve growth. Mayo Foundation for Medical Education and Research, USA, WO, 2008008288, 2008, p. 53.

181. Carmichael, R. and B. C. Fox. Nerve regeneration device. Bonwrx, Inc., USA, WO, 2008063878, 2008, p. 37.
182. Myers, M. and P. W. Reginald. Collagen-based drug delivery device for providing local analgesia, local anesthesia or nerve blockade. Innocoll Tehnologies Limited, Ire., US, 2008241245, 2008, p. 12.
183. Au, J. L. S. and M. G. Wientjes. Tumor-targeting drug-loaded particles. USA, WO, 2004089291, 2004, p. 61.

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