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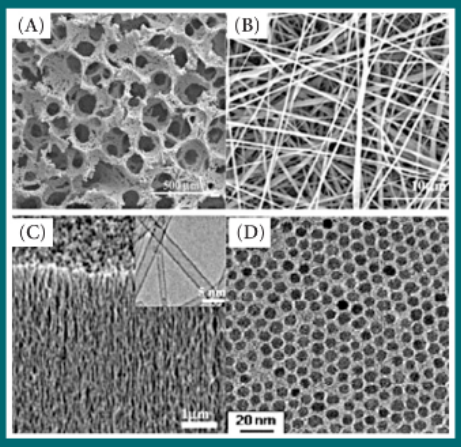

Bioactive materials in medicine

Design and applications

Edited by X. Zhao, J. M. Courtney and H. Qian

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Bioactive materials in medicine

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Foreword

The utilisation of biomaterials is an established feature in a wide range of medical applications and the consequent importance of biomaterials is readily acknowledged. However, it is a continuing goal to design, develop and utilise biomaterials capable of improving existing procedures and promoting the use of novel procedures. In this respect, it is relevant to consider bioactive materials.

A significant property of bioactive materials is the exhibition of a biological activity, thereby enabling control of the biological system response. The possible influence of bioactive materials covers tissue–biomaterial bonding, cell proliferation and adhesion, and tissue regeneration. Options for the application of bioactive materials include the control of biomaterial surface properties, the preparation of biomaterials with a bulk nanostructure, the release of bioactive molecules and the utilisation of the bioactive material as a biomatrix for tissue regeneration.

In this book, experts in their fields from both the UK and China have provided an overview on basic concepts for designing bioactive materials in medicine, including chapters in Part I to cover the process of designing bioactive materials, nanotechnology and tissue engineering. Chapters in Part II focus on the different applications of bioactive materials in medicine. The clinical applications discussed include applications in orthopaedics, in the circulatory system and as antibacterials for medical devices. The final chapters focus on the uses of these materials in gene therapy, plastic surgery and body reconstruction, and in drug delivery systems.

From a demonstrated benefit in orthopaedics to a potential use in association with stem cells, bioactive materials represent an important and exciting field of study. Current and possible future applications ensure that bioactive materials have a high academic, clinical and industrial importance.

Professor Xiaobin Zhao
Professor Jim Courtney
Dr Hong Qian

1

Introduction to bioactive materials in medicine

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Abstract: In this chapter, the comparison between bio-*inert* materials and bio-*active* materials is introduced, in order to understand the definition of bioactive materials. The current definition extends well beyond the original, and bioactive materials are now considered to be those materials which exhibit biological activities to stimulate the response of the biological system, when the materials are required to have clinical effects. The bioactive materials in this book range from traditional bioactive glass, bioactive ceramics in different forms for hard tissue repair to bioactive molecules–materials combination. In order to develop the clinical applications, assessment of the specific bioactivities is required. The principle of designing bioactive materials is required to take into account basic industrial safety and clinical efficacy. In addition, a bioactive material as the key element of a biomatrix in tissue engineering, utilised in conjunction with stem cells, offers future promise in regenerative medicine.

Key words: bioactive materials, tissue engineering, design of bioactive material.

1.1 Definition of bioactive materials

Bioactive materials represent a new generation of biomaterials, which are different from the traditional bio-inert biomaterials. Traditionally, a

> biomaterial is considered to be a non-viable material used in a medical device intended to interact with biological systems. Biomaterials may be distinguished from other materials in that they possess a combination of properties, including chemical, mechanical, physical, and biological properties that render them suitable for safe, effective and reliable use within a physiological environment [1].

However, by 1999, a biomaterial was defined as 'a material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body' [2]. This shows there is an increasing trend for a biomaterial to shift from a traditional bioinert material to a bioactive material.

Bioactive materials were originally discovered to react with the surrounding tissue to form a mechanically strong interfacial bond between a host tissue and an implant [3], with reference mainly to bone tissue repair and implant replacement. By definition, a bioactive material is 'one that elicits a specific biological response at the interface of the material that results in the formation of a bond between the tissues and the material' [4]. Nowadays, the term bioactive materials generally refers to biomaterials which have the capability to induce and conduct the response to the biological system upon interacting. They have the following bioactivities or functions to:

- stimulate cell differentiation and proliferation;
- stimulate gene and tissue regeneration;
- release bioactive molecules to respond body actively and effectively for restoring and repairing the impaired functionality of the organs.

For example, an ideal bone graft material needs to have all the characteristics of osteoconductivity, osteogenicity and osteoinductivity. Osteoconductivity refers to the situation in which the bone graft substitute supports the attachment of new osteoblasts and osteoprogenitor cells, providing an interconnected structure through which new cells can migrate and new vessels can form. Osteogenicity refers to the situation when the osteoblasts that are at the site of new bone formation are able to produce minerals to calcify the collagen matrix that forms the substrate for new bone. Osteoinductivity refers to the ability of a bone graft to induce non-differentiated stem cells or osteoprogenitor cells to differentiate into osteoblasts [5, 6]. To design a bioactive material to fulfil all these requirements is always a challenge when bone material is considered. Other than the application of bioactive materials in the traditional orthopaedics field, bioactive materials have become a most important part of biomedical engineering, and have been widely used in tissue engineering and artificial organs.

1.2 History of bioactive materials

The concept of a bioactive material was first suggested by Larry Hench in the late 1960s, when he found that certain glasses had the capability of bonding to living bone [3]. Since that time, more than ten groups around the

Table 1.1 History of the development of bioactive materials for bone tissue repair [13–15]

	Composition	Year of report	Commercial products/authors
1	Hap (dense)	1971	Durapatite®
2	45S5 bioactive glass (SiO_2, Na_2O, P_2O_5, CaO-*Quaternary* component)	1972	BioGlass®
3	Hap (porous)	1973	Calcitite®
4	Bioglass + Hap	1973	Ceravital®
5	Metal coated with Al_2O_3	1976	
6	Metal coated with Hap	1980	
7	Bioglass + Hap + P	1982	A/W glass-ceramics
8	Bioglass + Hap + W	1982	A/W glass-ceramics
9	Metal fibre /Hap composite	1982	
10	Hap + PE composite	1985	Bonfield / Hapex®
11	$MgO–CaO–SiO_2–P_20_5–CaF_2$ glass	1987	Machinable A/W glass-ceramics
12	Ternary bioactive glass, three components (SiO_2, CaO and P_2O_5)	1992	Li *et al.* [16]
13	Binary bioactive glass, two components (SiO_2, CaO)	1991	Kokubo *et al.* [17]
14	45S5 glass dissolution/soluble silicon / calcium activate six families of genes in old bone cells that then form new bone cells	2001	Hench and co-workers [18]
15	Binary monoliths	2003	Saravanapavan and Hench [19], TheraGlass®

world have shown that glasses containing SiO_2, CaO, P_2O_5, Na_2O and other smaller amounts of oxides in various compositions bond to bone [6–12].

The history of bioactive materials can be reviewed via the discovery and use of various bioactive materials, such as 45S5 BioGlass, bioactive glass-ceramics, such as Ceravital®, A/W glass-ceramics®, or machineable glass-ceramics, further developed to dense hydroxyapatite (Hap), such as Durapatite® or Calcitite®; bioactive material composites, such as polyethylene (PE)–Hap composites, Palavital® and metal-fibre-reinforced bioglass, as shown in Table 1.1. It can be seen that by varying the composition of the bioactive glasses, combining bioactive glass with inorganic ceramics or synthetic polymers, or surface treatment of the metal implant with bioactive materials, many different bioactive materials can be produced for clinical applications. Nano-bioactive materials are now receiving considerable attention, owing to the nanoscale effect on the interaction with the biological system.

In addition to the traditional bioactive materials listed above, bioactive materials can now be extended to most of the biologically active materials, such as controlled release systems containing bioactive molecules (Chapter

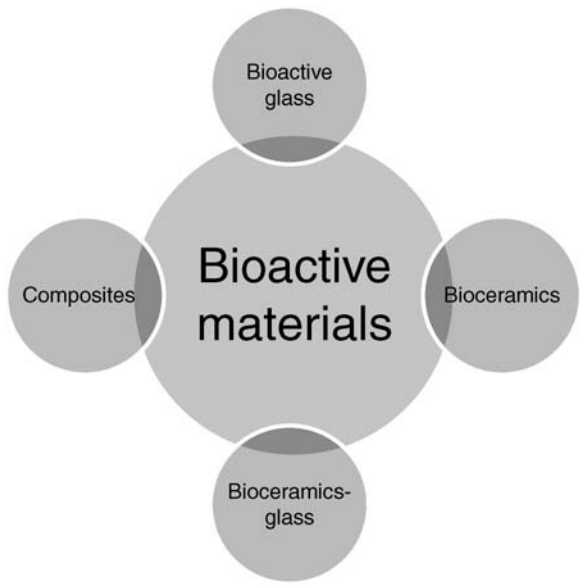
10), gene therapy using bioactive materials (Chapter 8), and bioactive material scaffolds for tissue regeneration (Chapter 4).

1.3 Medical applications of bioactive materials

Historically, medical applications of bioactive materials have more or less been focused on hard tissue repair in the dental and orthopaedics fields, with implants of bioceramics and bioglass, based on the principle of the formation of a hydroxyl carboxyl apatite (HCA) layer at the interface of hard tissue and the implant containing the bioactive materials. The HCA layer eventually leads to the formation of a strong bond between the implant and the tissue, which can hold the implant in place.

With the advancement of biomaterials, the concept of bioactive materials has been extended well beyond this scope. By molecular design of a biomaterial, a bioactive material can be formed to be capable of responding to the surrounding tissue, thereby achieving the designed functionality for specific medical applications. The traditional bioactive materials are summarised in Fig. 1.1, while some of the other bioactive materials covered in this book are summarised in Fig. 1.2.

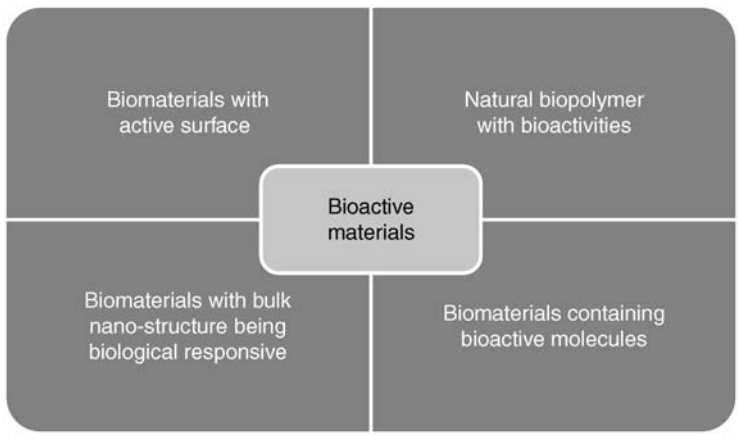
At present, the medical applications of bioactive materials cover almost every field, including tissue engineering and regenerative medicine (Chapter 4), orthopaedics (Chapters 2, 3 and 6), the circulatory system (Chapter 7), gene therapy (Chapter 8), plastic surgery and body reconstruction (Chapter 9), drug delivery (Chapter 10), and diagnostics, such as biosensors. Table 1.2



1.1 Historical examples of bioactive materials.

Table 1.2 Medical applications of bioactive materials

	Bioactive materials	Medical applications		Chapters/references
1	Hap (hydroxyapatite)	Hard tissue repair (orthopaedics)	Bone defect repair	References [13–15]
2	Bioglass		Spine/bone graft	
3	TCP (tricalcium phosphate)		Joint replacements	
4	Hap/TCP		Bone replacement materials (synthetic bone substitute/ graft)	
5	Metal/polymer containing bioactive molecules, such as antibacterial substances		Dental applications	Chapter 5
6	Collagen	Soft tissue	Augmentation as cosmetic surgery	Chapter 9, [20]
7	Chitosan		Wound tissue repair	
8	Gelatin		Implants for urinary incontinence	
10	Hap/collagen/ chitosan		Tendons and ligaments	
11	n-Hap/collagen		Vascular grafts	Chapter 7
12	Demineralised bone matrix (DBM) containing bone growth factors	Tissue engineering	Cartilage re-pair	Chapter 4 , [20]
13	Biodegradable polymer + Hap + cells		Skin substitutes	
14	Biodegradable polymer + bioglass		Bone tissue repair	



1.2 New scope for bioactive materials.

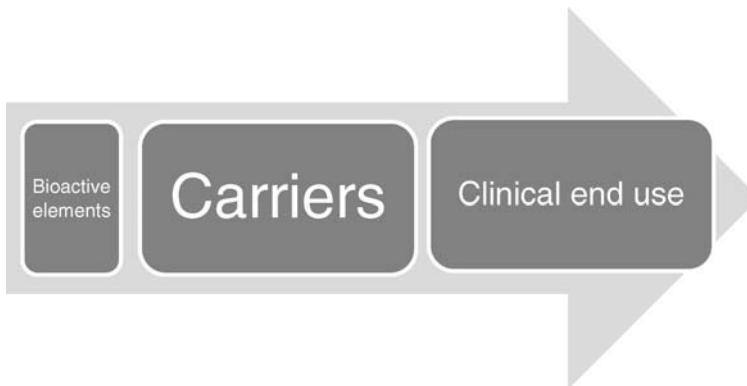
gives an overview of the medical applications of bioactive materials. The detailed clinical applications of bioactive materials are discussed in the relevant chapters.

1.4 Design and commercialisation of bioactive materials

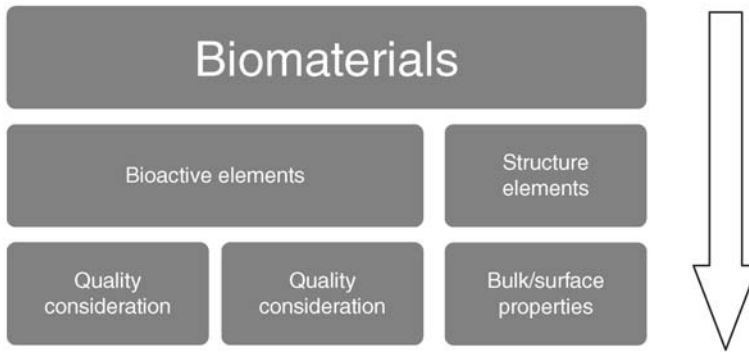
The term bioactive material covers a wide range. Consequently, in this book it is not possible to provide a complete list of bioactive materials. However, in principle, the design of a bioactive material needs to be closely related to the end clinical use, when the bioactive elements or moieties are incorporated into the material carriers or structures (Fig. 1.3). For soft tissue repair such as a wound care product, for example, the bioactive elements include growth factor, fibrinogen, enzyme, hydrogen peroxide, silver, or other anti-infection/antibacterial substances. To ensure that the end requirments are met, the carriers can be natural bioactive polymers, such as collagen, gelatin, chitosan, alginate and other polysaccharides; synthetic polymer fibres or foams; silica nanofibres, or bioactive glass powders.

For hard tissue repair, such as synthetic bone substitutes, the bioactive elements include various compositions of bioactive materials, for example, bioactive glass, the release profile of the ions, the nanostructures of the pores and the surface areas; these elements will determine the bioactivity of the materials upon contacting the hard tissue. In addition, by controlling the structure, the bioactivities can be adjusted.

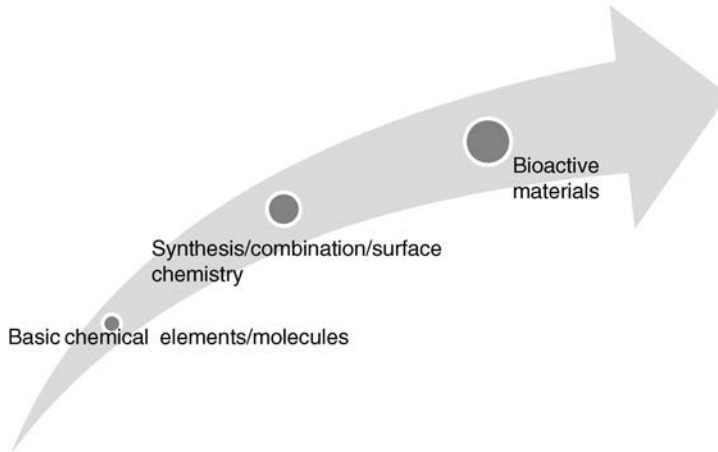
In Chapter 2, the molecular design of a bioactive material is discussed in more detail, based on two approaches: the top-down approach and the bottom-up approach. The 'top-down' approach is based on existing well-accepted biomaterials, either bio-inert or bioactive by addition of bioactive



1.3 Consideration of the molecular design of a bioactive material.



1.4 Design of a bioactive material based on the 'top-down' approach.



1.5 Design of a bioactive material based on the 'bottom-up' approach.

elements, to meet the clinical requirements, as shown in Fig. 1.4. The 'bottom-up' approach is designing a bioactive material from the molecular level by processing, to produce bioactive materials, as shown in Fig. 1.5.

Commercialisation of bioactive materials can be a challenge, as normally they will be fabricated and produced as implants for end users. Commercialisation of implants as medical devices for market realisation needs to follow a well-regulated system to ensure the product's safety and efficacy. According to UK law and EC Medical Devices Directives, there are currently four sets of Medical Device Regulations, implementing all of the Medical Devices Directives and amendments to date; Directive 2007-47-EC covers the placing on the market and putting into service of medical devices; Statutory Instruments 2002 no. 618 (consolidated legislation) and 2003 no. 1697 (amendments to cover the reclassification of breast implants and

additional requirements covering devices utilising materials from transmissible spongiform encephalopathy (TSE) susceptible animal species) and Medical Devices Regulations 2007 no. 400 (amendment to cover the reclassification of total hip, knee and shoulder joints) [21].

For advanced therapy medicinal products (ATMPs), which cover the gene therapy medicinal product, a somatic cell therapy medicinal product and a tissue-engineered product, the new regulation no. 1394/2007 entered into force on 30 December 2007 [22]. Therefore, when bioactive materials contain the above biological moieties, they will be regarded as ATMPs and the manufacturers must follow the new regulation.

To date, there are hundreds of bioactive material-based products commercially available, some of which are listed in Table 1.3, as examples. The table clearly indicates that many commercial players are focusing on developing bioactive materials. For example, Inion aims to be a medical device company, which can develop and commercialise successfully innovative and unique biodegradable and bioactive surgical implants in selected high-value markets. The company is currently developing the next-generation bioactive/biodegradable implant – NMP – built on the Inion Optima™ platform [23].

Table 1.3 Key players in the bioactive materials field and their commercial products

Manufacturers of bioactive materials-based products	Products/technology/medical applications	Type of materials
Advanced biomaterial systems	Poly (methyl methacrylate) (PMMA) bone cement/ *twist* or PrePack®	Polymers
aap Implantate	Ostim® – 100% synthetic, nanoparticular, phase hydroxylapatite bone matrix in paste form	Bioceramics
Aesculap	Novocart 3D (biophasic matrix) – autologous chondrocyte transplantation (collagen-based matrix)	Biomatrix / Tissue engineering
Arthrosurface	Resurfacing arthroplasty – HemiCap® implant for repairing cartilage damage	Metal
Anulex Technologies	Inclose™ surgical mesh to repair soft tissue at the site of injury of annulus	Polymer
ArthroCare	BILOK TCP/PLLA screw (absorbable); PLLA (2–3 years) GraftLock screws	Composites
Artimplant	Artelon implant – biodegradable polyurethane urea for tissue repair	Polymers
AxioMed Spine	Artificial disc – Freedom® lumbar disc using a viscoelastic polymer	Polymers

Table 1.3 (cont.)

Manufacturers of bioactive materials-based products	Products/technology/medical applications	Type of materials
Arthro Kinetics	New generation of collagen matrix for repairing cartilage, degenerative disc disease	Biopolymer
Biomerix	Polyurethane foam, co-foam for vascular occlusion device	Polymer
Biorthex BoneSupport	Actipore – porous Nitinol for bone repair CERAMENT™ injectable bone substitute materialsCERAMENT™ (bone void filler) CERAMENT™ (spine support) (calcium sulphate + hydroxyapatite)	Metal Bioceramics
Cologne	High-density, long carbon fibre-reinforced polymer (LCFRP) for spinal defect repair	Composites
co.don	ACT-3D (three-dimensional chondrocyte transplantation) for cartilage repair/AOT autologous osteoblast transplantation for bone repair	Biomatrix
DePuy	DuoFix™ Hap (hydroxyapatite coatings)	Bioceramics
Disc Dynamics	DASCOR® device – artificial disc	Polymer
Exactech	Cemex®, antibiotic-impregnated bone cement; OpteMx®	Polymer
Gentis	DiscCell / to restore the function of a degenerated intervertebral disc; NuBone® DBM putty; NuBone® DBM gel (gelatine)	Biomatrix
Inion	Inion Optima™ platform, BioRestore (BioGlass) for spine/special orthopaedics	Glass
Integra Life Sciences	Mozaik™ osteoconductive scaffold (80% highly purified beta-TCP granules + 20% highly purified type-1 collagen); Polyactive (1000PEGT70PBT30); OsSatura BCP (biphasic calcium phosphate) and OsSatura TCP (synthetic bone void fillers)	Composites
Intrinsic Therapeutics	Barricaid ARD: a woven mesh to be used to reconstruct the anulus in the region of the herniation	
Kensey Nash	Porous Tissue Matrix™ (PTM) technology based on: polylactides, polylactide-co-glycolides, polycaprolactones, polycarbonates (e.g. TMC), polyurethanes	Polymers
Lanx	OsteoSponge® Block and Filler, 100% cancellous form of DBM derived from donor bone. OsteoWrap is 100% human cortical bone	Biomatrix
MEDICREA International	Medicrea® OSMOSYS® sticks (60% Hap + 40% β-TCP)	Bioceramics
Nexgen Spine	Elastomer: polycarbonate polyurethane (PCU)	Polymers

Table 1.3 (cont.)

Manufacturers of bioactive materials-based products	Products/technology/medical applications	Type of materials
Orthofix	Origen DBM with bioactive glass OsteoMax synthetic bone graft for enhanced bone regeneration • Provides optimal osteoconduction • Reduces the time for the bone to regain its full structural function	Composites
Orthovita	Vitoss®; Vitagel® – control bleeding and facilitate healing • Vitagel is composed of microfibrillar collagen and thrombin in combination with the patient's own plasma which contains fibrinogen and platelets Cortoss®* is a high-strength, bonding, self-setting composite; bioactive glass-ceramic particles • Glass-ceramic particles release alkali into the immediate environment, due to ion exchange with body fluid • Alkali environment induces deposition of calcium phosphate • Calcium phosphate is slowly converted to bone	Biopolymer, bioceramics
Osteotech	Osteotech's Plexur™ – bone void filler. An osteoconductive biocomposite of cortical fibres suspended in a resorbable, porous polylactide-co-glycolide scaffold, Plexur P bone void filler contains calcium, phosphate, trace elements and extracellular matrix proteins; necessary components for bone healing	Composites
Pioneer Surgical Technology	NanOss™ – nanostructured apatites as orthopaedic biomaterials E-Matrix – a porcine collagen gelatin-dextran	Bioceramics, biopolymer
Replication Medical	Neudisc™ – tubular, fabric-reinforced hydrogel designed to mimic the behaviour of a normal disc nucleus	Composites
Ranier Technology	Precision polyurethane manufacture (PPM)	Polymers
Scient'x	Bioscorp – cervical bioresorbable corpectomy implant (polyester tube coated with PLLA)	Polymers
SeaSpine	OsteoSponge® demineralised bone matrix (DBM)	Biomatrix
Spinal Restoration	BIOSTAT BIOLOGX – fibrin sealant	Biopolymer

Table 1.3 (cont.)

Manufacturers of bioactive materials-based products	Products/technology/medical applications	Type of materials
SpineSmith	FUSIONARY™ – FUSIONARY™ process promotes the regeneration of tissue in spinal procedures by delivering a highly concentrated amount of autologous adult stem cells	Tissue engineering and regenerative medicine
Spine Wave	NuCore® injectable nucleus	
Synthes	chronOS is a fully synthetic cancellous bone graft substitute, consisting of pure β-tricalcium phosphate	Bioceramics
Theken Group (Integra LifeSciences)	Collagen guided tissue repair membranes Collagen/ceramic bone graft substitutes Demineralised bone matrix putties/gels	Natural biopolymer
US Spine	40% β-TCP + 60% Hap	Bioceramics
Wright Medical Group	CELLPLEX® TCP graft	Bioceramics
Zimmer	CopiOs™ bone void filler	Bioceramics

1.5 Future trends

Traditional bioinert biomaterials, such as synthetic polymers and metals, have been playing a very important role in medical applications for decades. However, with the advancement in the biomaterials field, bioactive materials have achieved fast growth in the medical industry. The fastest growing areas for the application of bioactive materials are orthobiologics, spinal and trauma, owing to an increased ageing population. According to a market research report, the world orthopaedic market has a highly positive outlook in the medical device industry. It is estimated that the global orthopaedic market was worth approximately US$29.1 billion in 2006, following a growth of 8.8% over the previous year [24]. In particular, the spinal market has surpassed the knee implant market in 2006 for the first time and will see a continuous growth trend in the future [25].

The gold standard for orthopaedic bone graft is still the autologous bone graft. In order to create a synthetic bone substitute material to mimic natural bone material, the biomimetic approach involving nanotechnology, tissue engineering and regenerative medicine has been attracting great attention. This is described in Chapters 2, 3 and 4. For other medical areas such as the circulatory system, wound care and plastic surgery, bioactive materials with controlled bioactivity in order to meet the clinical requirements are described in Chapters 2, 5, 7 and 8. In order to improve upon the brittleness of the bioceramics for load-bearing use, the composites

approach will dominate the market for the next 20 years, while the emerging tissue-engineering technology will be the next step for using bioactive materials in clinical applications [26–28].

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2

Molecular design of bioactive materials with controlled bioactivity

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Abstract: In this chapter, bioactivity is defined and described, and factors influencing bioactivity are discussed. These factors include types of material and the configuration of those materials. Molecular design of bioactive materials with controlled bioactivity, based on the principle of biological recognition, is introduced. Bioactivities include the bonding capabilities of hard and soft tissues to materials; delivery of growth factors to stimulate cellular adhesion, proliferation and differentiation; tissue regeneration capability; antibacterial properties; release of DNA or RNA in gene therapy; release of bioactive molecules for improving biocompatibility; control of material surface properties for biomimetics; and control of sensing elements for diagnostics. Based on these bioactivities, bioactive materials have found widespread application, which are described also with a view to future development trends.

Key words: bioactive materials, influencing factors, design, tissue engineering, medical applications.

2.1 Definition of bioactivity and bioactive materials

Bioactivity is an ability that can cause the normal mechanism of cells to change, or have an effect on, or elicit a response from living tissue. Bioactivities include the bonding capability of hard tissue to materials, the bonding capability of soft tissue to materials, the delivery of growth factors to stimulate cellular adhesion, proliferation and differentiation, the tissue regeneration capability, the release of DNA or RNA in gene therapy, the release of bioactive molecules for improving biocompatibility, and the control of the material surface properties for biomimetics, as well as the

Table 2.1 Types of bioactive compounds

Class	Application
Enzyme	Biosensors, active packaging, biomaterials, bioreactors, microanalytical devices
Peptide	Tissue engineering, antimicrobial surfaces
Polysaccharide	Tissue engineering, haemocompatible materials, antimicrobial surfaces
Phospholipid analogue	Biocompatible/haemocompatible materials
Antibody	Biosensors
Polyethylene glycol	Biocompatibility
Antimicrobial agent	Active packaging, antimicrobial textiles
Oligonucleotide	Microarrays, biosensors

control of drug delivery. With the bioactive materials containing bioactive molecules, those bioactive molecules are mainly represented by growth factors, which are polypeptides that play a major role in regulating extracellular matrix (ECM) formation, and may well revolutionise the treatment of bone and articular cartilage by administration of therapeutic agents [1]. It has been shown that there are a number of essential growth factors providing regulatory effects on chondrocytes or stem cells involved in chondrocyte maturation [2]. Several classes of bioactive compounds and their applications are listed in Table 2.1.

Based on their bioactivities, the bioactive materials have found wide applications in biomedical engineering. Suitable bioactive materials not only promote the adhesion of cells but also facilitate the growth of transplanted cells. In tissue engineering, the strategies mainly utilise the combination of cells, biodegradable scaffolds and bioactive molecules to reproduce natural processes of tissue regeneration and development. Natural and synthetic polymers, inorganic materials and their composites have been formulated into bioactive materials in the form of porous scaffolds, microparticles, nanofibrous membranes and hydrogels [2].

A composite material, consisting of two or more chemically distinct phases (metallic, ceramic, or polymeric) which are separated by interface(s), is designed to have a combination of the best characteristics of each of the component materials. Being composed of two or more types of materials, composites may carry an enhanced probability of causing adverse tissue reactions [3]. Over the last two decades, various bioactive composites have been studied for the purpose of tissue replacement and tissue regeneration. Each of these composites has its distinctive characteristics and may be used in specific clinical situations. With new knowledge being gained of natural tissues and the human body, and rapid progress in composite science and technology, novel and improved composite materials, in conjunction with

advanced life science and biomedical engineering, will become available for substituting diseased, damaged, or worn out body parts.

2.2 Influencing factors on bioactivity

The bioactivities of bioactive materials in clinical applications are influenced by numerous factors. The structure of materials and the micro-environment associated with the materials, which have effects on the release of bioactive molecules, the activities of molecules and the interface between materials and biological systems are the key factors requiring consideration when bioactive materials are designed for clinical application.

2.2.1 Factors influencing bioactivities of bioactive molecules

In order to have effective clinical uses for those bioactive materials incorporating bioactive molecules, the bioactive molecules need to preserve their bioactivity during the period of treatment. Integrins, laminin and RGD (arginine–glycine–aspartate) proteins were shown to be essential for cell attachment to material surfaces [4–7]. During *in vivo* studies, certain growth factors were shown to be osteoinductive [8]. Delivery of bioactive molecules, such as growth factors, to stimulate cellular adhesion, proliferation and differentiation, thus promoting tissue regeneration, is a strategy in tissue engineering. However, systemic administration of growth factors is often unpredictable, owing to their lack of long-term stability *in vivo*, short biological half life and tissue specificity. Carrier incorporated with growth factors can act as a local regulator to control doses and release growth factor at localised orthopaedic sites, thus increasing their potential retention time at therapeutic concentration levels [2]. The composition, porous structure and surface properties of carriers, media conditions, and the specific applications which have effects on the bioactivities of bioactive molecules, will influence their bioactivities.

2.2.2 Factors influencing cell functions

The geometry of a scaffold is an important factor, owing to its potential regulation of cell activity. Recent studies have found that cells on substrates with controlled topography exhibit different behaviours, suggesting that cells can distinguish the geometry of the substrates, such as shape [9] or extent of roughness. Li *et al*. [10] showed that chondrocytes seeded on poly (L-lactide) scaffolds respond differently to the different sizes of micro- or nanofibres. The results of the study demonstrated that the chondrocytes on the nanofibres had a rounded morphology and showed high levels of sulfated glycosaminoglycan, while having a well-spread fibroblast-like

appearance on the microfibrous scaffold, inconsistent with a chondrocytic phenotype.

2.2.3 Factors influencing interface of materials and biological system

For bone tissue engineering, the bioactive property has been attributed to the similarity to the surface composition, crystal structure and the mineral phase of natural bone. The other critical parameter for maintaining the bioactivities of biomaterial is the overall structure: density, pore shape, pore size and pore interconnection pathway [11]. A porous structure has an important effect on cell cellular attachment, proliferation, migration and tissue growth, and pore interconnectivity plays an important role; a complete pore interconnection provides pathways for biofluids and blood vessel invasion. Optimal pore diameter lies in the range 200–800 μm, with a porosity of 90%, which can minimise the resorption time and is compulsory for cell penetration and a proper vascularisation of the ingrown tissue [12, 13]. However, this optimum depends on the size of the bone substitute: larger pieces require larger pores [11].

The restricted size range can allow optimum vascularity development. The space between the larger particles is more readily filled up within a wide size range of particles, thereby obstructing the tissue repair. A highly porous, appropriate, interconnected pore structure generally favours tissue regeneration [14]. Pores, and pockets which resemble pores, have an effect beyond that produced by growth factors and other signalling molecules. The protective space in excavations or inside pores is essential to stimulate differentiation of precursors to osteoblasts. As composite scaffold materials combine advantageous properties of two or more types of materials, they can meet more efficiently the mechanical and physiological demands of the host tissue. The degree of bioactivity is controlled by the volume fraction, shape and size of the composites, and the arrangement of inclusions [15, 16]. Increased volume fraction and higher surface-area-to-volume ratio of inclusions can exhibit higher bioactivity. Therefore, in some applications, the incorporation of fibres instead of particles is favoured [17].

The material's surface has the most critical influence on the bioactivity of materials because the response of the host organism in macroscopic, cellular and protein levels to biomaterials is closely associated with the materials' surface properties [18]. The surface properties are determined by chemical constitution, surface charge and hydrophilicity/hydrophobicity. One way of obtaining an ideal surface property is via surface modification to alter the interactions between the biomaterial, proteins and cells.

Various factors affecting bioactivity of biomaterials are discussed in more detail in the following section on the design of bioactive materials.

2.3 Design of bioactive materials

The design of a bioactive material is based on the principle of biological recognition, where the growth factors and other bioactive molecules are the forceful regulators to determine cell behaviour. The biomaterial acts as a carrier to control doses and kinetics of release of those molecules and serves as a temporary substrate and three-dimensional matrix for cellular infiltration, in which cells can grow and become specific tissue types after the completion of the degradation of the carrier material. A major strategy in the design of novel bioactive biomaterials is to ensure biocompatibility and functioning in the biological system.

2.3.1 Design of bioactive materials for drug delivery

During the second half of the 20th century, extensive research work has been carried out by scientists to develop materials for controlled drug delivery. It is well known that the problems frequently facing many drugs are poor solubility, low bioavailability, short *in vivo* stability (half life), strong side effect on targeted delivery and insufficient *in vitro* stability. An ideal drug delivery system should deliver a drug to a specific site in a specific time and release profile. Incorporation of a drug into a particulate carrier can protect it against degradation *in vitro* and *in vivo*. Via a well-designed carrier, the release profile can be controlled and targeted release is possible. To achieve this, it is critical that the drug carrier needs to have a well-controlled biodegradation rate or releasing network to ensure the drug reaches its intended destination, securing the release of high local concentrations of the drug at the target [19]. Investigations in the past few years indicate that gene expression profiles, drug resistance and signalling pathways can be altered when bioactive agents are coupled with biomaterials. More recently, new ideas are being articulated about the use of nucleic acid-based materials for drug delivery. Proteins and nucleic acids are well-defined diverse building blocks for new bioactive materials, which can be new sources of biomaterials for drug delivery. In this chapter, some main designs of drug delivery systems are described.

Hydrogel system

Polymeric hydrogels have long been of interest in biomedical applications because of their excellent tissue compatibility, easy manipulation and solute permeability in general [20, 21]. These features of hydrogels are attractive

for local, sustained delivery of proteins and other biological pharmaceuticals, when the delivery system can be formulated and administered under mild conditions. Considerable interest has focused on hydrogels that exhibit a phase transition in response to environmental stimuli, such as solvents, temperature [8, 9], pH, ionic concentration [10], electric field [11], and light irradiation [22].

Numerous investigations have been reported on the volume phase transition of different hydrogel systems, in response to different environmental stimuli [23], and hydrogels with these properties have potential application in controlled drug delivery [24]. It is reported that a poly (acrylamide-co-acrylic acid) hydrogel system can be used to control the delivery of drugs or other active molecules because of its ability to undergo reversible optical transition and swelling behaviour, driven by the formation and dissociation of hydrogen bonds with changing temperature. Because the formation and dissociation of hydrogen bonds in the limited domain of the hydrogel are related to the network structure, the hydrogel can swell or collapse with changing temperature. Both optical transition and volume swelling are reversible, responding to temperature stimuli. This special property can be designed as a controlled release system for drugs or other active molecules [22].

In order to control their release, drugs are encapsulated into systems, which are expected to provide a certain site with a predetermined amount of drug over a well-defined period of time. A multi-component drug delivery hydrogel matrix, consisting of 2-hydroxyethyl methacrylate crosslinked by ethylene glycol dimethacrylate, in which drug-loaded biodegradable microcarriers are dispersed, has been designed as an implantable device, with long-term activity, as required by contraceptive and hormone replacement treatments. The microcarriers were biodegradable poly-e-caprolactone (PCL) microspheres in which active molecules, such as levonorgestrel (LNG), were encapsulated. Because of its composite structure, the developed device has the ability to combine several release mechanisms, leading to drug release obeying zero-order kinetics for most of the time. This ability of the hydrogel can be further developed by performing the copolymerisation of 2-hydroxyethyl methacrylate (HEMA) with a hydrophobic co-monomer, such as methyl methacrylate. As the hydrogel synthesis does not use organic solvents or high reaction temperatures, it is possible to encapsulate various drugs, including those which are temperature- and organic solvent-sensitive.

Currently, most therapeutic proteins are administered systemically by frequent injections, because of their instability and very short half-life *in vivo*. The effectiveness of therapeutic protein is strongly affected by the level of protein concentration in the bloodstream. Ideally, the concentration of the therapeutic protein should be maintained continuously within the

therapeutic range for a prolonged time period to ensure the optimal therapeutic effects, without toxicity and unfavourable side effects. A controlled delivery system has been designed to form a polymer matrix in situ from an injected aqueous polymer solution and the hydrogel produced is used as storage for sustained release of the incorporated therapeutic substance, thus avoiding an invasive surgical placement. A new poly (ethylene glycol) (PEG)-based copolymer, containing multiple thiol(–SH) groups along the polymer backbone, can be utilised as the drug carrier. This copolymer is capable of forming a polymer hydrogel from aqueous solution in the presence of protein drugs. A subcutaneously injected polymer hydrogel reservoir can be formed in situ and can deliver protein drugs for a prolonged time period, ranging from days to several weeks. Another functionalised, injectable hydrogel for controlled insulin release can be designed, using poly(β-amino ester) (PAE) as a duo-functional group to synthesise a novel sensitive hydrogel for controlled drug/protein delivery. PAE is used as a pH-sensitive moiety to conjugate to the temperature-sensitive biodegradable triblock copolymer to prepare a pH/temperature-sensitive, injectable hydrogel of pentablock copolymer PAE–PCL–PEG–PCL–PAE. As a result, the delivery of drug/protein from this hydrogel device can be controlled by the degradation of copolymer. This system has the following advantages: direct injection without any surgical procedure, straightforward drug loading to the polymer solution, no clogging during injection, simple dose adjustment, dry powder form, easy to dissolve, easy sterilisation by ultraviolet (UV) light, system biocompatibility with no inflammatory reaction, as well as no requirement for the use of organic solvents during fabrication [25].

Recently, a new design idea for sustained release drug has been suggested to encapsulate PEGylated drug into the thermogelling material. The attachment of a PEG chain to a protein, a liposome, or an organic drug, so-called PEGylation, has so far been used to prolong the circulating time of many drugs in the body. PEGylation can lead to a stealthy liposome. This stealthy property is beneficial for alleviating protease degradation and immunogenicity. The biodegradable thermogelling poly (lactic acid-co-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-co-glycolic acid) (PLGA–PEG–PLGA) copolymers as a sustained drug carrier have been used to control the release of MPEG (monomethoxy-poly(ethylene glycol))–CPT (camptothecin), as a model of a PEGylated drug. The thermosensitive physical gelling of PLGA–PEG–PLGA aqueous solution could be extended as a good injectable biomaterial and a long-term sustained release carrier of a PEGylated drug [26]. A similar design for the delivery of water-insoluble drugs is PEGylated nanographene oxide (NGO). Various potent, hydrophobic molecules (many of them aromatic) used clinically are often less effective due to their poor water solubility. Attaching

various types of insoluble, aromatic drug molecules onto NGO–PEG via simple adsorption has been a smart strategy for many hydrophobic drugs to achieve an improvement in their aqueous solubility. It has been verified that the NGO–PEG–SN38 (a water-insoluble aromatic molecule) complex exhibits excellent aqueous solubility and retains the high potency of free SN38. Other drugs that are successfully loaded onto NGO–PEG by simple adsorption include different camptothecin analogues and Iressa(geftinib), a potent epidermal growth factor receptor (EGFR) inhibitor. Graphitic nanocarriers, including nanographene sheets and carbon nanotubes, provide strong non-covalent binding with aromatic drugs via simple adsorption [27]. Thus, the biocompatible nanographene sheets become novel promising materials for biological applications.

Presently, natural cyclodextrins (CDs) and their derivatives with supramolecular structures have been designed as drug delivery systems to enhance solubilisation, stabilisation and adsorption because of the unique capability of forming inclusion complexes in the inner cavities, together with other favourable physicochemical and biological properties. Numerous newly developed drug delivery systems based on CDs have been used for the delivery of nasal drugs peptides and proteins, ophthalmic drugs, and in many other areas [28]. Research on inclusion complexes formed between CDs and polymers led to the interesting development of supramolecular hydrogels as potential biomaterials for controlled drug delivery. CD-containing cationic polymers and cationic polyrotaxanes have been used for gene delivery. Supramolecular hydrogels based on the self-assembly of the inclusion complexes formed between CDs and biodegradable block copolymers could be used as injectable drug delivery systems for sustained, controlled release of macromolecular drugs. Biodegradable polyrotaxanes, with drug-conjugated CDs threaded on a polymer chain with degradable end-caps, are interesting supramolecular prodrugs for controlled and targeted delivery. CD-containing cationic polymers as gene carriers revealed lower cytotoxicity than the non-CD-containing polymer counterparts. More importantly, the polyplexes of CD-containing cationic polymers with DNA could be PEGylated through a supramolecular process, using inclusion complexation between the CD moieties and a modified polyethylene oxide (PEO). Finally, new cationic polyrotaxanes, composed of multiple oligoethylenimine-grafted CDs threaded and end-capped on a block copolymer chain, were designed and synthesised as a novel type of polymeric gene delivery vector [20].

System of functionalised polymers

Hydrophilic and hydrophobic drugs are designed to treat different diseases, and these drugs need to be selectively absorbed by different material systems

according to their solubilities. In addition, the release of drugs from biomaterials should be performed in a sustained controlled manner to enhance the therapeutic effect and prevent deleterious side-effects [29].

Some novel functionalised polymers have been designed for the development of nanoparticle drug delivery systems. A polymer backbone consisting of two ester-linked, non-toxic, biological monomers, glycerol and adipic acid, was prepared and then further functionalised. These functionalised polymers are able to self-assemble into well-defined small particles of relatively small size and high homogeneity, with a very low toxicity. They have the ability to entrap a water-soluble drug, dexamethasone phosphate, with a high efficiency. The drug loading varies with the polymer specification. These novel functionalised polymers could be developed into useful nanoparticulate drug delivery systems [30].

A new protease-responsive matrix, which contains a protease-sensitive motif and two weak β-sheet forming peptides sequences, could be designed for controlled delivery of drugs in response to proteolytic degradation. Anticancer drugs, for example, have been linked to polymer backbones through protease-specific peptide spacers, enabling selective drug release at tumour sites with high protease activity. Because the degradation of the matrices proceeds via dose-dependent enzymatic cleavage and dissolution, the degradation pattern can be adjusted by varying the ratios of different components of the gels, and thus controlling the rate of matrix degradation. This approach could be used to produce controlled or enhanced release preparations, especially in the preparation of hybrid materials for drug delivery. These types of biomaterial will respond to physiological stimuli and have the ability to release an adaptable dosage, which could have far-reaching applications for targeted and functional drug delivery [31].

Most of the current drug delivery systems can only load a single drug, since each drug has different properties, such as solubility and hydrophilicity. However, in practice, two dissimilar drugs might need to be released in different stages, in a controlled manner, to enhance the therapeutic effect and prevent deleterious side-effects. In this situation, an ideal dual-drug release system will be expected to release the first drug in a low-pH environment, followed by the release of the second drug in a neutral pH environment. For this purpose, the mesoporous bioactive glass/PBLG-g-PEG [poly(γ-benzyl-L-glutamate)-poly(ethylene glycol) graft copolymer] nanomicelle composites can be selected as a dual-drug delivery system. A pH-controlled release of individual drugs was achieved by the predominant release of water-soluble drug from mesoporous bioactive glass in an acid environment and fast release of fat-soluble drug in an alkaline environment from polypeptide nanomicelles. This type of mesoporous bioactive glass/polypeptide graft copolymer nanomicelle composite can be designed as a

dual-drug delivery system, and the individual drug release can be controlled by the pH of the surrounding environment [29].

There are other designs based on functionalised carriers for sustained and controlled drug release systems. For example, hydrogen-bonding layer-by-layer (LbL)-assembled biodegradable polymeric micelles can be utilised as nanometre-sized vehicles for delivery of hydrophobic, neutral therapeutics under physiological conditions [32]. Polymersomes are novel and valuable tools for both disease therapy and diagnosis. Co-encapsulation of two drug molecules in the same polymersome enables combination therapies and eliminates the need to administer individually two separate drug formulations. Moreover, polymersomes may not only become more effective in treating recurrent, resistant, or residual tumours, but may also be more convenient for patient administration and treatment tolerance. It is also possible to make separate polymersome formulations, each with different drugs or with different dosing that deliver drugs in a sequence to treat a particular disease [33].

System of encapsulation

Nanosized carriers are prime candidates for the release of hydrophobic and/or highly toxic therapeutic agents. These delivery vehicles have the potential to augment the pharmacodynamic and pharmacokinetic profiles of drug molecules, thereby enhancing the therapeutic efficacy of the pharmaceutical agents. In addition, encapsulating the drug molecule in this delivery system can increase *in vivo* stability, extend its half-life time in blood and provide a means for controlling the release of the agent [1]. Moreover, the delivery system can alter the biodistribution of the drug molecule, enabling the agent to accumulate at the tumour site either passively or actively.

For example, one effective design approach is the encapsulation of peptides and proteins using lipid- or polymer-based carriers. The delivery systems include polymeric nanoparticles (NPs), microspheres, micelles and liposomes [34]. It has been found that delivery systems with mean diameters in the range of hundreds of nanometres have a greater ability to penetrate the epithelia, when compared to particles in the micrometre size range.

In recent years, chitosan (CS) has been investigated extensively as a carrier for mucosal drug delivery, owing to its muco-adhesive property, as well as an absorption enhancer, owing to its function of increasing the transport of molecules across mucosal barriers [34]. Chitosan nanoparticles (CS NPs), with a controlled size (below 100 nm) and narrow size distribution, were prepared through the process of ionic gelation between CS and sodium tripolyphosphate (TPP). In one study, the model protein, bovine serum albumin, was encapsulated into the NPs. The results show that the CS NPs can provide sustained release of this protein in simulated

intestinal fluid (pH 7.5) over a six-day period. These small, uniformly-sized particles are suitable as a delivery system for the mucosal delivery of vaccines, peptides and proteins [35].

The ability to encapsulate and release drugs in response to an acidic environment is an interesting area, as many tumour sites present as acidic. Several pH-sensitive polymer-based drug encapsulated nano- or microspheres have been designed for effective targeting of cancer- or antigen-presenting cells (APCs). Drug delivery systems constructed by linking drugs through a pH-cleavable bond, such as hydrazone or acetal, to amphiphilic polymers to produce pH-responsive micelles or dendrimers, have been utilised for oncology indications. Since the pH in a tumour site is more acidic (pH 5.5–6.5) than in normal tissues, pH-responsive drug-encapsulated nanospheres can be used to enhance the efficacy of current cancer therapeutics by reaching close to the cancer cells or getting inside these cells [36]. Nanospheres made of poly(ortho esters) or their derivatives are typical examples of pH-mediated drug release in an acidic environment, in which the ortho ester part of the polymer backbone acts as an acid-labile functional group for degradation [37].

2.3.2 Design of bioactive materials for tissue engineering

Tissue engineering is an emerging therapy that has the potential to treat a wide variety of body defects. Commonly, tissue engineering employs three fundamental 'tools', namely cells, scaffolds and growth factors [38, 39], to reproduce natural processes of tissue regeneration and development. High porosity and pore interconnectivity are key requisites for increasing the specific surface area available for cell attachment and tissue in-growth, thereby facilitating the uniform distribution of cells and the adequate transport of nutrients and cellular waste products [40]. The scaffold primarily acts as a local regulator to control doses and kinetics of released growth factor, thus increasing their potential retention time at therapeutic concentration levels [2]. In addition, the biomaterial scaffolds have to provide biological signals to guide and direct cell function through a combination of matricellular cues exposition and growth factors sequestration and delivery. There are general criteria for an ideal scaffold that will stimulate the body's repair mechanisms to regenerate diseased or damaged bone to its original healthy state. These include: a pore network large and open enough for cells and blood vessels to penetrate; and the ability to bond to bone [41, 42]. Therefore, the selection of the most appropriate material to produce a scaffold is very important in the construction of a tissue-engineered product, since it determines the success of the tissue–implant interaction.

Bioactive bioceramics-based scaffolds for bone tissue replacement

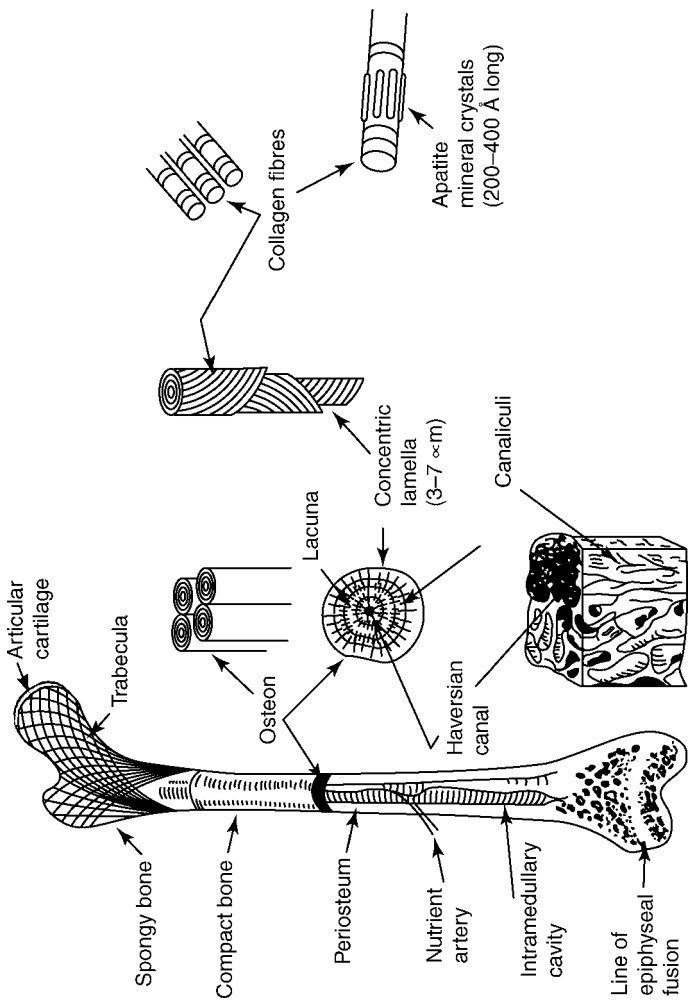
An ideal scaffold designed for using in bone tissue engineering needs to meet the following requirements: it must act as a template for bone growth; it must promote osteogenesis, inducing cell adhesion, proliferation and producing new materials for tissue replacement; it must form a strong bond between the tissue and the implant after implantation; and it must possess mechanical properties matching those of natural bone. It should be possible to tailor the scaffold to match the shape of the bone defects. In general, two levels of composite structure are taken into consideration when developing bone substitutes (shown in Fig. 2.1).

The properties of materials play an important role in determining the performance of scaffolds. Hydroxyapatite (Hap), tricalcium phosphate (TCP) and bioactive glass and glass ceramics (BG) used in the medical field, which are usually grouped together and termed 'bioceramics' [2], have been widely studied and recognised as the most promising materials for scaffolds devoted to bone regeneration, owing to their osteoconductive properties, ability to integrate with the bone tissue and absence of immune response.

Hap has been extensively used for hard-tissue repair, because it possesses excellent biocompatibility and osteoconductivity, resembling bone apatite, and as it is a member of the calcium phosphate family that forms part of the bioactive bioceramics group [3]. It has been used clinically in the form of powder, porous structure, or dense body. The most successful use of Hap is as a bioactive coating in total hip prostheses. Calcium phosphate (CaP) salts, such as β-tricalcium phosphate (β-TCP) or β-calcium pyrophosphate (β-CPP), can act as Hap precursors and have usually been adopted as fillers for small bone cavities in orthopaedics and dentistry. It has also been used for bone repair in the form of ceramic blocks, granules, or calcium phosphate cements. Bioglass® and apatite–wollastonite (A–W) glass–ceramic have been successfully used for tissue replacement, owing to their ability of bonding to both hard and soft tissues.

However, compared with natural bone tissue, these bioactive materials, exhibit lower mechanical properties. In particular, the low fracture toughness limits their application as scaffold structures in load-bearing situations. Therefore, one of the most important challenges is the design and fabrication of high-load-bearing scaffolds, capable of maintaining the applied loads for the required time, without showing symptoms of fatigue or failure [43].

For the development of new bone-tissue-engineering materials, many novel design strategies of scaffolds have been studied and developed. It is reported that a novel porous inorganic scaffold, created using bioactive glass–ceramic powders as a matrix and polyethylene particles as thermally removable pore formers, is expected to be used as a bone tissue replacement. The composition of the glass–ceramic was designed to induce the nucleation



2.1 Structural organisation in a human long bone.

of highly biocompatible and bioactive fluoroapatite crystals in the final scaffolds. The three-dimensional network with interconnected macropores ($> 100\,\mu m$) can promote *in vivo* blood vessel access, and cell adhesion, proliferation and migration. Moreover, the fluoroapatite-containing glass-ceramic scaffolds showed a mechanically orthotropic behaviour and a compressive strength, and, therefore, can be proposed as effective candidates for load-bearing applications in orthopaedics and for bone tissue replacement [42].

Concurrently, some studies have reported silica as an essential element for bone development and formation. Silica-based bioactive glass plays an important role in the surface bioactivity by the exchange of ions at the glass–tissue interface, which results in the formation of a carbonated Hap layer, similar to the mineral phase of bone. A silica-based bioactive ceramic with dual advantages of both Si-O and Hap, composed of a porous Hap ceramic coated with calcium silicate, Hap and tricalcium phosphate (HASi), has been developed as a scaffold for cell-based tissue-engineering applications. Hap endowed with a silica-containing coating encouraged cell proliferation and osteogenic differentiation of human bone marrow-derived stromal cells. This newly developed indigenous material is proposed as a suitable candidate material for cell-based tissue-engineering applications. It is found that controlled release of biologically active Ca and Si ions from bioactive glasses leads to the up-regulation and activation of seven families of genes in osteogenic cells [44] that give rise to rapid bone regeneration. The bioactive response appears to be under genetic control and the genes encode transcription of numerous proteins that control the cell cycle, proliferation and ultimately the differentiation of the cells towards the mature osteoblastic phenotype. This discovery can be used to design a new generation of bioactive materials, especially for tissue engineering and in situ regeneration of tissues. Recent findings also indicate that controlled release of lower concentrations of ionic dissolution products from bioactive glasses can be used to induce angiogenesis and thereby offer potential for the design of gene-activating glasses for soft tissue regeneration [45].

Design of polymer-based bioactive materials

The highly complex degree of intrinsic properties of the natural tissues imposes the need to define appropriate strategies for the design of three-dimensional scaffolds with tailored properties, adapted to be effective in tissue-engineering applications [46]. One of the key issues for the design of bioactive materials used in tissue engineering is the employment of biomaterials that integrate biodegradable scaffolds with growth factor delivery devices, to better guide cellular activities and enhance tissue neogenesis [47], while possessing higher strength and stiffness than their

polymer counterparts at the initial stages of cell seeding and subsequent tissue growth [2]. Therefore, the development of polymeric scaffolds with a high degree of porosity but, simultaneously, with good control over the pore size and morphology, is crucial for bone regeneration, dental repair and orthopaedic fixation devices, as well as many other biomedical applications [48].

A number of polymers are being studied for the purpose of developing tissue-engineering scaffolds. These include synthetic polymers, such as polycaprolactone, poly(lactic-co-glycolic acid), poly(ethylene glycol), poly (vinyl alcohol), polyurethane [49] and polysulfone, or their copolymers with additions of inorganic particles or fibres (mainly bioactive glass or Hap) [50, 51]. Each of these presents different biological and mechanical properties, allowing a choice of the correct polymer for the appropriate application [52]. Among them, biodegradable polymers and their copolymers are widely used for many biomedical applications.

One idea of bone tissue engineering is to create a bioactive scaffold that provides a local release of osteogenic factors to influence the healing bone [53]. Degradable polymeric scaffolds are the optimal choice, because drugs or bone-influencing proteins can be either covalently bound within the polymer and released as it degrades, or 'trapped' between polymer chains, giving an initial release of osteogenic factors on implantation. Encapsulated drugs that are released on degradation of a scaffold that forms a physical barrier to diffusion are also utilised.

Recent approaches to integrating fundamental concepts of drug delivery for enhancing the ability to trigger biological signals favourable for complex tissue morphogenesis [54] have been pursued to design scaffolds for bone tissue engineering. These include direct interspersion of growth factors within the scaffold or their encapsulation in micro-depots interspersed in the scaffold structure [55]. A bottom-up approach can be utilised in the formation of multifunctional polymer scaffolds with predefined pore size and interconnection, incorporated with protein-loaded polymeric micro-carriers, acting as local chrono-programmed point source generation of bioactive signals. Bioactive scaffolds are created by the thermal assembly of protein activated poly (ε-caprolactone) (PCL) microspheres. These matrices offer the possibility to modulate concurrently and control the size and extension of the porosity, mechanical properties and the spatial–temporal distribution of multiple bioactive signals [47].

Injectable bioactive materials are another attractive design for treating irregularly shaped defects with minimum tissue dissection [56]. Degradable ceramic particulates can be added to degradable polymers for the improvement of their compressive strength. Some degradable macromers, such as poly(anhydride), and poly(propylene fumarate), have been used as injectable scaffolds for tissue regeneration [57], but their degradation profile

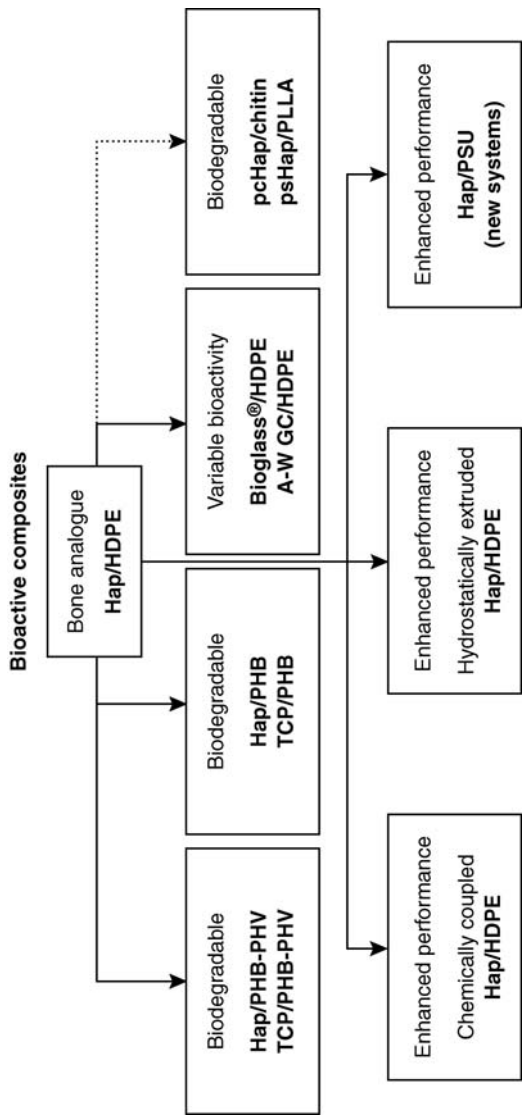
cannot be tailored to a particular application. To meet clinical requirements, unsaturated crosslinkable lactide-co-glycolide macromers (cMLGAs) can be considered for designing the fabrication of in situ hardening scaffolds for tissue regeneration. Low molecular weight lactide-co-glycolide chains are functionalised with fumarate unit and covalently attached with the integrin-binding RGD amino acid sequence for obtaining higher expression of osteogenic markers, such as osteopontin, collagen Iα and osteonectin. In the result, cMLGA exhibits linear mass loss with incubation time *in vitro* (constant degradation rate) and becomes attractive as an in situ cross-linkable macromer for fabrication of biologically functional scaffolds, with degradation characteristics that can be tailored to a particular application.

The development of prototype scaffolds for either direct implantation or tissue-engineering purposes and featuring spatiotemporal control of growth factor-release is a highly desirable design strategy. Biomedical prototype silk fibroin (SF) scaffolds with embedded poly-(lactide-co-glycolide) microparticles (PLGA MPs) loaded with an osteoinductive growth factor can be produced as an ideal drug delivery system by combining insulin-like growth factor I (IGF-I) to stimulate the growth and proliferation of osteoblasts and the synthesis of new bone matrix, during the initial stages post implantation [58]. Embedment of PLGA MPs in SF scaffolds led to more sustained release rates compared with free PLGA MPs. Such a construction is expected to be favourable for tissue-engineering applications, with the potential to mimic physiological patterns more precisely, based on opportunities arising from temporal control of growth factor delivery, and from spatial control of growth factor deposition within the scaffolds. Certainly, the developed scaffold prototype offers promise for promoting tissue regeneration [59].

Design of bioactive composite materials

Beginning with Bonfield's pioneering work of using Hap as the bioactive and reinforcing phase in high-density polyethylene to produce a bone analogue, many bioactive composite systems, consisting of bioceramics and biomedical polymers, have been investigated and designed as scaffolds for medical purposes. Some systems are shown in Fig. 2.2.

Numerous materials have been used for the preparation of scaffolds, from metals to ceramics and polymers. Both bioactive metal matrix composites and bioactive ceramic matrix composites have their attractions and disadvantages as tissue replacement materials. When utilised in bone tissue engineering, metal matrices can provide the necessary strength and toughness and bioactive ceramics are most likely to be the matrices, and the incorporation of a glassy material or metal fibres leads to the toughening of the ceramics. A number of polymers have also been studied for the



2.2 Development of bioactive composites for medical applications.

purpose of developing tissue-engineering scaffolds. These include synthetic polymers, such as polycaprolactone, poly(lactic-co-glycolicacid), poly (ethylene glycol), poly(vinyl alcohol) and polyurethane, and natural polymers, such as alginate, collagen, gelatin, chitin and CS [49]. Among them, natural polymers have found favour for use in scaffolding because of their non-toxic, mucoadhesive, biocompatible, and biodegradable properties [60]. However, they do not present inherent bioactivity. Some ceramics and glasses exhibit osteoconductive properties but with poor mechanical properties and a very slow degradability, which does not appear to be very attractive for tissue engineering [61]. Being composed of two or more types of material, composites have the advantage of overcoming the problems of brittleness of bulk bioceramics, while maintaining a bioactive response *in vivo*. Consequently, composites are proposed as promising bioactive materials that can be designed for tissue replacement and tissue regeneration purposes, through a combination of the best characteristics of each of the component materials, to meet better the mechanical and physiological demands of the host tissue.

The classification of biomedical composites can be based on the matrix materials or on the bioactivity of composites, in which at least one of the constituent materials should be bioactive, which may render the composite bioactive; in some cases, two or all of the constituent materials are bioactive. Using the matrix material as the basis for classification, there are three types of biomedical composites: polymer matrix composites, metal matrix composites and ceramic matrix composites [2].

Outwith the above basic methods, some new design ideas have been reported in recent studies. To overcome the lack of bioactivity of the polymers and the poor mechanical properties of the ceramics, the preparation of composite matrixes, containing Hap-based calcium phosphates (CaP) and Bioglass®, can be regarded as a promising approach [62]. A starch-based blend (SPLA50), impregnated with different concentrations of Bioglass® and processed by a supercritical assisted phase-inversion method, has been proposed for a wide range of bone-related therapy applications, such as tissue-engineering scaffolds [63] or bone cements. The natural origin, together with interesting mechanical properties and biocompatibility, supports the potential of starch-based materials in the biomedical field. A trace amount of silicon species has been reported to stimulate cellular activities in that the proliferation of human osteoblasts was enhanced in a culture medium containing silicon ions, prepared by dissolving Bioglass® in the medium. The excellent bone bonding ability of Bioglass® has been attributed to its ability to release silicon and the ability of hydroxycarbonate apatite formation in living body. Therefore, a biodegradable composite with silicon species releasability is expected to be designed as a scaffold material for bone tissue engineering, in which silicon species released from the

composites stimulate the cells to proliferate and differentiate, and then enhance the cellular functions [64]. Another composite scaffold containing nanosilica can be prepared using chitin hydrogel. Incorporation of silica into the chitin scaffold can produce a bioactive scaffold, which can be used for tissue-engineering applications. Organic modification of calcium silicate, that is an essential constituent of bioactive ceramics, is reported to provide a novel design of various bioactive organic–inorganic hybrids.

Some investigations have revealed that the bioactive filler containing bioactive glass (BAG) and bioactive glass ceramic (BGC) particles can serve as a reinforcing component to enhance the stiffness of polymer composites, because BAGs or BGCs have a much better performance in bone tissue engineering than Hap. Nano-sized particles have a large specific surface area compared with micron-sized bioactive ceramic particles and can form a tighter interface with the polymer matrix in composites. Thus, introduction of nano-sized BGC particles into polymeric materials cannot only endow polymer scaffolds with biomineralisation capability but also increase the stiffness of polymer material without greatly losing the mechanical strength [65, 66].

Studies by Changsheng Liu's group showed that increasing the specific surface area and pore volume of biomaterials for tissue repair may greatly accelerate the kinetic process of apatite deposition and, therefore, enhance the bone-forming bioactivity [67]. Incorporation of WS into PCL could improve the hydrophilicity of the composites, and mesoporous wollastonite (m-WS) could improve the hydrophilicity of the composites more effectively than conventional wollastonite (c-WS) at the same content. These findings suggest that the m-WS/PCL composite scaffold is promising in terms of its mesoporous structure and bioactivity, and has potential application for bone repair [68].

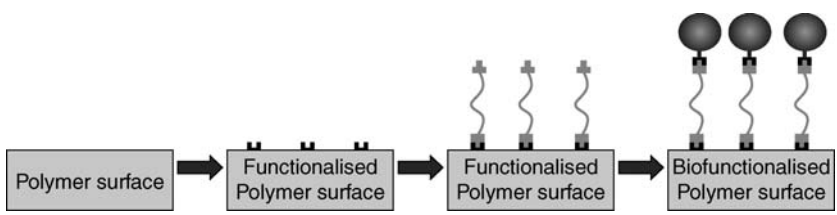
2.3.3 Design of bioactive materials for control of the material surface properties

It has long been recognised that the response of the host organism in macroscopic, cellular and protein levels to a biomaterial is, in most cases, closely associated with the material's surface properties, with the surface of the material having a critical influence on the biological response. Most biomaterials interact with moieties via ligand/receptor binding pathways and resist non-specific adhesion. In contrast, synthetic materials are generally thrombogenic and do not offer a favourable environment for the growth of fully functional tissues [69], but their drawbacks can be rectified or improved through surface modification. By incorporating recent advances in cellular biology into implant design, material surfaces that are bioactive have been

created to promote specific biological responses from host tissues [70]. Thus, the design of bioactive materials for control of the material surface properties is a promising way to construct biocompatible implants.

Generally, two main design strategies in surface modification of biomaterials are employed. The first one is that the material surface properties such as chemical composition, hydrophilicity/hydrophobicity, surface charge, and roughness, are modulated to a state that the adsorbed proteins can maintain their normal bioactivities. However, this method cannot induce specific cell behaviour, owing to the non-specific protein adsorption. The second strategy is to immobilise directly certain biomolecules on the biomaterial surface and, thereby, induce a specific cellular response [18]. Reactive groups such as -COOH and -NH_2 are usually introduced onto material surfaces as coupling sites for the covalent attachment of proteins.

In medical applications, surface modification focuses mainly on those materials with inert surfaces, in particular synthetic polymers and metals. Bioactivity can be induced on bioinert surfaces by introducing bioactive molecules that can promote cell adhesion, proliferation, viability and enhanced ECM-secretion functions, and then achieve active interaction with the surrounding biology. Covalent immobilisation of bioactive compounds onto functionalised polymer surfaces is an effective approach, as the end use of the biofunctionalised polymer varies with each application; Fig. 2.3 shows this concept of biological surface modification. A number of polymers have been selected as a matrix for biomolecule immobilisation, intended for a variety of medical applications (Table 2.2). In order to improve the adhesion and retention of cells to these polymer scaffolds, most polymeric materials must be functionalised before bioactive peptides or proteins are immobilised on their surfaces. A variety of means can be used for this purpose, which include synthesising a polymer that has active functional groups present on the surface, with subsequent surface modification by plasma treatment, ozone oxidation [71], surface graft polymerisation and site-specific reactions. Ideally, such treatments should be targeted solely at the surface layers so as not to compromise the bulk properties of the material, which may already have been optimised for a specific application [72].

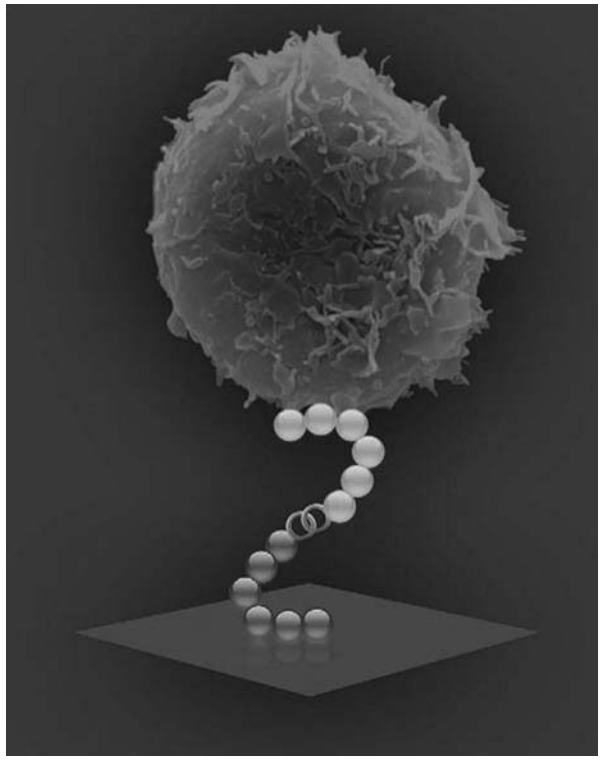


2.3 Concept of biological surface modification.

Table 2.2 Polymer substrates in bioconjugation

Polymer	Abbreviation	Application
Poly(styrene)	PS	Bioanalytical assays, antimicrobial surfaces, tissue culture, haemocompatible materials
Poly(tetrafluoroethylene)	PTFE	Biosensors, haemocompatible materials, immobilised enzymes, MEMS
Poly(ethylene terephthalate)	PET	Biocompatible/haemocompatible materials, antimicrobial surfaces, textiles, tissue engineering
Poly(ethylene)	PE	Drug delivery, biomedical devices, biocompatible materials, active packaging
Poly(a-hydroxyacids)		Tissue engineering
Poly(lactic acid)	PLA	
Poly(glycolic acid)	PGA	
Poly(lactic-co-glycolic acid)	PLGA	
Poly(propylene)	PP	Antimicrobial surfaces, textiles, haemocompatible materials, active packaging
Poly(pyrrole)	PPY	Biosensors, MEMS
Poly(dimethyl siloxane)	PDMS	Biosensors
Poly(methyl methacrylate)	PMMA	Tissue engineering, microarrays, biosensors, immobilised enzymes, MEMS
Methacrylate copolymers	Various	Enzyme reactors, DNA hybridisation

Currently, peptide-based functional coatings, termed 'interfacial biomaterials' (IFBMs), have been developed to achieve material surfaces with desired biological activities. These peptides contain at least one material binding domain and at least one bioactive functionality (Fig. 2.4). Unique peptide sequences that bind specifically and with high affinity to various materials or biological targets were identified using a combinatorial phage display screening process. These affinity peptides were subsequently conjugated to a functional moiety with distinct biological activity, providing a biomimetic bridging macromolecule, which is able to promote specific interactions with cells and biomolecules at the surface of a material. This new concept of an IFBM can be spatially and geometrically confined to create patterns that resist washing and submersion in buffer, while retaining their biofunctionality, which allows for a range of biological patterning applications [73]. Also, the combination of gene therapy and tissue engineering is a promising approach, which may sustain release of functional plasmid DNA from the surfaces of materials that support cell adhesion for tissue formation, and has been applied in bone, cartilage, wound, urogenital and nerve tissue regeneration [74, 75, 76]. Lu *et al.* [77] fabricated layer-by-layer (LBL) assembled multilayer film from a degrad-



2.4 Schematic diagram of an IFBM at the interface between the biological and material realms.

able cationic poly(2-aminoethyl propylene phosphate) and plasmid DNA that performed long-term release of plasmid DNA, with gradual degradation, under physiological conditions. It has been demonstrated that such multilayer films facilitated osteoblast cell adhesion, with enhanced cellular alkaline phosphatase activity and calcium accumulation, and prolonged gene expression, which provides a model for further biomaterial surface modification and tissue-engineering application, with long-term localised gene delivery.

In addition, LBL polyelectrolyte multilayer (PEM) films have attracted special interest for biomaterial surface modification, since proteins and bioactive moieties can be non-covalently immobilised on material surfaces and then effectively control cellular function. Selection of polycationic and polyanionic components and deposition conditions can be used to control the interfacial properties. This technique makes it possible for bioactive macromolecules, such as proteins and polysaccharides, to be incorporated into PEM films via weakly interacting hydrogen bond acceptors/donors with polymers of complementary polarity. PEM deposition is a versatile

system allowing nanoscale control over composition and molecular architecture and is not restricted with respect to the types, sizes and shapes of substrata [78].

It is well known that calcium phosphate (Ca-P) coatings show excellent osteoconductive ability and simultaneously offer an opportunity to incorporate protein molecules without compromising their activity, as they are generated under physiological conditions. Therefore, these Ca-P coatings can be used as a tool for the controlled release of biologically active molecules, such as bone growth factors (bone morphogenetic proteins (BMPs) and other osteoinductive proteins). With this methodology, bioactive proteins can be directly integrated into the structure of Ca-P coatings, maintaining their conformation close to their native form, and thus improving the functionality of the inorganic layer at the implant interface. This coating can be applied in a biodegradable polymer. Such a combination should also integrate within tissues, and progressively be degraded and fully replaced by bone material [79]. Also, the formation of an apatite layer onto the surface is important in the development of osteoconductive biomaterials in orthopaedic applications. It has been reported that silanol (Si–OH) groups on polymeric materials can induce apatite formation in acellular simulated body fluids (SBFs), with ion concentrations nearly identical to those of human blood plasma. Therefore, incorporation of Si–OH groups into polymeric substrates, such as CSMPs, can provide bone-bonding ability to the MPs, accelerating the tissue integration, which leads to the unique strength of such interfaces. Some study results on this subject have indicated that the incorporation of Si–OH groups and the water-uptake properties of CS, which allow it to absorb calcium ions from the calcium silicate solution, give it the ability to induce the formation of an apatite layer on their surfaces. This type of system is expected to be designed as an injectable biomaterial system that can also provide drug delivery [80]. Another similar study successfully developed smart biodegradable surfaces. These are prepared by grafting CS, a pH-sensitive biodegradable and natural polymer, to the surface of poly(L-lactic acid)/Bioglass® films, which respond to pH, and that could be used to control the biomineralisation process. This approach could be applied to the development of smart coatings of different minerals and produced in substrates with more complex geometries, using other methodologies (e.g. chemical activation of the substrate surface) instead of plasma for coupling CS to the surface [81].

At the present stage, a novel approach to the fabrication of porous scaffolds with surface-immobilised nano-hydroxyapatite (N-Hap) has been developed for effective bone tissue engineering. The discrete nano-level anchoring of N-Hap exposed on the pore surface of CS scaffolds expressed high specific surface area, excellent protein/cell binding ability and

osteoconductivity, and showed much improved surface properties for cell adhesion and growth. Nano-scale engineering may serve as a useful approach for the generation of bioactive cell-compatible/tissue-regenerative surfaces of many types of biomaterial, with a wide range of shape and dimensions [82].

Apart from polymers, metal-based alloys have been widely used as artificial implants, examples being orthopaedic and dental implants, mini-implants and metal plates. Among these, titanium and titanium alloys have found wide application as implant materials and bone plates. However, when these materials are used as bone implants they are bioinert and generally encapsulated by fibrous tissue, which isolates them from the surrounding tissue after implantation into a living body, and finally leads to clinical failure [77]. Therefore, many approaches have been developed to treat titanium and increase its surface bioactivity. Coating metals with polymer films is a valuable strategy to modify the properties of the metal surface because they can act as barrier films against corrosion and also possess bioactivity [83]. Hap, α- and β-tricalcium phosphate, tetracalcium phosphate, octacalcium phosphate, fluorapatite and amorphous calcium-phosphate phases have been studied and used as coatings on titanium and its alloys due to their excellent osteoconductive properties [84]. Biochemical methods of surface modification using proteins, such as collagen and growth factors, represent another clever design for improving the biocompatibility of titanium-based implants [85]. A new titanium-based biomaterial with covalently attached biologically active molecules (i.e. RGD-peptides, growth factors) able to improve osteoblasts responses has been developed. The strategy followed was based on a preliminary coating of the implant material by an adherent thin polymer film, such as pyrrole-3-acetic acid (Py-3-acetic) and polyacrylic acid, on which the presence of -COOH groups grown onto the implant devices surface could conveniently attach biologically active molecules, capable of promoting positive interface reactions (bioactivity) with the surrounding biological system. It is suggested that the presence of carboxylic groups on these polymer films, adherently grown on the surface of the implant device, could be conveniently exploited to graft more complex biomolecules, which could promote positive interface reactions with the surrounding biological system [83, 86].

Several studies have indicated that the oxide structure and porous morphology of the titanium (Ti) surface are responsible for apatite deposition. It was found that coating of crystalline Ti oxide structure with submicron-sized porous morphology onto Ti surfaces could promote the peri-implant bone healing response around endosseous Ti implants with micro-roughened surface at the early healing stage. It is suggested that a porous Ti oxide surface may be effective in increasing the osteoconductivity

of micro-roughened Ti implants, thereby enhancing early bone responses and shortening the healing period. A synergistic effect on osseointegration, related to biomechanical interlocking and the bioactive Ti oxide surface, can be expected when combined with micron-scale surface properties of implants.

By incorporating recent advances in cellular biology into implant design, a model system has been developed for altering the surface chemistry of nickel–titanium (NiTi) shape memory alloy by covalently attaching self-assembled peptide amphiphile (PA) nanofibres, presenting the biological peptide adhesion sequence Arg–Gly–Asp–Ser, on pretreated NiTi substrates using an intermediary aminosilane layer. The bound PA nanofibres are very important to the substrate, creating robust coatings and leading to a confluent cell monolayer. This strategy to create modified NiTi surfaces can be used to produce biomedical implants with enhanced capabilities to facilitate cell adhesion, proliferation and potentially a variety of implant-specific cellular responses.

2.4 Future trends

Designs of bioactive materials cover many aspects, for example surface modification, incorporation of bioactive molecules into substrates, formation of hydrogel system or encapsulation, and functionalisation of polymers. which endow the biomaterials with excellent biocompatibilities and gain much attention in biomedical applications. From the viewpoint of materials science, the present challenge in tissue engineering is to design and fabricate reproducible bioactive and bioresorbable three-dimensional scaffolds with optimal porosity and pore structure, which are able to maintain their structure and integrity for predictable times, even under load-bearing conditions.

The covalent immobilisation of bioactive compounds onto polymer surfaces has seen rapid growth in the past decade in the biomedical held; many procedures based on surface modification have been suggested to improve the biocompatibility and biofunctionality of inert biomaterials. However, a number of aspects still remain to be worked on and numerous challenges must still be addressed.

First, the activity of a bioactive compound often changes after immobilisation, most likely owing to differences between the bulk solvent and surface microenvironmental conditions. For example, surface charge may produce an effect on pH activity. Enzymes immobilised on polycationic surfaces have had their optimal pH shifted to a more acidic value [87], and those immobilised on polyanionic surfaces have shifted to a more alkaline value. The chemistries may be useful in controlling the microenvironment at

the polymer interface, in turn influencing the activity of the immobilised active compound [87, 88].

However, microenvironmental effects continue to be a challenge, and they serve as a reminder that conditions observed in the readily measurable bulk solution may not be representative of those occurring at the polymer surface. Additionally, functional groups immobilised on an inert polymer surface may be unstable and lose reactivity over time. The kinetics of surface restructuring [89] can be quantified by measurement of changes in contact angle and surface composition. Therefore, surface rearrangement can influence results of surface analyses that are not conducted promptly after functionalisation. It is important to control and quantify bioactivity of the polymer including bioactive compound conjugate, because surface charge and hydrophilicity are often changed during the process of functionalisation with a multi-step reaction. In this way, optimising the reaction conditions to improve bioactivity can benefit from understanding the extent to which a biomolecule has lost activity.

For the design of a drug delivery system, it is necessary to consider a number of factors that create barriers to ultimate clinical approval of the drug-loaded system. These factors include biocompatibility of the device, cytotoxicity, FDA approval, efficiency, inconvenience caused to patients and cost effectiveness. Only after these factors are examined can a drug delivery system be therapeutically acceptable [90]. Nanoparticles and nanoformulations provide massive advantages in terms of drug delivery and release, drug targeting and, in addition, possess the potential to combine diagnosis and therapy. They have still greater potential for many applications, such as anti-tumour therapy, AIDS therapy, gene therapy and radiotherapy, in the delivery of proteins, antibiotics, virostatics and vaccines, and as vesicles to pass the blood–brain barrier. However, there are disadvantages which have to be overcome. These include biodistribution of active compounds, stability in the biological environment, drug loading, targeting, transport, release and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remain a major problem, and improvements in biocompatibility are obviously a main concern of future research. Three avenues of new research are conceivable, which cover advancing the clinical utilisation of a new generations of delivery systems, understanding potential alterations in mechanisms of action of therapeutic agents when they are attached to or incorporated into biomaterials, and designing novel materials with improved properties.

Tissue engineering may be viewed as the next step, when the damage is too extensive: the current concept of tissue engineering lies in the belief that tissues can be regenerated *in vitro* and implanted. Skin tissue engineering has found its way into the clinical practice of restoring skin tissue. Methods to regenerate tissues of the musculoskeletal system, such as bone, teeth and

cartilage tissue, are still in the preclinical phase and far from being applied clinically on a large scale to humans. It should take from 5 years for cartilage, for which the technique has been mastered at the laboratory scale, to at least 10 years for bone, for which only few reports are available [52]. Success in engineering orthopaedic tissue depends on the optimised design of scaffolds that not only act as a carrier for growth factors at therapeutical levels during tissue regeneration processes, but also allow initial cellular infiltration and subsequent integration with native tissue [2]. Further advances in bioactive material design are required to achieve an optimised repair of tissue defects. Improvements should include prolonged release, protection of proteins from degradation and the targeting of certain cell types in the wound space. Furthermore, simultaneous delivery of multiple growth factors in a timed manner and at the right concentration might enhance the therapeutic effect, by targeting more than one step in the repair cascade [1].

More recently, ideas are being articulated about the use of nucleic acid-based materials for drug delivery because signalling pathways, gene expression profiles and drug resistance can be altered when bioactive agents are coupled with biomaterials [48]. Proteins and nucleic acids are well-defined yet diverse building blocks of new materials. It is anticipated that tissue-engineering scaffolds created by biodegradable polymer matrices with bioactive inorganic phases will play a vital role and perhaps they will be the 'scaffolds of choice' in combination with stem cell seeding [8]. Furthermore, it should be kept in mind that most of the studies on biomaterials were performed using animal cells (mostly young adult or even fotal), and not with cells from elderly osteoarthritis patients. Therefore, extensive research will be needed to determine if the results can be extended to the human situation and used in a clinical setting for treating human tissue defects [1].

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3

Bioactive materials and nanotechnology

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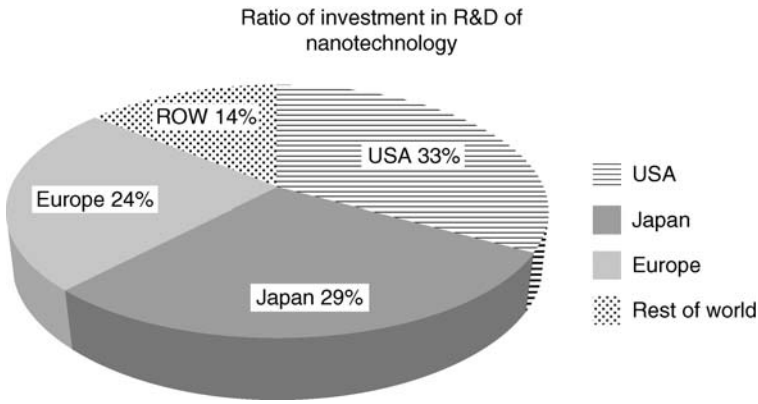
Abstract: Bioactive materials with nanoscale structure, including nanomedicine, nanodevices, nanomaterials, such as nanofibres and nanocomposites, can be designed and produced from natural biopolymers, synthetic polymers and inorganic substances. The applications include tissue engineering, wound dressings, immobilised enzymes, and controlled delivery of drugs (genes). Nanocomposites have become another prominent area of current research and development in nanotechnology. The nanocomposite-based scaffold allows for cell growth, yielding a unique composite system. Limitation remains with the difficulties of commercialisation, due to the scalability and the cost/benefits ratio. Nanofibre is still produced on a small scale. New development in the large-scale production of nanofibre and nanocomposites will have a future impact on the commercialisation of nanotechnology. The trend also includes the enhanced efforts to understand the interface between cells, tissues and the bioactive materials at the nanoscale level for improved material design and enhanced clinical performance.

Key words: nanotechnology, nanomedicine, nanomaterials.

3.1 Introduction

Nanotechnology is an attractive subject, receiving increasing public and private investment on a worldwide basis. The total global spend was estimated at US$6.25 billion in 2004 and this amount has risen annually. In the USA alone, funding in this field has been almost US$3.7 billion in the period 2005–2008. In Japan, between 2001 and 2003, US$800 million has been invested, while within Europe, a total of US$1.25 billion is currently spent annually on nanotechnology research and development, with a UK government allocation of about US$81.9 million per year from 2003 to 2009

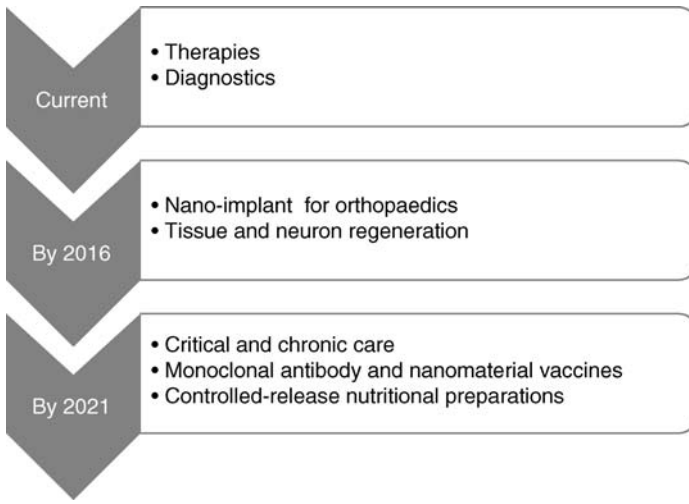


3.1 Research and development investment in nanotechnology by country.

[1]. In China, around RMB 8000 million has been invested by the National Science Foundation from 1999 to 2000 [2]. The current ratio of investment for each country in the total worldwide nanotechnology research and development is shown in Fig. 3.1 [3].

The first industrial commercialised nanomaterials were carbon black and fumed silica, which date back to the early 20th century. The first use of the concepts in 'nanotechnology' was in 'There's plenty of room at the bottom', a talk given by physicist Richard Feynman at an American Physical Society meeting at Caltech on 29 December 1959. The term 'nanotechnology' was defined by the Tokyo Science University Professor Norio Taniguchi in a 1974 paper [4] as follows: ''Nanotechnology' mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or by one molecule.' Nanotechnology and nanoscience were initiated in the early 1980s with two major developments: the birth of cluster science and the invention of the scanning tunnelling microscope (STM). The evolution of nanotechnology has led to nanotechnology being defined as the design, characterisation, production and the application of structures, devices and systems by controlling shapes and size at the nanometre scale.

Nanotechnologies have found wide application, including as structural materials, skin cosmetics, personal care products, in information and communication technology, biotechnology, instruments, sensors and environmental protection, such as water treatment. In this respect, the demand for nanomedicine and other nanotechnology-based medical products is very significant. According to a market report [5], US demand for nanomedicines, nanodiagnostics and nanotech-based medical supplies and devices, based on the science of using molecular-scale technology to diagnose, treat and prevent disease, will increase by over 17% per year to



3.2 Perspective on medical applications of nanotechnology.

US$53 billion in 2011 and will boost demand to more than US$110 billion in 2016 [5]. Applications will range from general therapeutic and diagnostic uses to critical and chronic care, such as cancer treatment and preventive medicine, in the near future (Fig. 3.2). Examples include: contrast agents incorporating nanoparticles for greatly improved imaging (e.g. nanomagnetic materials); bone replacement materials incorporating nanostructured materials, enabling improved integration in the body (e.g. hydroxyapatite (Hap) and bioactive glass); nanostructured biomaterials for use in scaffolds for regenerative medicine (e.g. nanofibres); wound dressings incorporating antibacterial nanoparticles (e.g. nanofibres with nanosilver); orthopaedic implants with nanocontoured surfaces to improve fixation in bone (e.g. metals with bioactive material surface coating).

3.2 Bioactive materials under nanoscale (nanomaterials)

The definition of nanomaterials is the study of how materials behave when their dimensions are reduced to the nanoscale. It can also refer to the materials themselves that are used in nanotechnology. Nanomaterials can also be defined as materials that have structured components, with at least one dimension less than 100 nm. Materials that have one dimension in the nanoscale are layers, such as thin films or surface coatings, or are called nanofilms. Materials that are nanoscale in two dimensions include nanowires and nanotubes. Materials that are nanoscale in three dimensions are particles, for example precipitates, colloids and quantum dots (tiny

particles of semiconductor materials). Nanocrystalline materials, made up of nanometre-sized grains, also fall into this category [6].

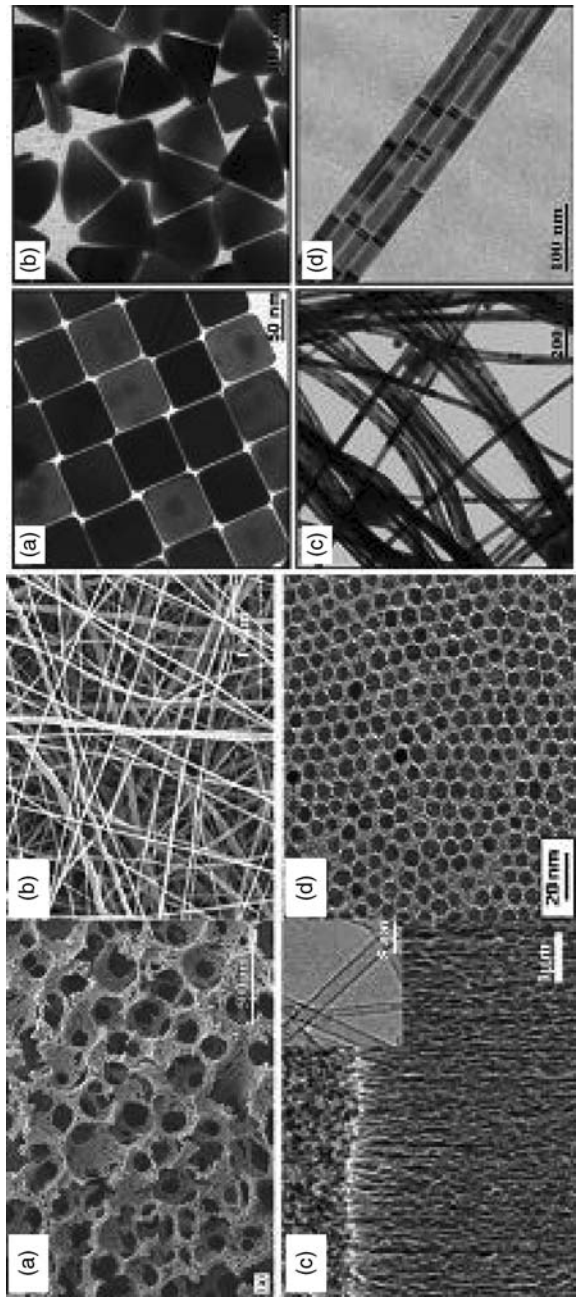
Bioactive nanomaterials have well-defined nanostructures in terms of the size of the material, the shape, the channels, pore structure and the surface domain (Fig. 3.3). They include nanoparticles, nanotubes, nanofibres, nanogels, nanofilms, and nanofoams.

Two principal factors cause the properties of nanomaterials to differ significantly from other materials: increased relative surface area and quantum effects. These factors can change or enhance properties, such as reactivity, strength and electrical characteristics. As a particle decreases in size, a greater proportion of atoms are found at the surface in comparison to those inside. For example, a particle of size 30 nm has 5% of its atoms on its surface, at 10 nm 20% of its atoms, and at 3 nm 50% of its atoms. Thus, nanoparticles have a much greater surface area per unit mass compared with larger particles. As growth and catalytic chemical reactions occur at surfaces, this means that a given mass of material in nanoparticulate form will be much more reactive than the same mass of material made up of larger particles [9]. In comparison to conventional materials, nanomaterials will typically have a defined surface morphology, which will have a significant influence on the interaction between the materials and the biological system. In addition, the presence of the nanoscale (nanopore, nano-pattern) on the bioactive materials creates a biomimetic feature towards most of the proteins at the nanoscale, which leads to a significantly enhanced response to further biological reactions, such as cell adhesion, proliferation and new tissue formation.

Miller *et al*. [10, 11] examined fibronectin interactions with nanomaterials having various nanoscale surface features under atomic force microscopy, and demonstrated for the first time how proteins respond differently to such nanoscale surface features. Specifically, fibronectin (5 mg/mL) adsorbed to poly(lactic-co-glycolic acid) (PLGA) surfaces with 500 nm spherical bumps demonstrated little or no interconnectivity; fibronectin (5 mg/mL) adsorbed to PLGA surfaces with 200 nm spherical bumps demonstrated a higher degree of interconnectivity; fibronectin (5 mg/mL) adsorbed to PLGA surfaces with 100 nm spherical bumps demonstrated well-spread fibronectin molecules, with the highest degree of interconnectivity, leading to a masking of the underlying PLGA nanometre surface features.

For example, nanostructure materials with sizes 1–100 nm can act as new and effective constituents of bone materials, because bone also consists of nanosized organic and mineral phases. Several studies have reported improved osseointegration on nanostructure surfaces created from a wide range of materials, including ceramics, metals, polymers and composites [9–13].

A surface with nanometre structure has been demonstrated to have the



3.3 A few examples of nanomaterials and nanodevices under nanoscale. Left: (a) Scanning electron microscopy (SEM) image of poly(L-lactic acid) (PLLA) nanofibrous scaffold with interconnected spherical macropores created by a phase-separation technique. (b) Electrospun polycaprolactone/hydroxyapatite/gelatine (PCL/Hap/gelatin, 1:1:2) nanofibres, which significantly improved osteoblast functions for bone tissue engineering applications. (c) Densely aligned single-wall carbon nanotube (SWCNT) forest grown with novel water-assisted chemical vapour deposition in 10 min. (d) Transmission electron microscopy (TEM) image of monodispersed magnetic Fe_3O_4 nanoparticles (6 nm) deposited from their hexane dispersion and dried at room temperature [7]. Right: TEM images of silver nanoparticles: (a) cubes; (b) triangles; (c) wires; (d) an alignment of wires (reproduced from reference [8] with permission from the American Chemical Society).

capability of promoting osteoblast proliferation and the formation of new bone. Most nanoscale bone implant materials, such as ceramics (alumina, titania and Hap) [9, 10], polymers (PLGA and polyurethane) with nanostructure [9], and metals (Ti, Ti6A14V and CoCrMo alloys) [11], have been shown to enhance bone cell response and functions, including cellular adhesion, proliferation, synthesis of alkaline phosphatase and calcium deposition [9, 11]. For example, Park *et al.* [14] prepared nanostructured PLGA by chemically etching PLGA in 1 N NaOH for 10 min. Results demonstrated that NaOH-treated PLGA three-dimensional scaffolds enhanced chondrocyte functions, such as adhesion, growth, differentiation and extracellular matrix synthesis, compared to non-treated, traditional PLGA scaffolds. Specifically, viable chondrocyte numbers, total intracellular protein content and the amount of extracellular matrix components, such as glycosaminoglycans and collagens, were significantly greater when chondrocytes were cultured on NaOH-treated, as opposed to non-treated PLGA scaffolds [14].

For nanosized bioactive particles, Gutwein and Webster [12] investigated the viability of osteoblasts *in vitro*, when cultured in the presence of nanoalumina and titania particles for 6 h, and demonstrated that ceramic nanoparticles were safer to osteoblasts than conventional, micron-sized, ceramics particles.

Recently, size-controlled nano-Hap particles were synthesised and their size effect on osteoblast-like cells evaluated. The results demonstrate that both cell proliferation and cell apoptosis are related to the size of the Hap particles. Np20 (Hap with 20 nm particle size) was the most effective at promoting cell growth and inhibiting cell apoptosis. This work provides an interesting view of the role of nano-Haps as ideal biomedical materials in future clinical applications [15].

Elastin, a structural protein distributed in the extracellular matrix of vascular tissues, is critical to maintaining the elastic stability and mechanical properties of blood vessels, as well as regulating cell-signalling pathways involved in the vascular injury response and morphogenesis. In the tissue-engineering field, Kothapalli and Ramamurthi [16] found copper nanoparticles can enhance the crosslinking of elastin matrices by adult vascular smooth muscle cells (SMC) for utilisation in tissue-engineering vascular replacement.

The incorporation of gold nanoparticles into polyurethane has been found to be capable of changing the morphology of the polymer surface, which could influence the migration of endothelial cells cultured on these materials [17].

For nano-shaped nanomaterials, such as nanofibre and nanotubes, study has shown that 60 nm diameter carbon nanofibres (CNFs) significantly increased osteoblast adhesion and concurrently decreased competitive cell

(fibroblast, smooth muscle cell) adhesion, in order to stimulate sufficient osseointegration [14]. Other research efforts have also demonstrated that carbon nanotubes (CNTs) are suitable for promoting osteoblast functions [18].

Biologically based materials, such as collagen, have found general acceptance for application as implant materials. Inspired by the phenomenon of hierarchical assemblies of nanofibrils in nature, several self-assembled and organised nano-Hap/collagen composites under different biomimetic conditions have been synthesised [19–22]. In the self-assembly process, collagen fibrils are formed by self-assembly of collagen triple helices. Hap crystals then grew on the surfaces of these fibrils, and finally mineralised collagen fibrils aligned parallel to each other to form mineralised collagen fibres. These nanocomposites exhibited high osteoconductivity and good bio-integrativity. Recently, a gradient collagen/nano-Hap composite scaffold, with a Ca-rich side and a Ca-depleted side, has been developed by a biomimetic diffusion–precipitate method. This method is able to precipitate nano-Hap crystallites in the interior of a collagen scaffold to form a compositional and structural gradient composite scaffold by careful control of the precipitation parameters, such as the concentration of ions and the porosity of the collagen matrix [23].

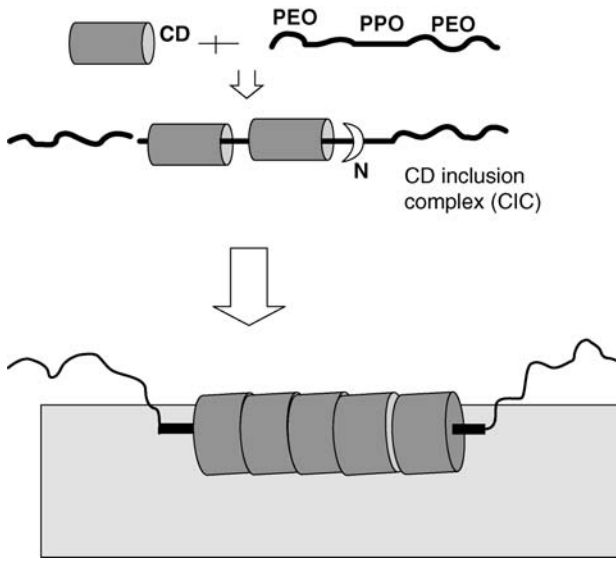
A chitosan–b-glycerophosphate formulation was combined with bioactive glass (BG) nanoparticles in order to prepare novel thermo-responsive hydrogels, exhibiting a bioactive character. The BG particles were spherical in shape, with nanometre-scale diameters, which guarantee the efficient injection of these systems through small-gauge needles into bone defects. The gelation points of the developed organic–inorganic injectable systems are adequate for intracorporal injection, in particular BG-40% and BG-50%, which have gelation points of 36.9 and 36.8°C, respectively. However, it was seen that the gelation temperature could decrease by increasing the deacetylation degree of the chitosan employed. After immersion in simulated body fluid (SBF), the materials containing BG nanoparticles induce the formation of bone-like apatite clusters that are well integrated in the hydrogel organic structure. The density of the apatite precipitates increased with increasing BG content and soaking time in SBF. Although the concept of bioactive and biodegradable thermo-responsive gels was introduced in this work, more research is required to validate fully the use of such systems in the bone regeneration context, possibly by combining them with cells and growth factors. These bioactive in situ gel-forming systems might also be incorporated into three-dimensional scaffolds, with the aim of approaching the bone's required natural biomechanical properties and positioning the cells inside the porous structure. It has been recommended that *in vitro* and *in vivo* experiments should be performed in the future to

corroborate the non-toxicological profile of the BG nanoparticles and evaluate their effect on the osteogenic differentiation of progenitor cells [24].

Carbon nanotubes are attractive as additives in fibre-reinforced composites, due to their high aspect ratio, strength and electrical conductivity. In one study, solubilised collagen type I was polymerised in the presence of dispersed single-walled carbon nanotubes (SWNT) and human dermal fibroblast cells (HDF) to produce collagen–SWNT composite biomaterials, with HDF embedded directly in the matrix. The resulting constructs, with SWNT loadings of 0 (control), 0.8, 2.0 and 4.0 wt% SWNT, were cultured and the electrical properties were evaluated in the frequency range 5–500 kHz at days 3 and 7. All collagen–SWNT hydrogel matrices underwent HDF-mediated gel compaction over time in culture, but the presence of SWNT significantly decreased the rate and extent of gel compaction. Viability of HDF in all constructs was consistently high and cell morphology was not affected by the presence of SWNT. However, cell number at day 7 in the culture decreased with increasing SWNT loading. Electrical conductivity of the constructs varied from 3 to 7 mS cm^{-1}, depending on SWNT loading level. Conductivity increased uniformly with increasing wt% of SWNT ($R = 0.78$) and showed a modest frequency dependence, suggesting that the electrical percolation threshold had not been reached in these materials. These data demonstrate that the electrical conductivity of cell-seeded collagen gels can be increased through the incorporation of carbon nanotubes. Protein–SWNT composite materials may have application as scaffolds for tissue engineering, as substrates to study electrical stimulation of cells, and as transducers or leads for biosensors [25].

Another intriguing new field of great promise is supramolecular chemistry, which is concerned with developing molecular assemblies for biological applications, based on macromolecular architectures that mimic nanoscale systems or mechanisms in nature. An excellent example of a supramolecular system of molecule interaction is the formation polyrotaxanes, which are polymers, comprising cyclic compounds that are threaded onto linear polymeric chains, capped with bulky end groups, for example biodegradable polyrotaxanes in which a-cyclodextrins (a-CDs) were threaded onto a PEG chain capped with amino acids. By bonding drugs to the a-CDs, a controlled release system can be created. The hydrolytic enzymes could first attack the peptide bonds of the macrostructure, degrading the terminal moieties and releasing the drug-immobilised a-CDs, and a second enzyme could then attack the a-CDs and release the drugs as nanomedicine [26].

A recent development by Zhao and Courtney [27, 28] is to use a biomimetic approach for surface modification of biomedical materials, such as plasticised poly (vinyl chloride) (PVC-P) for improved blood compat-



3.4 Anchor modification of a polymeric biomaterial surface using supramolecules.

ibility. By the utilisation of oligosaccharides, such as cyclodextrins and cyclodextrin/ poly(ethylene oxide) (PEO), cyclodextrin/PEO poly (propylene oxide) (PPO) combinations, a nanoscale modified polymer surface has been configured at the surface. This modification is called anchor modification (Fig. 3.4). According to this modification, PEO-PPO-PEO and CD inclusion complexes (nanomaterials) were blended with PVC-di(2-ethyl hexyl) phthalate (DEHP) for surface modification. The significant reduction in fibrinogen adsorption at the surface indicates the effect of anchoring of the complex on the alteration of the surface properties, which leads to an improved blood compatibility.

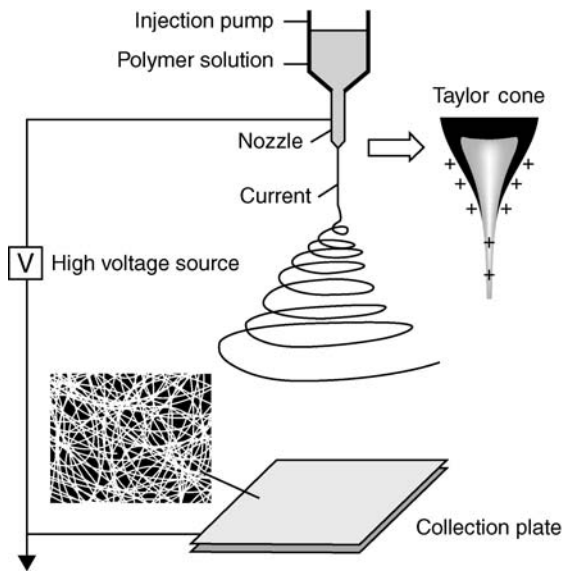
3.3 Nanofibres

Nanofibres are ultra-fine solid fibres, with notable features of very small diameter (lower than 100 nm), large surface area per unit mass and small pore size. Nanofibre has recently attracted considerable attention, owing to its extremely high surface-to-volume ratio, tunable porosity, malleability to conform over a wide variety of sizes and shapes, and a similarity to extracellular matrix (ECM) structure, making it suitable as a bioactive material for tissue engineering [29].

Nanofibres can be produced by electrospinning of natural biopolymers, synthetic polymers and composites to obtain fibres at the nanoscale. By adjusting the material composition, the surface properties can be altered to

interact with cells and tissues. Owing to their greater structure accessibility towards biological elements in comparison to any other scaffolds, electrospun nanofibrous scaffolds have demonstrated great promise and potential for many biomedical applications, including tissue engineering, wound dressings, immobilised enzymes and controlled delivery of drugs and genes.

Electrospinning technology is not new. As early as 1934, Formhals [30] patented a process and device to utilise electrospinning technology in the production of fibres. In 1966, a non-woven fabric was developed, based on electrospun nanofibres [31]. In 1981, melt-electrospinning technology was developed for producing synthetic polymer nanofibres, such as polyethylene and polypropylene [32]. In principle, electrospinning uses an electric field to draw a polymer melt or polymer solution from the tip of a capillary to a collector. A voltage is applied to the polymer, which causes a jet of the solution to be drawn toward a grounded collector. The fine jets dry to form polymeric fibres, which can be collected on a web as illustrated in Fig. 3.5. The electrospinning process has been documented using a variety of polymers, such as polymetaphenylene-isophthalamide, polyetherimide, PEO, polyethylene terephthalate, polyaniline, polycaprolactone and poly-L-lactic acid [33–37]. Biopolymers, such as collagen, elastin, gelatin, fibrinogen and silk fibroin, and polysaccharides, such as chitosan or chitosan/polyvinyl alcohol (PVA), can be selected for nanofibre production [29, 38].



3.5 Schematic representation of the electrospinning process for nanofibre production.

Many researchers have been working on understanding the relationship between the nanofibre structures and processing conditions, including crosslinking [39]. By selection of different polymers, solvent used for dissolution and the templates for the formation of the final forms of the product, both nano-hollow-fibres and three-dimensional tubes can be produced [40] (Fig. 3.6). Aligned nanofibrous scaffolds based on poly(D, L-lactide-co-glycolide) (PLGA) and nano-hydroxyapatite (nano-Hap) have also been synthesised by electrospinning [41]. The biomimetic nature of nanofibre and its similarity to the biomatrix for tissue regeneration promote the research and development of nanofibres for medical applications.

3.4 Nanocomposites

Nanocomposites have become another prominent area of current research and development in nanotechnology. A nanocomposite can be formed by mixing synthetic polymers or biopolymers with nanomaterials as fillers to form the composite. The size of the filler or at least one dimension should be nanoscale. The applicability to biomedical/biotechnological applications of nanocomposites is a rapidly emerging area of development. This is reflected in a recent marketing report by Frost and Sullivan [42]. According to this report, the global nanocomposites market had revenues of US$33.7 million in 2006 and it was estimated that this figure will reach US$144.6 million in 2013.

There are three major advantages of using nanocomposites to fabricate bioactive materials. First, improved mechanical properties of the materials can be achieved [43]. Second, the presence of the nanomaterials within the polymer base can produce a barrier effect, creating a tortuous path, and thus reducing the rate of active agent release. Third, cell interactions with the surface of the polymer nanocomposites can be modified without modifying the bulk chemistry of the base polymer [44].

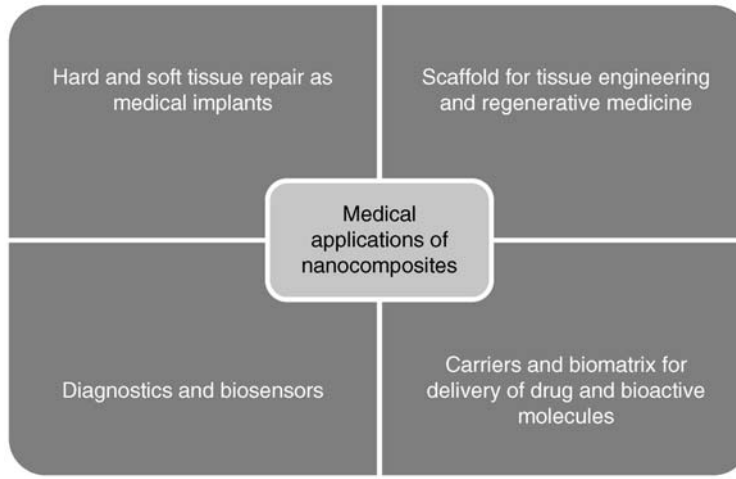
In the medical field, the nanocomposite-based scaffold allows for cell growth, yielding a unique composite system for clinical applications. For example, traditional polymer/inorganic composites, including polymers such as polylactic acid, polyglycolic acid, poly-e-caprolactone, polyethylene, polyetheretherketone and polyurethane, have been used with Hap as composite materials for tissue engineering [45–52]. The PLLA/Hap and PLGA/Hap scaffolds have been developed with improved mechanical properties and osteoconductivity [53, 54]. In contrast, to mimic the organic/inorganic nature of the bone matrix, polymer/nano-Hap scaffolds offered not only improved mechanical properties, but also exhibited significantly enhanced protein adsorption over microsized Hap/polymer scaffolds [55, 56]. The enhanced protein adsorption benefits cell adhesion and function [57].

3.5 Applications of nanomaterials

Nanotechnology has become one of the most active research areas in terms of both theoretical interest and practical applications. In particular, nano-bioactive materials have been widely used as novel medical implants and medical devices in the fields of tissue engineering, regenerative medicine, novel drug delivery systems and diagnostics (Table 3.1).

Table 3.1 Selected nanomaterials and biomedical applications

Nanomaterials	Biomedical applications	Featured properties/ references
Equiaxed forms gold nanoparticles	Cancer diagnostics and cancer therapy	Strongly enhanced surface plasmon absorption and scattering
Titania nanoparticles	• Orthopaedic coatings • Reinforcing phase in composite materials for TE scaffolds	[58]
Dendrimers	Efficient multi-drug delivery system	[59]
Quantum dots	Fluorescent contrast agent, amplifiers and biological sensors/diagnostics	Superior transport and optical properties [60]
Carbon nanotubes	Novel drug carriers/drug delivery systems	[61]
Nanofibres	Tissue engineering, wound dressing, drug delivery, artificial organs, vascular grafts	[62]
PLLA	Neural TE	[63, 64]
PLGA	Soft tissue	[65]
PCL	Cartilage TE	[66]
PLLA-CL	• Smooth muscle and endothelial cells • Blood vessel engineering	[67, 68]
Nano-hydroxyapatite	• Bone/cartilage tissue engineering in orthopaedic implants • Drug carriers for various bone diseases • Reinforcing phase in composite materials for TE • Immunotherapy	[69–72]
Biodegradable polymer nanoparticles	• Delivery systems for TE	[63]
Nanofibre (silk + Hap + BMP-2, chitosan + PVA, collagen, fibrinogen, chitosan + PEO)	• Bone repair • Bone tissue engineering • Cardiovascular	[73–77]
(PVA)/nanosilver / nanofibre-based non-woven webs	• Wound dressing	[78]



3.6 Medical applications of nanocomposites.

For nanocomposites, as shown in Table 3.1, the great advantages of using nanocomposites are modification of both the mechanical properties and the interfacial response between the materials and the biological system, while they are used as implants for tissue engineering and drug delivery. Medical applications of nanocomposites are summarised in Fig. 3.6.

3.6 Limitations of nanomaterials

Although there is great potential for nanomaterials to be used clinically, many challenges remain. First, nanotechnology is a multidisciplinary subject which requires the collective efforts of material scientists, bioengineers, biologists, clinicians, medical scientists and technology transfer. From the commercialisation point of view, nanomedicine will boost economical growth in the field of the health care industry and the time is appropriate for the transfer of laboratory research on the use of nanomaterials in biomedical applications to clinical utilisation. This is clearly lagging well behind public expectations, owing to the limitation of the multidisciplinary approach.

Second, there are still many technical challenges involved in working with nanomaterials for medical applications. For example, there is no doubt that electrospun materials are going to take a major place in the future for biomedical applications, but methods and materials have to be provided on a large scale, and produced in a controlled and reproducible manner. With nanoparticles as carriers for drug delivery, it is still necessary to understand the potential risks of nanoparticles, circulating in the body and functioning *in vivo*. With nanocomposites as scaffolds for tissue engineering, there are

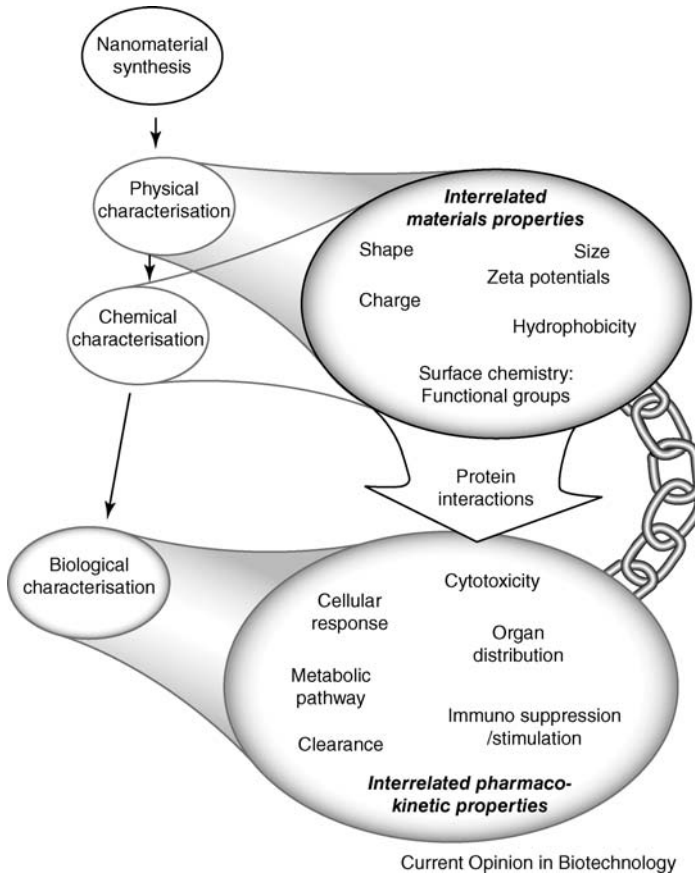
still many regulatory issues regarding the development of advanced therapy when tissue and cells are involved, in addition to the processes (dispersion, blending, foaming and sheeting), control of the structure, functions and biodegradability of the bioactive materials.

Third, nanotoxicity is now a major focus of interest in nanotechnology [62, 79]. Retention of nanomaterials to reduce the potential risk of the materials to human beings and the environment has become an important research topic. Our understanding of the potential human health and environmental implications of nanomaterials has improved with the increasing number of nanotoxicity studies in the past few years. Bulk TiO_2 particles (> 100 nm) are known to be harmless to humans and animals [80, 81]. Although nanoscale TiO_2 was classified recently as a possible carcinogen (if inhaled) by the International Agency for Research on Cancer (IARC) [82], its potential ingestion via water is not expected to be a major concern, as reflected by its use in toothpaste and sunscreens. No evidence has been found on the toxicity of nAg to humans [83]. The only known negative health impact of nAg is darkening of the skin and mucous membranes, resulting from long-term exposure to high silver concentration. However, some nanomaterials, such as nC60, have been shown to exhibit toxicity to mammalian cells [84]. ZnO nanoparticles have also been shown to reduce the viability of human T-cells at an elevated concentration (5 mM) [85].

3.7 Future trends

In this chapter, bioactive materials as nanomaterials have been reviewed. Clearly, when materials are reduced to the nanoscale, there are many outstanding properties, resulting in an outperformance of traditional biomaterials. Owing to the great potential of nanotechnology in medical applications, it is expected that a great deal of effort will be directed to the following areas.

- Continuing attempts will be made to achieve a full understanding of the interface between nanomaterials and the biological system [85] (see Fig. 3.7).
- There is a necessity to develop novel high-resolution imaging and analysis tools, which enable easy sample preparation and in situ monitoring. The creation of such high-resolution nano tools would be of great benefit in understanding cellular functions of nanomaterials, a critical requirement for advancement in this field.
- Utilisation of a biomimetic approach is a key area for research in the field of advanced therapy (tissue engineering and regenerative medicine). Proper selection of biomaterials is required in terms of mechanical properties and degradation time, which is dependent on the type of



3.7 The nanostructure physico-chemical property relationship to *in vivo* responses [85].

scaffold, type of tissue to be regenerated and the tissue regeneration time.

- There is a requirement for development and modification of a process for mimicking of extracellular matrix, thereby providing enhanced proliferation and differentiation of cells.
- Scale-up and manufacture of nanofibres and nanomedicines is required.
- Formulation of a full *in vivo* 'life cycle' characterisation framework is needed, in which systematic evaluation of the size, shape and surface chemistry of nanostructures, and their correlation to *in vivo* behaviour is obtained. This permits the mapping of the fate, kinetics, clearance, metabolism, protein coating, immune response and toxicity of nanostructures to the nanostructure's physical properties. This would allow the development of predictive models of nanostructure toxicity [85].
- Clinical applications of nanomedicines will be investigated.

In nature, materials with nanostructured surfaces can create super-hydrophobic character (lotus leaf) and exceptional adhesion (gecko foot). As the secrets of nature's methodology for optimisation of material properties by nano-level construction are unlocked (biomimetics), it is believed that advanced 'artificial' nano-bioactive materials will appear in medical practice, in the near future.

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Bioactive materials and tissue engineering

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Abstract: Tissue engineering has emerged as a revolutionary approach to the reconstruction and regeneration of lost or damaged tissue. Bioactive materials act as a scaffolding frame to deliver cells to the appropriate site, define a space for tissue development, and direct the shape and size of the engineered tissue. Bioactive materials have reached clinical use in a variety of orthopaedic and dental applications; bioactive glasses, ceramics, glass–ceramics composites, and inorganic/organic hybrids can be used as hard tissue substitutes or substrates for synthetic orthopaedic graft materials. Additionally, injectable biomaterials that form scaffolds in situ are capable of taking the shape of a tissue defect, avoiding the need for patient-specific scaffold prefabrication.

Key words: bioactive materials, tissue engineering, application.

4.1 Introduction

The restoration of damaged or diseased tissue forms a significant part of modern medical practice. Tissue engineering has emerged as a revolutionary approach to the reconstruction and regeneration of lost or damaged tissue. The fundamental premise of tissue engineering is the regeneration of tissues through the implantation of cells/tissues or stimulating cells to grow in an implanted matrix. Tissue engineering has emerged from the use of biomaterials to repair or replace damaged or diseased tissue to the utilisation of three-dimensional scaffolds with controlled structure, in which cells are seeded prior to implantation. The scaffold acts to deliver cells to the appropriate site, define a space for tissue development, and direct the shape and size of the engineered tissue. The clinical success of the tissue-engineered construct is critically dependent on the bioactivity of biomaterials and three-dimensional (3D) scaffolds, which guide the growth of new tissue *in vitro* and *in vivo*, and on a suitable supply of cells. The scaffold must

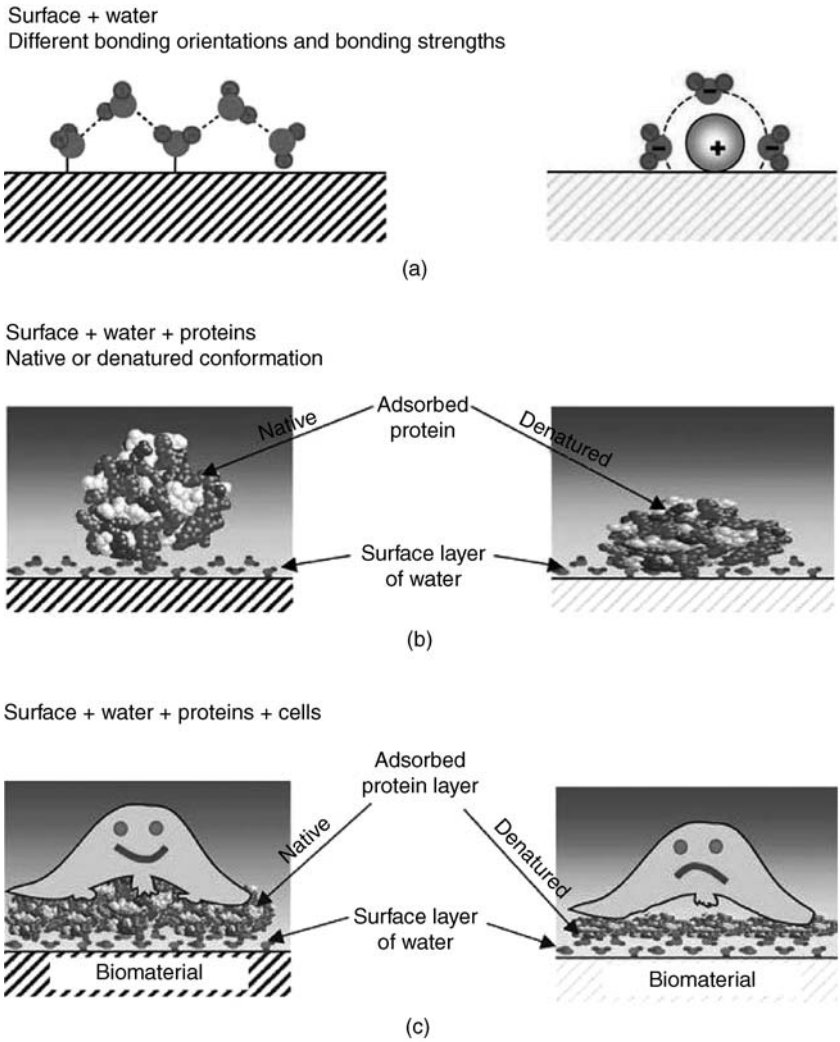
not only serve to provide the 'right' biological, structural and mechanical properties in the tissue-engineered construct for the repair or replacement of the damaged or diseased tissue, but must also be formed into the required geometry [1].

The general trend in biomaterials is to use and employ materials that play an active role in tissue regeneration. Therefore, understanding how a material interacts with the surrounding environment, including cells and tissue fluid, allows material design to be tailored so that implants can be constructed to promote a specific biological response, enabling an improved performance of their function [2].

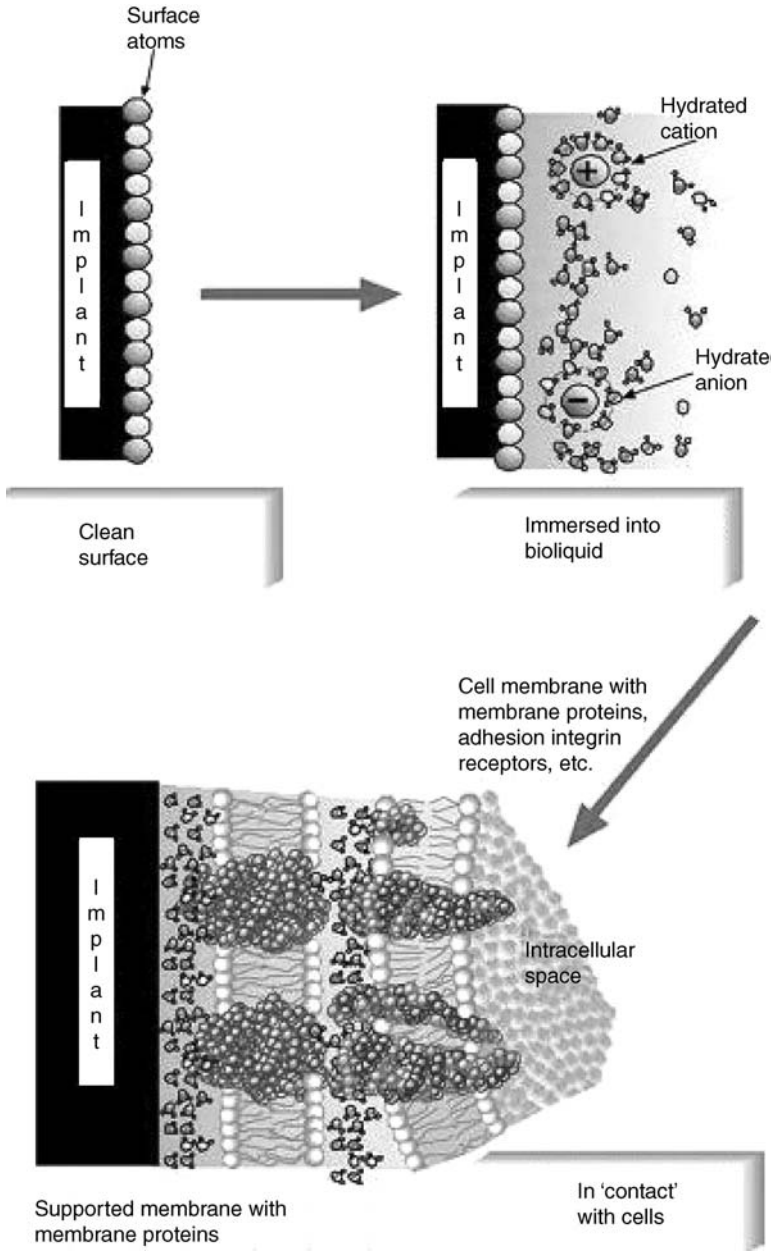
4.2 Interaction between bioactive materials, cells and surrounding tissue

The success of the scaffold depends on the ability of the implanted cells to attach to the surrounding environment and to stimulate angiogenesis (new blood vessel formation). The combination of high cell density and an appropriate substrate is needed to induce cooperative cell–cell and cell–matrix interactions. To programme scaffolds with biological instructions, cells and growth factors need to be integrated into the scaffold fabrication for tissue engineering so that the bioactive molecule can be released from the scaffold and trigger or modulate new tissue formation [3]. The responses at the interface of the implanted material and in the surrounding environment are important events in determining the biocompatibility of the implant. The biological response to an implant can be attributed to acute and chronic inflammatory changes, with concomitant formation of a fibrous capsule, which are seen over a period of time.

There is an interesting and unique synergistic connection between the nanometre and the micrometre length scales when cells are present, as, for example, in the cases of medical implants, tissue engineering and cell-based bioelectronics. This is schematically illustrated in Figs. 4.1 and 4.2. Figure 4.1 [4] shows the sequence of events after a biomaterial has been placed in a biological environment containing cells, when cells arrive at a protein-covered surface whose protein layer has properties that were initially determined by the preformed water shells. Thus, cell–surface interactions become ultimately an interaction between cells and surface-bound proteins (or other biomolecules). The latter is illustrated in Fig. 4.2, where the clean surface in the illustration is deliberately covered with a native or artificial biomembrane, containing embedded receptors that can specifically interact with cells approaching the surface. Figures 4.1 and 4.2 emphasise the above-mentioned large size range that the functional units in biology cover, from water molecules and small proteins at sub-nanometre sizes, via cell



4.1 Schematic illustration of the successive events following implantation of a medical implant. (a) The first molecules to reach the surface are water molecules (ns time scale). The water shell that is formed affects the protein interaction starting on the micro- to millisecond time scale, and continuing for much longer times. (b) The water shell on the surface affects the protein interaction. Eventually cells reach the surface. (c) There surface interaction takes place via the protein coating whose properties are determined by the surface and water adlayer properties.



4.2 A conceptual approach to convert a synthetic surface to a biomimetic surface by coating it with a supported biomembrane with built-in functional membrane-bound proteins.

membranes and supra-molecular complexes in the 10 nm range, to cells in the micrometre range and finally fully organised tissue and organs at the macroscopic size level.

Although various materials have been used widely in tissue engineering for a long time, the understanding of the cellular responses to biomaterials is incomplete. Many factors contribute to the biological response to implanted materials. These are related to the surface chemistry, size, shape, site of implantation, and duration of the implant material in humans and in animal models. The inflammatory reaction which occurs may be related to the composition and structure of the biomaterial, but the response, in general, is that of acute and chronic inflammatory changes. The events that lead to these changes can be ascribed to the interactions of cells with the matrix and the myriad of mediators produced by these cells. As mentioned above, the surfaces of biomaterials play a key role in controlling cellular behaviour [5]. Cell activity can be affected by surface properties, such as surface roughness, hydrophobicity and specific interaction with the cell surface. The modulation of cell activity through substrate interaction can generate a significant effect on biomaterial-based therapies. Tissue-engineered constructs, *ex-vivo* cell propagation and cell encapsulation all require some type of interaction between cells and supporting material for growth, function and/or delivery [6]. The identification of biomaterials that allow for appropriate levels of cellular attachment is of central importance in tissue engineering and cell therapy [7].

Materials that are only reactive on the surface could be used in the body as implant materials for bone repair. These materials, also called bioactive materials, will not significantly change their mechanical properties, while still reacting and bonding chemically with the surrounding tissues. Clinically, the use of such materials would be a more practical solution, since the joint can be functional much sooner after implantation. Macrophages, endothelial cells, fibroblasts, osteoblasts, osteoclasts and foreign body giant cells are involved in the cell–biomaterial relationship. Macrophages may influence the fibrogenic response to polymers by regulating the extracellular environment and remodelling the connective tissue; they are viewed as a control cell in the inflammatory reactions involving implanted biomedical polymers. Macrophages can also play a central role in a very complex interaction involving inflammatory mediators, chemotactic factors, growth factors, clotting factors, lymphokines, prostaglandins, leukotrienes and complement components. These factors interact to alter cell activation, proliferation and synthetic abilities of such cells as fibroblasts, osteoblasts and osteoclasts. Furthermore, the macrophage interacts with T-lymphocytes to activate them, allowing elaboration of lymphokines from these cells [8]. With implantation of a material onto or into bone, there is a sequence of events that takes place,

involving the interaction of tissue ingrowth of mesenchymal cells (fibroblasts, endothelial cells and bone cells) with the implanted material, especially with the use of porous material implants and bone cements. In one study, Maguire *et al*. [8] have shown that severe foreign body reactions occurred in patients receiving cemented total hip arthroplasty. They found microfragments of polyethylene and poly(methyl methacrylate) in tissue obtained from these patients, and evidence of foreign body giant cells, particularly osteoclasts. It is suggested that poor cement fixation, micromovement and debris caused loosening and eventual bone destruction probably related to osteoclast stimulation, macrophage activation and eventually bone resorption.

The interaction between cells and substrates is a complex situation involving cell–cell and cell–biomaterial interactions. The response of tissues at the implant site involves a myriad of mediators including chemotactic substances and signalling molecules, such as cytokines, and growth factors that modulate cell functions, such as activation, proliferation and protein production. Although some evidence of biomaterial-induced mediator release has been reported, cell–biomaterial interactions are still incompletely understood [9]. It remains of interest to speculate how this interaction is controlled by the structure and chemistry of biomedical implants. The bioactive material must provide the appropriate biomimetic environment to ensure cell survival. Also, the material can be used to direct desired cell behaviour, such as orientation and migration, to ensure that the appropriate cells migrate to and/or adhere to the implant [10]. Therefore, knowledge of the basic mechanisms of cell adhesion and activation, mediator release and resultant sequelae of cell–cell and cell–matrix interactions should lead to the development of improved biomaterials. The modulation of bioactivity through the rational design of materials has been widely investigated. Tissue-engineered constructs, *ex-vivo* cell propagation and cell encapsulation all require some type of interaction between cells and supporting material for growth, function and/or delivery. Bioactive material-based control of cellular function is a potentially powerful tool, which has the potential to differentiate into many tissue types; for example, a self-assembling peptide-based biomaterial that can specifically direct the differentiation of neural progenitor into neurons [11]. Much research is currently focused on the development of bioactive materials through the incorporation of ligands, and encapsulation of DNA and growth factors [12, 13].

4.3 Bioactive materials as a scaffolding frame used in tissue engineering

One of the most critical elements of tissue engineering is the ability to mimic the body's natural scaffold that normally serves to organise cells into tissues. According to this concept, cells need to be isolated from the patient, expanded *in vitro*, and delivered to the defect within a 3D construct designed to provide space for efficient delivery of the cells, control cell function and overall tissue shape [14]. The selection of the most appropriate material as a scaffolding frame is an important step toward the construction of a tissue-engineered product, since it determines the success of the tissue–implant interaction. The matrices have to provide several requirements, of which biocompatibility is the most important. Furthermore, the surface structure of the matrices should not only support cell adhesion but also assure the supply of nutrition and the removal of metabolic waste. It is necessary to ensure that neovascularisation and cell–matrix interactions can take place [15–17]. In addition to the shape and composition of the scaffold, the influence and regulation of cell interactions by bioactive components can be crucial [18].

4.3.1 Bioactive materials as solid scaffolding frame

Tissue engineering has emerged as a revolutionary approach to the reconstruction and regeneration of lost or damaged tissue. Bioactive materials act as a scaffolding frame, including solid and injectable systems, to deliver cells to the appropriate site, define a space for tissue development, and direct the shape and size of the engineered tissue. Inorganic, inorganic–organic hybrids and 'nanostructured' materials have become promising materials as scaffolds for solid systems used in tissue engineering because of their excellent characteristics, such as enhanced mechanical and biological properties. Injectable materials hold promise for tissue-engineering applications, as they offer some advantages over prefabricated scaffolds for certain indications. Crystalline ceramics, such as hydroxyapatite (Hap), β-tricalcium phosphate and combinations of these, have also been extensively studied as solid scaffolding materials [19, 20], owing to their similarity with the inorganic component of bone. More recently, glasses or glass–ceramics represent a very attractive alternative and have been investigated as candidates for bone grafts, as they are biocompatible, bioactive and even osteoproductive, can bond to both bone and soft tissue, and can stimulate bone growth [21]. Bioactive glass has been selected to prepare solid scaffolds, especially as bone tissue implants, owing to its osteoproductive properties and its potential to stimulate osteoblast proliferation with its dissolution products. The bonding ability of bioactive glasses is well known

and has been ascribed to their capability of forming a surface layer of microcrystalline Hap, when placed in contact with simulated body fluid.

Bioactive glasses, silicate-based in particular, have been the subject of intense interest for the last three decades as bioactive materials for tissue regeneration applications. It has been discovered that the ionic dissolution products released from bioactive silica-based glasses up-regulate seven families of genes found in osteoblasts [22]. When exposed to physiological fluids *in vivo*, they form a surface apatite layer. This layer has the capacity of bonding to collagen synthesised by connective tissue cells, such as osteoblasts. One commercially available bioactive glass is Bioglass®, which has a composition known as 45S5, corresponding to 45.0 wt% SiO_2, 24.5 wt% CaO, 24.5 wt% Na_2O and 6.0 wt% P_2O_5 [23, 24]. Today, the 45S5 composition is used as a benchmark by which the performance of new silicate-based bioactive glasses is measured. Such glasses have achieved great success in many clinical applications, especially in the dental and orthopaedic fields. In addition, it has been reported that the dissolution products of bioactive glasses exert a positive effect on the expression of genes regulating osteogenesis [25].

More recently, scaffolds from bioactive glasses have been developed, using the foaming technology based on sol–gel derived glasses. The nanoparticles assemble into a silica network [26], which provides an interconnected nanoporous structure throughout the glass. After decreasing material size into the nanoscale, thereby dramatically increasing surface area, surface roughness and surface area to volume ratios can be created to ensure superior physiochemical properties (i.e. mechanical, electrical, optical, catalytic and magnetic properties). Therefore, nanomaterials with such excellent properties have been extensively investigated in a wide range of biomedical applications, in particular regenerative medicine. In tissues such as bone or cartilage, the surface topography is on the macroscale. However, collagen, a major component of connective tissue, basal membranes and the extracellular matrix (ECM), is approximately 300 nm long and 0.5 nm wide and presents a nanostructured surface to the cells adhering to it [27]. Specifically, 70% of the bone matrix is composed of nanocrystalline Hap, which is typically 20–80 nm long and 2–5 nm thick [28]. Other protein components in the bone ECM are also nanometre-sized in dimension. Moreover, cartilage is a low regenerative tissue composed of a small percentage of chondrocytes, but dense nanostructured ECM that is rich in collagen fibres, proteoglycans and elastin fibres. Clearly, advances in design and synthesis of nanostructures have paved the way for the development of nanocomposite scaffolds mimicking the underlying fibrous structure and specific chemistry of the tissue [29].

Inorganic–organic hybrids often show an excellent balance between stiffness and toughness and usually improved characteristics compared to

their individual components [30]. There are two classes of nanocomposites. One is a nanoscale version of a conventional composite, in which nanoparticles are dispersed in a polymer matrix. The second is the composite covalently bonded by inorganic and organic phases at a molecular scale during processing. These materials have the greatest potential of combining the desired properties of the constituent materials for bone regeneration [26]. There have been several attempts to combine bioactive glasses with biodegradable polymers to create a scaffold material with degradability, bioactivity and toughness. Composite scaffolds with ideal interconnected macropore networks have been produced by introducing bioactive glass particles into polylactide foams [31]. The aim of producing nanocomposites is to have a nanoscale interaction between the bioactive inorganic phase and the organic phase, which can allow bone cells to come into contact with both phases simultaneously. The material should degrade at a single rate, and in a more linear fashion than conventional polyesters or their composites.

Bioactive composites based on functionalised polymer, including the introduction of coupling agents, provide promising scaffold material for use in tissue engineering. One example is that of silica/poly (ε-caprolactone) hybrid discs [32, 33]. The hybrid with 60 wt% polymer showed desirable mechanical properties, which is in the range of cancellous bone. Star gels are a type of organic–inorganic hybrids, having an organic core surrounded by flexible arms, which are terminated in alkoxysilane groups. Manzano *et al.* [34] developed the first bioactive version of a star gel hybrid by incorporating calcium methoxy ethoxide into the sol. Monoliths (not macroporous) were shown to have a Young's modulus and compressive strength of 1 GPa and 50 MPa, respectively, comparable with that of human bone.

Injectable tissue-engineering methods are particularly attractive examples as they can offer some advantages over prefabricated scaffolds for certain indications. Injectable scaffolds eliminate the need for surgical interventions for delivery, and the minimally invasive procedure of injection reduces discomfort and complications for the patient [35]. Injectable bioactive materials are not used for tissue-engineering purposes but are rather volume fillers, with biological activity that is often limited, owing to encapsulation as part of the foreign body response. Many injectable materials have been studied as scaffolds for tissue engineering and other applications. CaP cement-based injectable biomaterials are currently used and regulated in clinical applications. These materials have achieved widespread success for bone defect repair and regeneration. CaP composites have also been used as soft tissue fillers, demonstrating the potential versatility of these materials.

Polymeric biomaterials are the most widely use as injectable scaffolds. In situ solidification of polymeric systems is typically achieved either through

phase separation or via polymerisation or crosslinking of reactive monomers and macromer chains. Radical initiator, chemical crosslinking or polymerisation strategies and biologically motivated systems can be used for the in situ formation of biomaterial scaffolds [36]. Materials that undergo physical gelation instead of chemical crosslinking are also being studied for use as injectable scaffold materials for tissue engineering. These materials can undergo physical gelation in response to one or multiple changes in their surrounding environment, including changes in temperature, ions, pH and pressure, or the presence of electrical and/or magnetic fields [37]. Injectable materials that self-assemble in situ undergo gelation or precipitation, often with the ability to form precise nano- or microscale structures. Many such systems use hydrophobicity, either of the bulk material for phase segregation or of certain molecular domains for amphiphiles, as the key means by which self-assembly occurs. Micro- and nanosphere injection for use as drug delivery vehicles and scaffolds are possible [38]. Salem *et al.* [39] prepared crosslinked biotinylated PLA–PEG microparticles using avidin in the presence of cells to create injectable cell-containing matrices with mechanical strength suitable for supporting bone regeneration *in vivo*. Most of the materials and techniques used in the development of injectable scaffolds for tissue engineering have concurrently been investigated as injectable drug delivery systems, including antibiotic delivery and growth factor delivery [36].

4.3.2 Fabrication technology (methods) of scaffolding frame

Tissue engineering is an interdisciplinary field that combines the knowledge and technology of cells, engineering materials and suitable biochemical factors to create artificial organs and tissues, or to regenerate damaged tissues. The scaffold provides a framework and initial support for the cells to attach, proliferate and differentiate, and form an ECM. Biomaterials used in tissue-engineering scaffold fabrication can be divided into broad categories of synthetic or naturally derived, with semi-synthetic materials rapidly emerging. A number of techniques are being pursued for the creation of scaffolds, of which the solid free-form fabrication (SSF) technique is being studied by a number of groups worldwide. The main advantage of this technique is the ability to fabricate highly reproducible scaffolds with fully interconnected porous networks and precise control of the scaffold architecture. It is also possible to integrate cell seeding within the scaffold fabrication process, thus avoiding the problem of poor cell infiltration into the scaffold that may be encountered with other techniques [3]. Such biomimetic internal architectures may prove valuable for multi-tissue and structural tissue interface engineering.

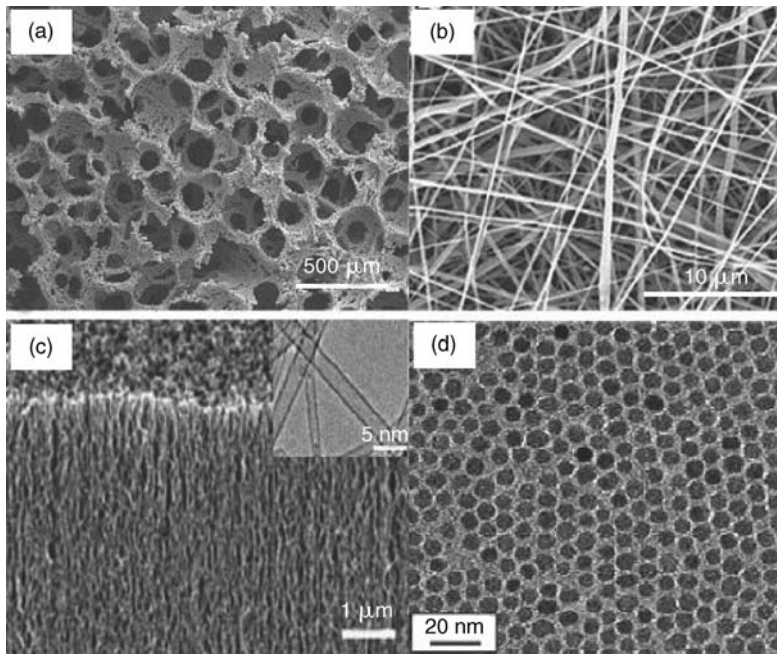
Fabrication of composite scaffolds is attractive because two or more types

of materials can be combined to meet more effectively the physiological and mechanical demands of the host tissue. The bioactivity degree is adjustable by the volume fraction, shape, size and arrangement of inclusions [40, 41]. There are numerous foaming techniques including sol–gel routes to obtain highly porous structures [42]. However, only relevant fabrication techniques lead to 3D composite scaffolds with highly interconnected pores.

A thermally induced phase separation (TIPS) technique can be used to produce 3D resorbable polymer scaffolds with very high porosities (~97%) and to control macro- and microstructures suitable as scaffolds for tissues, such as nerve, muscle, tendon, ligament, intestine, bone and teeth. The scaffolds produced possess a highly porous structure, with anisotropic tubular morphology and extensive pore interconnectivity. The microporosity of TIPS-produced foams, their pore morphology, mechanical properties, bioactivity and degradation rates can be controlled by varying the polymer concentration in solution, the volume fraction of the secondary phase and the quenching temperature [43]. Solvent casting of biocomposite scaffolds is a straightforward method of fabrication, without the need for specialised equipment. This process involves the dissolution of the polymer in an organic solvent, mixing with inorganic granules and casting the solution into a predefined 3D mould. The solvent is subsequently allowed to evaporate. The main disadvantages of this method include the limitation in the shapes, the possible retention of toxic solvent within the scaffolds and the denaturation of the proteins and other active molecules incorporated into the polymer, resulting from the use of organic solvents.

Microsphere sintering has been employed to fabricate composite scaffolds. In this process, microspheres of a ceramic and polymer composite are synthesised by the use of an emulsion/solvent evaporation technique. Sintering the composite microspheres produces a 3D, porous scaffold [44]. Lu *et al*. [45] have produced 3D composites of degradable polymers and bioactive glass by sintering composite microspheres. Bioceramic-coated porous scaffolds in the form of foams, fibrous bodies and meshes [46] can be obtained by slurry dipping or electrophoretic deposition (EPD). Roether *et al*. [47] were the first to develop composites of macroporous D,L-poly(lactic acid) (PDLLA) foams coated with Bioglass® particles (grade 45S5 with particle size $< 5\,\mu m$) by slurry dipping. A stable and homogeneous coating on the foam surface and infiltration of Bioglass® particles throughout the porous network were achieved. EPD is an attractive method to incorporate nanoparticles into porous structures, with potential use as tissue-engineering scaffolds [48].

Nanotechnology is a useful tool for creating scaffolds, with size range from the macroscale down to the nanoscale [26]. To date, numerous top-down and bottom-up nanofabrication technologies, such as electrospinning, phase separation, self-assembly processes, thin film deposition, chemical



4.3 (a) Scanning electron microscopy image of poly (L-lactic acid) nanofibrous scaffold with interconnected spherical macropores created by a phase-separation technique. (b) Electrospun polycaprolactone/hydroxyapatite/gelatine (PCL/Hap/gelatin, 1:1:2) nanofibres, which significantly improved osteoblast functions for bone tissue-engineering applications. (c) Densely aligned single-wall carbon nanotube forest grown with novel water-assisted chemical vapour deposition in 10 min. (d) Transmission electron microscopy image of monodispersed magnetic Fe_3O_4 nanoparticles (6 nm) deposited from their hexane dispersion and dried at room temperature.

vapour deposition, chemical etching, nano-imprinting, photolithography, and electron beam or nanosphere lithographies, are available for synthesising nanomaterials with ordered or random nanotopographies (see Fig. 4.3.) [28]. In addition, the sol–gel foaming process is widely used to produce ideal pore networks for bioactive glass scaffolds. The polymer network can form at the same time as the silica-based nanoparticles assembly by introducing polymer chains into the sol, while the inorganic chains are forming. Sol–gel derived bioactive glasses have a very useful surface chemistry, as they have a high concentration of silanol groups at the surface. They are also easily functionalised with groups, such as mercapto- and amino-groups, that can be used to bond covalently proteins to the surface of the glasses [49].

From the material science perspective, the present challenge in tissue engineering is how to design and fabricate reproducible bioactive and

bioresorbable 3D scaffolds with tailored porosity and pore structure, which are able to maintain their structure and integrity for predictable times, even under load-bearing conditions. The incorporation of biomolecules, such as growth factors, with the aim of accelerating local tissue healing, is promising and currently under extensive research. However, incorporating biomolecules during scaffold processing is not straightforward, as biomolecules are sensitive to elevated temperatures and extreme chemical conditions. A promising strategy is the immobilisation of proteins and growth factors in the post-processing phase via surface functionalisation of the scaffold [50].

4.3.3 Establish feasible processes for scaffold fabrication

A variety of techniques have been used already to process bioactive materials into scaffolds for regenerative medicine. The success of growing tissues depends on scaffold materials and their internal architecture, as this influences the cells' morphology following attachment, cell density and distribution, as well as cell–scaffold interaction. Therefore, some key factors, such as controlling scaffold architecture and tailoring scaffold–cell interactions, should be considered in developing tissue-engineering scaffolds.

Some tissues, such as liver and kidney, have very complex architectures, composed of multicells with different functions and abundant capillary networks, that are beyond the ability of current scaffold fabrication. Approaches to culture these tissues remain unsuccessful clinically. Simply producing a highly porous scaffold and seeding it with the appropriate types of cells does not reproduce the desired features of a normal tissue. Many tissues have a hierarchical structure at scales from the nanometre to millimetre level. The tissue scaffold must be designed to satisfy several requirements, including capillary networks, and maintain the hierarchical cellular architectures. Scaffold structures have an effect on the spatial cell arrangement and the transmission of mechanical and biochemical signals, which determine the ultimate shape of the newly grown soft or hard tissue. In order to resemble natural tissue, fabrication techniques must produce scaffolds with a completely interconnected pore network, a highly regular and reproducible scaffold morphology, and a microstructure which varies across the scaffold matrix. For bone tissue engineering, a scaffold with relatively large channels (0.5~1 mm) for rapid tissue penetration and local porosity for tissue development is required.

The successful scaffold should have the ability of enabling the implanted cells to attach to the surrounding environment and to allow the growth of blood vessels into the cell–scaffold composition. Such a design will provide the means for selective transplantation of parenchymal cells, which possess the necessary biological function, without transplantation of passenger

leucocytes and antigen-presenting cells. A challenge in developing tissue engineering scaffolds is to control architecture and overall surface chemistry in order to regulate precisely cell behaviour. This requires arranging the cells/tissue in an appropriate 3D configuration and presenting molecular signals in an appropriate spatial and temporal fashion, so that the individual cells will grow and subsequently form the desired tissue structures, both *in vitro* and *in vivo*.

There are various techniques/methods used to process biomaterials into scaffolds for tissue engineering. One of the efficient techniques is to apply the quality control and process analysis to each stage of scaffold production during or after manufacturing. Since controlling process variation is the key step, process improvement and optimisation are necessary for the scaffold design, in order to develop capable manufacturing processes for the generation of performance-consistent regenerative medical products.

4.4 Applications of bioactive materials in tissue engineering

Bioactive materials had obtained clinical application by the mid-1980s in a variety of dental and orthopaedic applications, such as various compositions of bioactive glasses, ceramics, glass–ceramics and composites [51]. There are three types of bioactive scaffolds being developed for tissue-engineering applications: bioactive foam scaffolds for bone tissue engineering; bioactive resorbable scaffolds for soft connective tissue regeneration and repair; and inorganic/organic bioactive composite scaffolds [52]. The clinical success of bioactive materials is particularly dependent on the tissue-engineered 3D construct, which supplies cells and guides the growth of new tissue *in vitro* and *in vivo*. The scaffold must not only provide the 'right' biological, structural and mechanical properties for reconstruction and regeneration of lost or diseased tissue, but must also be formed into the required geometry.

4.4.1 Hard-tissue-engineering applications

Bioactive inorganic materials are specially suited for use as hard tissue scaffolds, as they can provide significant mechanical support. Bioceramics are usually created as dental implants based on alumina and zirconia, Hap and resorbable calcium phosphates. Alumina has been widely used as a ceramic head in hip joint and knee prosthesis, owing to its good surface finish, leading to exceptionally low coefficient of friction and wear rates [53]. Bioactive glasses are used as middle-ear prostheses to restore the ossicular chain and treat conductive hearing loss, and as oral implants to preserve the

alveolar ridge from bone resorption. For hard-tissue-engineering applications, a number of bioactive glass systems have been developed by the addition of various metal oxides, such as Fe_2O_3, Al_2O_3, ZnO and TiO_2, into the parent glass [2]. Use of 45S5 Bioglass® particulate as an injectable for the treatment of urinary incontinence has also been tested *in vivo*. Use of the dissolution products of resorbable bioactive gel–glasses to stimulate cellular repair at a molecular level offers promise for creating scaffolds for bone tissue engineering [51]. A bioactive glass–ceramic with strong and tough mechanical properties is used for the replacement of vertebrae in patients with spinal tumours.

Some polymers have also been used as scaffold materials for bone tissue engineering. Polymers based on polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers (PLGA) are degradable, so the scaffold can be gradually replaced by a bone matrix [54]. The incorporation of a bioactive inorganic composition, such as bioactive glass and Hap, within polymeric microspheres may result in an enhanced osteogenic potential. Bioactive composites, with combined Hap in a polyethylene matrix, have been used in the repair and replacement of bone in the middle ear. Poly (3-hydroxybutyrate), poly (3-hydroxybutyrate-co-3-hydroxyvalerate), and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) are among the polymers which have been fabricated as composites, in combination with Hap, bioactive glass and glass–ceramic fillers or coatings [55]. The use of biodegradable porous polymer scaffolds coated with tailored Bioglass® layers can produce a new hybrid composite material. Acceptable mechanical and biological response of the composite is achieved by optimisation of the structure of the bioactive layer and of the polymer–Bioglass® interface [56]. Novel porous microspheres constituted by an apatite–wallastomite (A–W) bioactive ceramic phase have been developed for application in hard tissue regeneration [57].

Some studies have focused on the study of hybrid nanocomposites, in which the inorganic phase is incorporated into an organic polymeric matrix. For example, polymer/silica nanocomposites have been produced by physically mixing silica nanoparticles with polymers or by copolymerisation of an organic monomer in the presence of organically functionalised preformed silica nanoparticles [58]. The results strongly suggest that these nanohybrids are suitable substrates for the regeneration of dental mineralised tissue.

4.4.2 Soft-tissue-engineering applications

Studies in soft tissue engineering for improved tissue regeneration have focused on novel biodegradable materials with a specific and controllable bioactivity [1]. Bioactive glasses, silicate-based in particular, have been the

subject of intense interest for the last three decades, as materials for tissue-regeneration applications. A study of a 3D phosphate glass fibre construct, made from fibres with a composition of $(P_2O_5)_{62.9}(Al_2O_3)_{21.9}(ZnO)_{15.2}$, demonstrated that it could support the proliferation and differentiation of human masseter muscle-derived cell cultures. Biodegradable polymers and scaffolds with suitable mechanical properties have been developed for application in the engineering of soft tissues [59, 60]. An elastomeric poly (ester urethane)urea scaffold capable of releasing bioactive basic fibroblast growth factor exhibits a combination of mechanical properties and bioactivity, which might be attractive for use in cardiovascular and other soft tissue applications [61]. Giovanni *et al.* [62] developed a bioabsorbable polylactide–polyglycolide acid sponge with good biocompatibility and safety. This graft material acted as a barrier and did not allow the ingrowth of connective tissue from the overlying soft tissue flap following extraction. Finally, the grafted material was completely resorbed. The results suggested that the material is suitable for filling alveolar sockets following extractions, to prevent volume reduction and collapse of the overlying soft tissue flaps.

In biomedical applications, nanofibres have been developed as carriers for drug delivery and as wound dressing materials. As porous 3D scaffolds, nanofibres can be used to generate various tissues, such as skin, blood vessels, nerve, tendon, bone and cartilage, owing to their extremely high surface to mass ratio. This is mainly due to several novel properties, such as low density, high pore volume, variable pore size, and exceptional mechanical properties. Nanofibre scaffolds are extensively used as wound dressings, since they are able to protect the wound area from the loss of fluid and proteins, remove exudates, restrain exogenous microorganism invasion, improve appearance and provide excellent anti-adhesion properties. Bioactive materials for the production of such scaffolds include composites of nanofibres of collagen and polycaprolactone, electrospun silk fibroin (SF) nanofibres, electrospun chitin nanofibres, PLAGA-dextran electrospun nanofibres and nanofibres of poly(p-dioxanone-co-L-lactide)-block-poly (ethylene glycol). Blood vessels are tubular-shaped structures composed of oriented protein fibres and integrated cells, including both smooth muscle and endothelial cells. Electrospinning techniques have demonstrated the potential for mass production of such tubular scaffolds that would satisfy the functional parameters under mechanically active conditions [63].

Some other polymers, such as aligned poly(L-lactide-co-ε-caprolactone), P(LLA-CL:75:25) nanofibre gelatin grafting on polyethylene terephthalate (PET) non-woven nanofibre, hybrid scaffolds composed of polyglycolic acid (PGA) non-woven tubular fabric and a copolymer gel of L-lactide and ε-caprolactone P(CL/LA:50/50), have been used to prepare the scaffolds for blood vessel grafts. The integrated cells exhibited spread morphology and significantly high cellular proliferation. Knitted poly(lactide-co-glycolide)

(PLAGA) scaffolds can modulate bone-marrow-derived stromal cells (BMSCs) to exhibit a higher expression of collagen-I, decorin and biglycan, and chitin implants promote aggressive tissue ingrowth [63]. Hybrid nano-microfibrous scaffolds, composed of knitted PLAGA (90/10) covered with randomly oriented electrospun PLAGA (65:35) and dry non-woven chitin fabric, have been reported for use as tendon grafts. Several biodegradable polymers were successfully electrospun into nerve grafts and had the potential to stimulate axonal regeneration through its entire length. For example, the microbraided and aligned microfibre scaffolds produced by PLAGA nanofibres and microfibres could support neurite outgrowth and neural stem cells (NSCs) differentiated in the direction of fibre alignment, and the nanofibres blend, prepared by PCL and collagen/PCL (C/PCL 25:75 wt%), supported cell attachment, migration and axonal regeneration. Both bioactive materials are thought to be promising scaffolds for use in nerve grafts.

4.4.3 Other medical applications

In addition to the wide applications in hard and soft tissue engineering, bioactive materials can be also used to encapsulate cells for medical applications. Kraehenbuehl *et al*. [64] explored the *in vitro* potential of synthetic, cell-responsive hydrogels displaying vascular adhesion epitopes for encapsulation of vascular cells, while acting as a controlled drug release system at the same time. The physical incorporation of the small bioactive peptide, thymosin β4, in the PEG-based hydrogel can generate a 3D environment, conducive for human umbilical vein endothelial cell adhesion, survival, migration and vascular-like network organisation. These matrix metalloproteinase-responsive PEG hydrogels may thus potentially serve as a controlled co-encapsulation system of vascular cells and cytokines for in situ regeneration of ischemic tissues [65]. These researchers reported that a hydrogel scaffold of well-defined geometry was created and modified with laminin-derived peptides in an aqueous solution, thereby maintaining the geometry of the scaffold, while introducing bioactive peptides that enhance cell adhesion and neurite outgrowth.

4.5 Limitations of bioactive materials in tissue engineering

Although bioactive materials have been used as tissue substitutes or grafts with some success for several years, limitations remain for most clinical applications of tissue-engineering constructs. Because all tissues have a complex interdependence of cell types with an interconnected 3D construct,

and most tissue-engineered scaffolds involve only one-dimensional, or at most two-dimensional structure, with cell phenotypes grown primarily in a two-dimensional configuration, this limits the clinical viability of the constructs. Moreover, tissue-engineered scaffolds at present lack an interconnected network for a circulatory system to provide nutrition and eliminate waste products, when they are transplanted [66]. The problem of insufficient vascularisation must be overcome, as most tissues are strongly dependent on blood supply for growth [52].

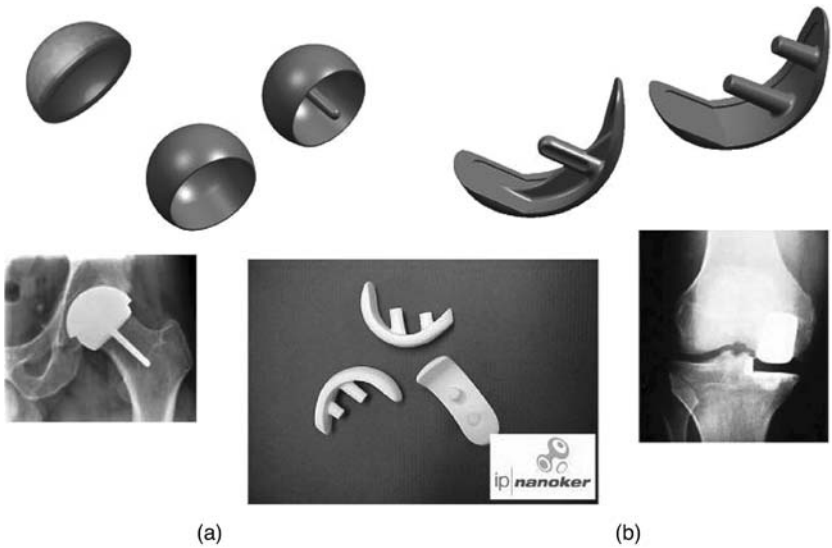
For specific bioactive materials, calcium phosphate ceramics and bioactive glasses have demonstrated good bicompatibility and clinical successes in some specific applications. However, calcium phosphates and bioactive glasses lack sufficient mechanical integrity, which limits their use for load-bearing applications. Since high porosity is essential for osseointegration, using ceramic–polymer composites is a better way to achieve less brittle bone implants. Ideally, the combined advantages of both polymer and ceramic characteristics can ensure a scaffold with stiffness and toughness similar to the replaced bone. Such composites can be based either on a polymer or a ceramic matrix [67]. The use of resorbable porous composite scaffolds, consisting of PCL, PLA, polysulfone or their copolymers with additions of inorganic particles or fibres (mainly bioactive glass or Hap) [68, 69], may produce optimal properties for the clinical application. However, polymers usually present lower modulus and creep resistance compared to bone, and, normally, the stiffness and strength cannot meet the expectations, owing to the lack of interfacial bonding strength between the inorganic phase and the polymer substrate. This would limit their clinical use for bone substitution [50].

Ideal scaffolds should have the ability to maintain mechanical loading and transmit it to the cells. Examples of minimally invasive, resurfacing ceramic orthopaedic implants currently developed in the framework of the Nanoker European integrated project to try to address this challenge [67] are shown in Fig. 4.4.

Limitations remain for tissue engineering with respect to the large, damaged organ or tissue. The incorporation of stimulatory factors, such as bioactive molecules, gene therapeutical molecules, physical factors (e.g. mechanical loading), or using bioreactors, can lead to enhanced tissue production. Ultimately, regulatory approval may present a challenge to clinical utilisation.

4.6 Future trends

To mimic and create tissue architectures using tissue engineering represents a major challenge to biomedical science. Although many bioactive ceramics have been used in bone tissue engineering, owing to their beneficial



4.4 Design of minimally invasive implants: (a) hip resurfacing and (b) unicondylar knee prostheses.

properties of stiffness and strength, potential still exists for major advances to be made in the future. These include improvements in the performance of biomedical coatings, in terms of their mechanical stability and ability to deliver biological agents, and development of improved bioactive composites. An improved understanding of the biological system, such as the exact bonding mechanism between bone mineral and collagen, needs to be achieved. Whether there is a more complex signalling process involved with the proteins in collagen, when constructs are implanted, remains unknown. If we were able to understand fully the fundamentals of the bone response to specific ions and the signals they activate, we would be able to design better bioceramics in the future [70].

The future trend in tissue engineering is to design and produce reproducible bioactive and bioresorbable 3D scaffolds, with interpenetrating pore structure and appropriate porosity, which are able to maintain their structure and integrity for predictable times, even under load-bearing conditions. The incorporation of biomolecules, such as growth factors, for local bone healing is currently under extensive research. A promising strategy is the immobilisation of proteins and growth factors in the post-processing phase via surface functionalisation of the scaffold [50]. Many current studies are focused on the design of bioactive materials with multiple patterns, micro- and nanoscale domains for specific tissue and organ patterning, which will be critical to complex tissue engineering [71, 72].

It should be feasible to design a new generation of bioactive materials that

are able to incorporate gene stimuli in biomaterials for an individual patient with a specific disease. Tissue-engineered constructs based on a patient's own cells may be produced to achieve an optimal pharmaceutical treatment [51]. The understanding of scaffold–cell interaction, and cell behaviour in an artificial 3D environment, will assist the design of scaffold materials for the regulation of cell growth.

4.7 References

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5

Antibacterial bioactive materials

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Abstract: Bioactive materials with antibacterial properties have significant medical interest. Antibacterial bioactive materials can be prepared by simple combination of antibacterial substances with materials such as hydrogels, ceramics, metals, and polymers, in different forms, such as fibres, foams, films or gels. The delivery of the antibacterial molecules will lead to the killing of bacteria. Another approach is to design the material itself to possess the antibacterial properties, especially at the surface of the material. The applications have been found in orthopaedics and cardiovascular grafts, as a means of reducing the incidence of infection. In the wound care industry, using antibacterial bioactive materials to control infection is always the first line for wound treatment. However, there are still considerable limitations, including the difficulty in treatment of the infection at depth, the control of the biofilm formation, and the development of generic and specific antibacterial bioactive materials. In future, bioactive materials based on biomimetic materials with antibacterial properties will be developed from natural resources to minimize the negative impact on the human body.

Key words: antibacterial materials, anti-infection materials, biofilm, wound care.

5.1 Introduction

Antibacterial medicine has been commonly used for the treatment of infections. The definition of an antibacterial bioactive material is a material possessing the activity to destroy bacteria or suppress their growth or their ability to reproduce. Over the past several years, infectious disease management has become an increasing challenge for physicians. Management of bacterial infection has become difficult due to the emergence of drug-resistant bacteria. There has been an alarming increase in the number of resistant Gram-positive organisms over the last 5 years.

These Gram-positive organisms include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Streptococcus pneumoniae*. Reports from several centralized agencies that follow bacterial resistance trends indicate that the prevalence of methicillin-resistant *S. aureus* (MRSA) has increased from 25 to 37% and vancomycin-resistant *E. faecium* (VRE) from 35 to 65% in the last 3 years alone. These resistant organisms represent a major cause of morbidity and mortality in hospitalized patients with hospital-acquired infections (HAIs). However, the problem of resistant Gram-positive organisms is not limited to the hospitalized patient. Outpatients have also been affected, with the emergence of penicillin-resistant *S. pneumoniae* (PRSP), a cause of community-acquired pneumonia [1].

Hospital-acquired infections and the healthcare environment have attracted considerable worldwide attention in the past few years, owing to many occurrences and outbreaks of MRSA and VRE caused morbidity and mortality. The search for methodologies to prevent and treat infections is currently an important clinical topic.

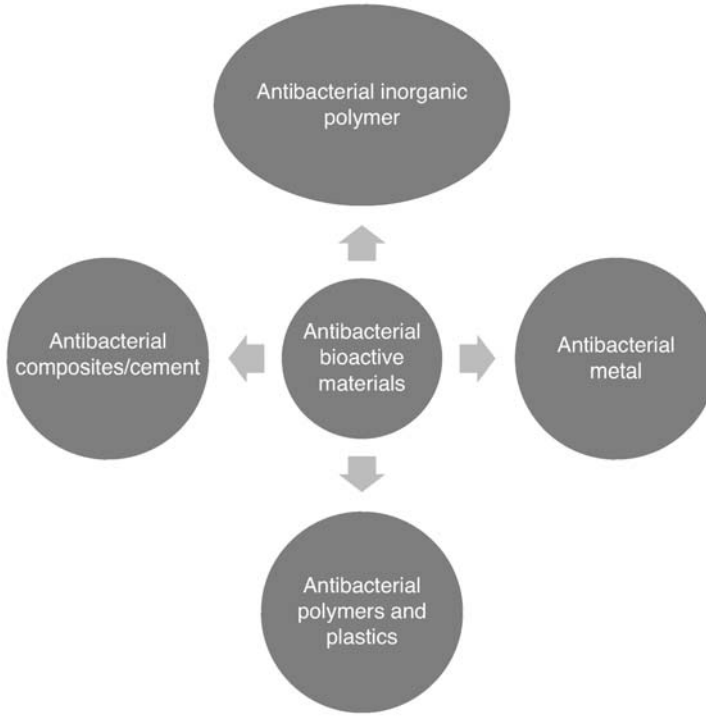
It has been estimated that the current global anti-infective market is valued at US$66.5 billion, with bioactive antibacterial agents accounting for over 50% of sales. The antibacterial market is set to grow to over US$45.0 billion by 2012, driven by the uptake of newer antibacterial agents, such as glycopeptides and carbapenems, which demonstrate resistance to MRSA and VRE, as well as other emerging strains. Pharmaceutical companies continue to develop a new generation of antibacterial agents, such as cephalosporins, macrolides, and quinolones, to overcome the major issue of drug resistance. In addition, a number of new drug classes, effective in multi-drug-resistant organisms, such as dihydrofolate reductase inhibitors (DHFRs), are under evaluation [2].

In this chapter, the focus is on bioactive materials with antibacterial functionality for use in the medical device related health care industry, for example, wound care [3], dental and orthopaedics [4, 5], and cardiovascular. The antibacterial materials discussed in this chapter include: antibacterial inorganic polymers, such as bioglass, ceramics, glass–ceramics, and zeolites; antibacterial composites, such as bone cement; antibacterial metal; antibacterial polymers and plastics (Fig. 5.1).

5.2 Antibacterial materials

5.2.1 Antibacterial inorganic polymer

Antibacterial ceramics have recently received great attention, because of their wide range of applications, including electronics and medical applications, and various forms, such as fibres, fabrics, building materials,



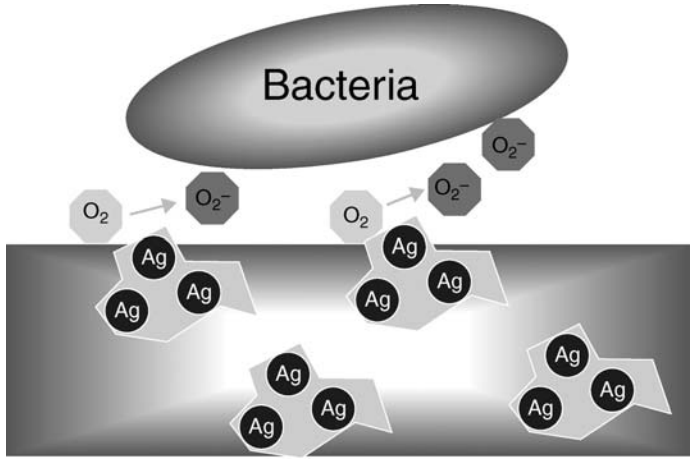
5.1 The classification of antibacterial bioactive materials.

storage containers, and devices. With or without the incorporation of certain metal ions into the ceramics, bioceramics, including bioglass, ceramics or glass–ceramics, can exhibit excellent antibacterial properties [6–8].

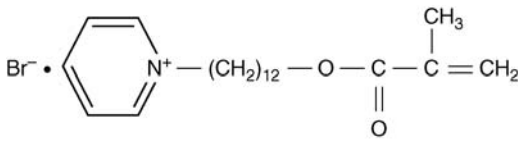
Among the metallic elements, heavy metals such as silver, zinc, copper, mercury, tin, lead, bismuth, cadmium, chromium, and thallium possess antibacterial properties and the exchange with these metals imparts antibacterial activity to inorganic polymers, such as zeolites and zirconium [9–12]. The antibacterial effects of silver-supported zirconium phosphate or silica gel are not due to the release of silver ion but to the activation of oxygen based on the catalytic action of silver [12, 13] (see Fig. 5.2).

5.2.2 Antibacterial composites – bone cement

It is a common practice to incorporate antibacterial materials into curable resins to obtain antibacterial composites for medical application. For example, loading polymethylmethacrylate (PMMA) bone cement with antibiotics to reduce infection rates has been proposed [14, 15]. Antimicrobials, such as chlorhexidine, have been incorporated into both



5.2 Schematic diagram of the antibacterial effects of composites containing non-releasing silver-supported powders. Activated oxygen is produced based on the catalytic action of silver in composites to show antibacterial effects [12].



5.3 Chemical structure of 12-methacryloyloxydodecylpyridinium bromide (MDPB).

glass ionomer cements (GICs) and resin-modified glass ionomer cements (RMGICs) to improve their antibacterial properties. This agent has been described as the gold standard for antibacterial application [16].

Other than the above approach of direct incorporation of antibacterial medicine into a bone cement system, a monomer such as 12-methacryloyloxydodecylpyridinium bromide (MDPB) (see Fig. 5.3) has the potential to be polymerized and incorporated into dental resin-based materials, such as dentin bonding primer/resin, to make a composite with bactericidal activity but having no adverse effect on biocompatibility [17, 18].

Composites based on biodegradable polymers and ceramics or bioglass have found wide application in bone tissue repair. The inclusion of antibacterial properties to combat bone tissue infection is an attractive approach in clinical application. The design of the bioactive materials can be achieved by simple blending and mixing of antibacterial materials or antibacterial molecules into the bulk to achieve a controlled release of the antibacterial substance. However, as the surface is normally the place where

there is contact with the body, a surface with anti-infection function is sometimes critical. Tokuda *et al.* [19] developed a method of blending PLA and calcium carbonate and siloxane with the mercapto groups to form a composite for guided bone regeneration. The mercapto groups have the capability to adsorb silver at the composite surface to ensure the antibacterial properties for the bone implant.

5.2.3 Antibacterial metal

Silver ions have long been recognized to possess strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities. Silver-doped titanium dioxide powder can show a marked antibacterial activity even without the presence of light. The antibacterial activity of the silver-doped titania material was influenced by the methods of preparation, such as sol–gel, ion-exchange, melting, and the effect of reactants (sulfate, chloride, and organic derivatives) and the calcination temperature [20].

Another method for producing antibacterial metal is to deposit noble metal at a surface of another material to form a thin film, using a process called reactive magnetron sputtering, which is a form of physical vapour deposition. For example, NUCRYST developed a technology to produce a nanosilver antibacterial thin metal surface. The process is reviewed as follows:

> in a vacuum chamber, pure silver is bombarded with positive ions to liberate or sputter individual atoms. The silver atoms are activated by an entity known as plasma, often referred to as a fourth state of matter. These silver atoms are then re-condensed to form new high-energy nanocrystalline structures on substrates – such as high-density polyethylene for non-adherent wound care dressings [21].

The nano-crystal silver used in NUCRYST Pharmaceuticals' existing medical devices and emerging pharmaceutical product line is between 1 and 100 nm and is being developed to target a wide range of potential pharmaceutical products [22–24].

Titanium dioxide (TiO_2) under ultraviolet A (UVA) has a well-recognized bactericidal effect on the treatment of bio-implant-related infections [25, 26]. Many commercial products have been developed based on this technology for antibacterial applications in hospital and other bacteria-prone environments.

Metals such as copper (Cu) and silver (Ag) have been deposited photocatalytically on TiO_2 coatings for the purpose of enhancing their antibacterial activity and to make these coatings work even in the dark. For example, antibacterial tiles based on this technology work effectively both under dark and illumination conditions, the effect being much higher under

light. This can be extended to fabricate photocatalytically modified Ag–TiO_2 coatings on silicone catheters and medical tubing, which effectively sterilize the microorganisms under dark conditions. Such coatings are useful for indwelling catheters, which are used inside the body, where guiding of light is a problem.

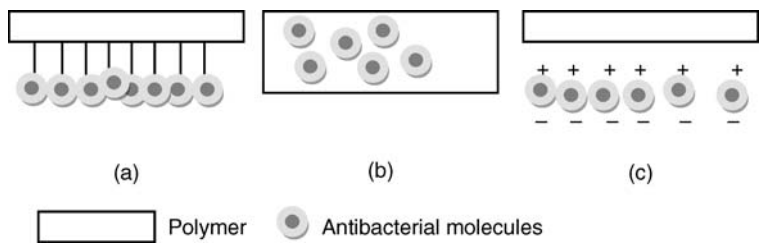
5.2.4 Antibacterial polymers

To design an antibacterial polymer, there are three approaches to have the antibacterial molecules incorporated into the system: covalent bonding, physical mixing, and physical complexation (Fig. 5.4).

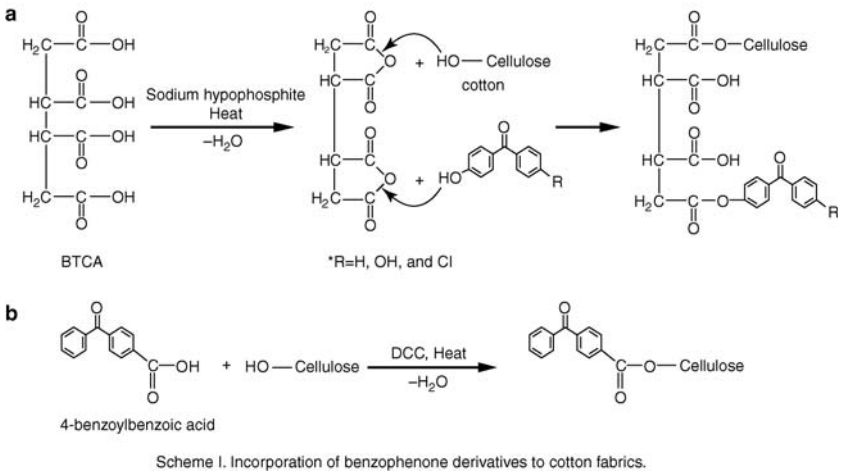
Covalent bonding

This is an approach to permanently attach the antibacterial moieties to the polymer system via covalent bonds. For example, antibacterial moieties, such as different benzophenone chromophoric groups, were incorporated onto cotton fabrics by reacting with 4-hydroxybenzophenone, 4, 4′-dihydroxybenzophenone, 4-chloro-4′-hydroxybenzophenone and 4-benzoyl-benzoic acid, and via a pad-dry-cure method. Antibacterial assessment of the benzophenone derivative-treated cotton fabrics was performed against *S. aureus* and *E. coli*. 4-Hydroxybenzophenone-treated cotton fabric demonstrated the most effective antibacterial ability, as shown in Fig. 5.5 [27].

Cellulose fabric can be chemically modified with the triazine derivatives containing the multi-cationic benzyl groups as shown in Fig. 5.6. The novel cellulose biomaterial containing the multi-cationic benzyl groups displayed excellent, durable antibacterial properties [28].

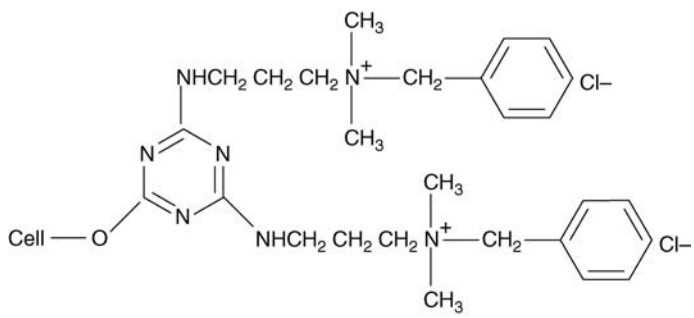


5.4 Design of antibacterial polymers: (a) covalent bonding antibacterial molecules on polymer surface; (b) polymer blending with antibacterial molecules; (c) complexing of antibacterial molecules with polymer via ionic or coordination.



Scheme I. Incorporation of benzophenone derivatives to cotton fabrics.

5.5 Covalent bonding antibacterial moieties on cotton fabrics.



Scheme 1. Chemical structure of the modified cellulose.

5.6 Covalent bonding antibacterial moieties onto cellulose.

Metal ion-containing polymers

Polymers having biocidal activities can be designed by introducing divalent transition metal salts metal ions, such as Zn^{++}, Cu^{++} into the polymer main chain to form a complete network [29–31]. These polymers have found application as antibacterial coatings [29] and they are soluble in dimethyl sulfoxide (DMSO), dimethyl acetamide (DMAc) and dimethyl formamide (DMF) [31]. In particular, poly(urethane–urea)s (PUUs) and poly (urethane–ether)s (PUEs) had satisfactory biocompatibility and biodegradability properties, which could potentially lead to a variety of blood-contacting applications. Other metal-containing polymers, utilizing sustainable resources, such as linseed oil-based polyesteramide, have also been developed for antibacterial purposes. It was found that minor incorporation

of zinc in linseed oil-based polyesteramide exhibited improved antibacterial activities against *E. coli* and *S. aureus* [32].

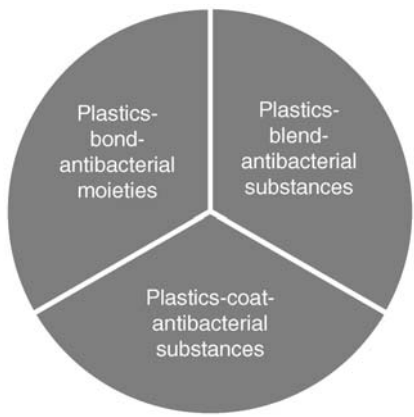
Antibacterial plastics

Plastics, combining low cost with good mechanical properties and easy processability, are widely used to prepare biomedical devices and food packaging, in which sometimes, antibacterial properties are essential. To obtain plastics with antibacterial properties, there are three general approaches, as shown in Fig. 5.7.

Water-insoluble antibacterial plastics via a chemical bonding approach is a type of environmentally friendly disinfection material, as it has no leaching of chemicals to the environment, and it has attracted much attention. For example, quaternary ammonium salts (QAS) moieties have been polymerized into plastics [33–36]. Organic–inorganic hybrid coatings containing QAS bonded to the organic–inorganic network were prepared from tetraethoxysilane and triethoxysilane terminated poly(ethylene glycol)-block-poly (ethylene) using a sol–gel process [37]. N-alkylated poly(4-vinyl-pyrdine) moieties [38, 39] have also been synthesized to demonstrate their effective long-term antibacterial properties.

Other than chemical bonding approaches, trials to achieve an antibacterial composite by simple blending can be carried out by two approaches.

1. Alterations to the resin components:
   (a) addition of soluble antimicrobial agents into the resin matrix [40–42];
   (b) immobilization of an antibacterial component into the resin matrix, utilizing an antibacterial monomer [43–45].



5.7 Approaches to the preparation of antibacterial plastics.

2 Alterations of the filler components:
   (a) addition of a component of silver as filler: silver-containing silica glass; silver zeolite/silver apatite; silver-supported zirconium phosphate/silver-supported silica gel [46–53];
   (b) addition of a non-biocide component as filler antimicrobial polymers and photocatalytic ingredients, which when exposed to light generate free radicals [42].

Antibacterial PVC composite

Polyvinyl chloride (PVC) is the most widely accepted biomaterial in medical applications. Microbial attack can be prevented by the incorporation of an effective biocide (also known as biostabilizer) into the plastic. There is a wide range of biocides available; this includes, among others: 10, 10′-oxybisphenoxarsine (OBPA), trichlorohydroxydiphenylether (Triclosan), n-octyl-isothiazolinone (OIT), 4,5-di-chloro-isothiazolinone (DCOIT), mercaptopyridine-n-oxide (pyrithione), butyl-benzisothiazolinone (butyl-BIT), metal-based biocides, such as organotin and silver, non-biocide additives, including inherently, antimicrobial polymers and photocatalytic ingredients, which when exposed to light generate free radicals.

To develop antibacterial PVC products, various types of antibacterial filler have been incorporated into the blending system to make composites for further processing into finished products. For example, zeolites containing Ag, Cu and zinc (Zn) powders have been incorporated into PVC blend to manufacture plastic products [52].

Other polymers such as thermoplastic olefins (TPO), thermoplastic elastomers (TPE), and polyurethanes, do not contain plasticizer, which can potentially provide a carbon source for microbial growth, but may still require protection from antimicrobials. Organic antimicrobial additives are compounded into the polymer, where they diffuse to the surface and destroy microorganisms by interfering with enzyme activity. As antimicrobial additive at the surface is used up or washed away, additive from the polymer matrix continues to come to the surface, providing extended performance [54, 55]. The strongest growth is for inorganic, silver-based biocides, with recent utilization in a broad range of polymers, applications and functions [53].

Antibacterial polymers have also been used as coating materials for the local delivery of antibiotics in implants. In general, they must be biocompatible and biodegradable, and the release profile of the active substance needs to meet the clinical requirements [56]. Commonly used coating materials are polyester urethane [57], polyester-polyurethanes containing different ratios of poly (lactic acid) diol and poly(caprolactone) diol [58], and other bioresorbable polymer, such as PLA and other coating materials [56].

5.2.5 Natural antibacterial materials

Researching alternative antibacterial materials to synthetic ones has been an attractive topic for many years. Natural antimicrobial peptides (AMPs) are the most popular natural biopolymers in terms of attention received. They can be cationic and anionic peptides. Typical examples are cecropins, defensins, thionins, amino-acid-enriched class, histone-derived compounds, beta-hairpin and lactoferrin, neuropeptide-derived molecules, aspartic-acid-rich molecules, aromatic dipeptides, and oxygen-binding proteins, such as bacteriocins [59].

Bacteriocins [60, 61] are usually non-toxic, odourless, colourless, and tasteless. Since their modes of action differ from those of conventional antibiotics, including their targeting of a much narrower range of bacterial species, cross-resistance of bacteriocins with systemically-administered antibiotics would be unlikely to develop. Also, because they are generally inactivated by one or more of the proteolytic enzymes present in the digestive tract of humans, they would be metabolized just like any other dietary protein. Finally, bacteriocins as natural products may have better public acceptance than synthetic chemical agents [59].

Lactoferrin (formerly known as lactotransferrin) is a glycoprotein, and a member of a transferrin family, which belongs to those proteins capable of binding and transferring $Fe^{3}+$ ions [62]. It was first isolated by Sorensen and Sorensen [63] from bovine milk in 1939. Lactoferrin affects the growth and proliferation of a variety of infectious agents, including both Gram-positive and negative bacteria, viruses, protozoa, or fungi [64].

5.2.6 Antibacterial nanomaterials

The rapid growth in nanotechnology has spurred significant interest in the medical application of nanomaterials. Many materials at the nanoscale exhibit superior antibacterial properties than their origins, which are not at the nanoscale. The most commonly reported antibacterial nanomaterials include: silver nanoparticles (nAg) [65–67], nanosilver-based nanocomposites [68–70], silver-liposome [71], chitosan-based nano-biopolymer [72], photocatalytic TiO_2 [73], fullerol [74], aqueous fullerene nanoparticles (nC60) [75], and carbon nanotubes (CNT) [76]. Among all the nanomaterials, nanosilver has been receiving the most attention.

Nair *et al.* [77] reported a one-pot synthesis of silver nanoparticle–polymer composites (Ag–PNCs) in water, involving the polycondensation of methoxybenzyl chloride (MeO-BzCl) directly on silver nanoparticle surfaces at room temperature, leading to highly soluble antimicrobial nanocomposites. The composites, which are soluble in a range of organic solvents, precipitate in the reaction vessel, making their separation simple. Solutions

of the composites can be cast directly on substrates or made into freestanding films. The material was found to be stable for nearly 2 years. A range of substrates have been shown to become antibacterial by direct coating application of this material. It was claimed that the simple one-pot approach of this type to produce organic-soluble antibacterial coatings could have wide implications.

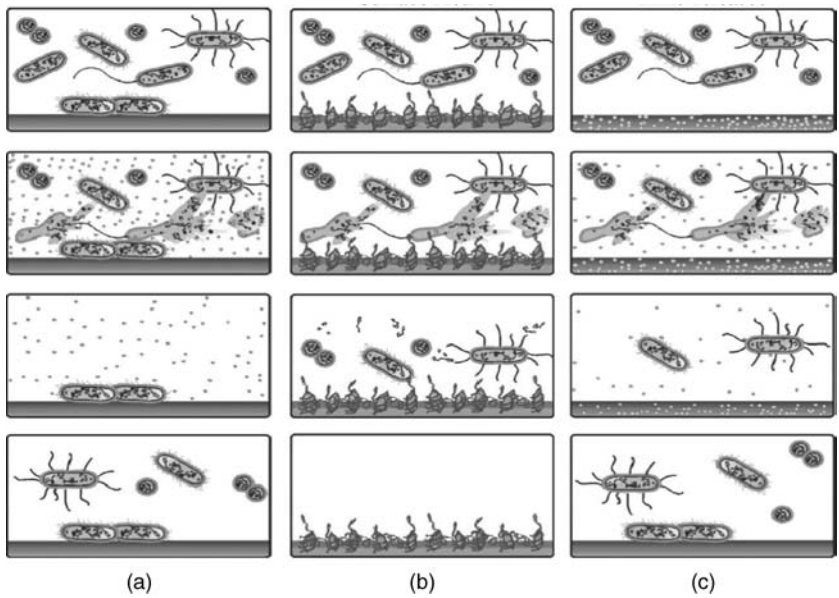
Barani *et al*. [71] reported a method for incorporating nanosilver particles into a liposome structure. The large silver-liposomes nanocomposites are transformed to the smaller silver-liposome nanocomposites (from 342 to 190 nm) through sonication treatment. The stabilized silver nanoparticles with various concentrations showed a good antibacterial activity against *Staphylococcus aureus*, a Gram-positive bacterium, and *Escherichia coli*, a Gram-negative bacterium.

Apart from liposome, silver (Ag) and silver sulfide (Ag_2S) nanoparticles can also be synthesized in a sago biopolymer, such as a starch matrix [68], hydrogel-based nanosilver nanomaterials [69], and other polysaccharide-based nanosilver systems, such as chitosan and alginate [70], where, at high concentrations, there is a release of silver nanoparticles from the composite in the water environment. In particular, for the hydrogel system, antimicrobial results show that these nanocomposite systems display a very effective bactericidal activity toward both Gram-positive and Gram-negative bacteria. However, the hydrogel does not show any cytotoxic effect towards three different eukaryotic cell lines. This is because the nanoparticles, immobilized in the gel matrix, can exert their antimicrobial activity by simple contact with the bacterial membrane, while they are not taken up and internalized by eukaryotic cells. This novel finding could contribute advantageously by responding to the growing concern over the toxicity of nanoparticles and facilitate the use of silver–biopolymer composites in the preparation of biomaterials.

5.3 Clinical applications of antibacterial materials

Despite the most stringent sterilization and aseptic procedures, bacterial infection remains a major impediment to the utility of medical implants, including catheters, artificial prosthetics, and subcutaneous sensors. Indwelling devices are responsible for over half of all nosocomial infections, with an estimate of one million cases per year (2004) in the USA alone. Device-associated infections are the result of bacterial adhesion and subsequent biofilm formation at the implantation site [78].

Much research has focused on developing a medical device surface that resists bacterial adhesion. In general, the mode of antimicrobial action of a surface may be (a) external, (b) surface active, and (c) time released (see Fig. 5.8). Each mode of action has its advantages and disadvantages. The



5.8 The three general modes of antimicrobial surface-mediated activity [79]: (a) external; (b) surface active; (c) time released.

external mode is represented by disinfectants as applied to surfaces that compromise the structural integrity of the microorganisms they contact. It is a blanket antimicrobial approach, where sufficient quantities kill the microorganism and may also affect humans. The surface-active mode can be represented by the selective transferral of antimicrobial surface agents into the microorganism until toxic accumulation occurs or membrane disruption occurs causing cellular leakage. Finally, the time-released mode consists of discharging antimicrobials in response to an environmental trigger, such as a change in surface pH, moisture, pressure induction, in which either of the latter conditions may be initiated by surface attachment of the antimicrobials, or a temperature change [79]. Most of the clinical applications of antibacterial materials are based on surface-active and time-release modes. However, in general practice, the first mode of disinfection is widely applied as a routine cleaning regime.

Typical applications of antibacterial materials range from products such as general hospital equipment (e.g. hospital beds and chairs), healthcare furnishings, medical packaging, and door handles, to high-grade medical devices like intravenous (IV) access systems, urological devices (catheters), bone cements, dental repairing materials, vascular grafts, and wound care products. These products with antibacterial properties can control the growth of bacteria on the surface of medical devices in an attempt to address the increasing problem of healthcare-acquired infections.

Table 5.1 Silver-based wound dressings

Product brand name	Manufacturers	Materials/structures	Modes of action
Acticoat	Smith & Nephew	Polyethylene mesh coated with nanocrystalline (<20 nm diameter) silver and two layers of rayon polyester	Sustainable release silver from nanocrystalline silver
Actisorb Silver 220	Johnson & Johnson	Activated charcoal dressing with bound silver	Adsorbing bacteria onto the charcoal component, where they are killed by silver
Aquacel-Ag hydrofibre	Convatec	70:30 sodium: silver carboxymethylcellulose hydrofibre	Sustainable release of silver ions
Arglaes	Maersk Medical UK/Medline	Silver/alginate	Sustainable release of silver ions
Contreet-H Contreet-F	Coloplast	Dense hydrocolloid /foam dressing bound with silver	Sustainable release of silver ions
SilvaSorb	Medline	Silver /hydrogel	Maintain a moist wound-healing environment with the benefits of sustained release antimicrobial silver
Silverlon	Argentum	Polymeric fabric coated with metallic silver	Sustainable release silver ions

5.3.1 Wound care

Wound care is a major healthcare market with an estimated value of US$10 billion in 2007, predicted to grow to US$12.5 billion in 2012 [3]. In this industry sector, the antibacterial materials play a very important role in combating wound infection. One development in the wound care market that has found favour with clinicians is the impregnation of products with an antimicrobial to reduce the risk of microbial infection.

The early use of silver in wound care was silver sulphadiazine (AgSD) cream, developed in the 1960s, for the treatment of burns [80]. Recently, a trend towards the use of wound cover dressings that contain silver has been evident [81], and today, a selection of foam, film, hydrocolloid, gauze, and dressings with silver technology – in which the wound dressings are impregnated with silver – are commercially available, as shown in Table 5.1.

Thomas and McCubbin [82, 83] compared the *in-vitro* effectiveness of various silver-containing products, using three methods – zone of inhibition, challenge testing, and microbial transmission testing – to demonstrate differences in the various dressings. Results against *Staphylococcus aureus*,

Escherichia coli, and *Candida albicans* suggested that polyethylene mesh had the most rapid antimicrobial effect due to its rapid release of silver. Hydrocolloid was similar but had a slower onset. Activated carbon had little activity on the surface, but organisms that were absorbed into the dressing were inactivated by the silver [82].

Iodine and polyhexamethylene biguanide (PHMB) are two popular antibacterial agents used in wound dressings. For example, company Smith & Nephew developed Cadexomer iodine (IodoflexAE and IodosorbAE), which is a three-dimensional starch lattice formed into spherical microspheres that trap iodine in the lattice. As fluid is absorbed, the pore size of the lattice increases, releasing iodine.

Polyhexamethylene biguanide (PHMB), also known as polyhexanide and polyaminopropyl biguanide, is a commonly used, fast-acting, and broad spectrum antimicrobial, providing activity against a wide range of bacteria (including MRSA, *Salmonella* spp., *Campylobacter* spp., *E. coli* 0157) and viruses (for example, VANTOCILTG antimicrobial has been independently shown to provide activity against FCoV, feline coronavirus at 0.2% product incorporation). PHMB-based wound dressing products include Kerlix AMD99, Excilon AMD99, and Telfa AMD99 (all from Tyco HealthCare Group, Mansfield, Massachusetts), XCellAE Cellulose Wound Dressing Antimicrobial (Xylos Corp, Langhorne, Pennsylvania) and COSMOCIL™ CQ antimicrobial (ARCH).

OXYZYME™ and IODOZYME™ active wound healing dressings are based on the biochemistry enzyme reaction system to generate a low level of hydrogen peroxide and iodine [84]. It has been claimed that the Oxyzyme dressing produces a peak surface concentration of iodine approximately 50 times lower than traditional 'iodine dump' dressings, such as those based on povidone-iodine. However, the concentration of the iodine is sufficient to produce an environment hostile to bacteria at the surface of the dressing.

5.3.2 Musculoskeletal and orthopaedics

Antibacterial materials have been widely used in dental and orthopaedic implants for many years, since bacteria are still a concern and a recurrent cause of failure for implants [85]. In the USA alone, the annual cost for the symptomatic treatment of dental infections in 1977 was estimated at over $11 billion , increasing to about $24 billion in 1984 and $34 billion in 1990 [4]. It was also reported that the infection rate of total joint arthroplasties is in the range 0.5–5% among over half a million implants used in the USA alone [86, 87]. The complication of infected implants quite often leads to surgical intervention at high health and social cost. For example, it was estimated that treatment of each single episode of infected arthroplasty costs over $50 000 [88]. Bone cements with antibiotics have been widely accepted

Table 5.2 Antibiotics-eluting bone cements [89]

Product brand name	Manufacturer	Materials/antibacterial substance	Clinical applications
Cobalt™ G-HV/ Palacos® G	Biomet	Gentamicin/PMMA bone cement	Arthroplasty
DePuy 1	Depuy	Gentamicin/PMMA bone cement	Arthroplasty
Cemex® Genta	Exactech	Gentamicin/bone cement	Fixation of prostheses to living bone for use in the second stage of a two-stage revision for total joint arthroplasty after the initial infection has been cleared
VersaBond™ AB	Smith & Nephew	Gentamicin/polymer powder and monomer liquid	Fixation of prostheses to living bone for use in the second stage of a two-stage revision for total joint arthroplasty after the initial infection has been cleared
Simplex® P	Stryker Orthopedics	Tobramycin / 75% methyl Methacrylate–styrene–copolymer/ 15% polymethyl-methacrylate/ 10% barium sulfate	Fixation of prostheses to living bone for use in the second stage of a two-stage revision for total joint arthroplasty after the initial infection has been cleared.

in clinical use, as shown in Table 5.2 [89]. Other than bone cements with antibiotics, coatings containing antibiotics in orthopaedics devices are also used clinically. For example, gentamicin, which has a relatively broad antibacterial spectrum, has been loaded on polymers for coating titanium implants [90, 91]. In addition, other antibiotics with broad antibacterial spectra, for instance, cephalothin, carbenicillin, amoxicillin, cefamandol, tobramycin, and vancomycin, have been used in coatings on bone implants [91–94].

5.3.3 Cardiovascular

Vascular graft infections represent one of the most challenging issues in surgery, having an incidence of 0.7–13%, with femoral site infections being the most common (13% incidence). Infection of vascular prosthetics implanted for arterial occlusive disease occurs in approximately 1–5% of patients, including early and late clinical presentation [95, 96]. Routine excision of infected peripheral arterial grafts and vascular reconstruction with extra-anatomic conduits are associated with mortality rates ranging from 10 to 30% and amputation rates of up to 70% [95]. Clinical data have

reported that in situ replacement with a rifampicin-bonded prosthesis has been accomplished successfully in a small number of patients and shows promising early results. Advances in the management of infected vascular prostheses over the last decade have led to improved mortality and decreased amputation rates with conventional excision and extraanatomical bypass. Newer methods, including in situ graft replacement with antibiotic-impregnated prosthetics, appear suitable for low-virulence *S. epidermidis* infection [96].

Stone *et al*. [97] implanted PMMA beads loaded with an antibiotic (vancomycin, daptomycin, or tobramycin/gentamicin, or a combination of these) to treat vascular surgical site (VSS) infections. Results indicate that antibiotic-loaded PMMA beads may be a useful adjunct in the contemporary surgical management of VSS infection involving a prosthetic graft. Another approach to treat methicillin-resistant *Staphylococcus aureus* (MRSA) or *S. epidermidis* prosthetic vascular graft infections has been carried out by in situ replacement with a rifampicin bonded Gelsoft graft [98].

An InterGard Silver (IGS) collagen and silver-acetate-coated polyester graft was used to replace an infected vascular prosthesis in situ. Preliminary results in this small series demonstrate favourable outcomes with IGS grafts used to treat infection in abdominal aortic grafts and aneurysms caused by organisms with low virulence. Larger series and longer follow-up will be required to compare the role of IGS grafts with other treatment options in infected fields [99]. A multicentre clinical study further demonstrated that the InterGard Silver graft is safe with no side effects. The primary patency rate was excellent, and the graft infection rate was low, despite a high incidence of nosocomial infections [100]. A comparison to show the efficacy of collagen silver-coated polyester and gelatin-sealed grafts with rifampin-soaked vascular grafts to resist infection from MRSA and *Escherichia coli* was carried out. The results indicate that collagen silver-coated grafts and gelatin-sealed grafts, both soaked in rifampin, provide resistance against MRSA and *E. coli*. There was a trend toward better resistance but without statistical significance against *E. coli* from the rifampin silver graft compared with the rifampin-soaked Gelsoft graft, without signs of inflammation from InterGard silver grafts [101].

Antibiotic retention on polytetrafluoroethylene (PTFE) grafts prepared using three antibiotic-bonding methods was compared following implantation into the arterial circulation. Ciprofloxacin or silver-ciprofloxacin was bonded to PTFE surfaces by surfactant-mediated or direct bonding methods. Bonding of silver-ciprofloxacin on PTFE grafts provided an effective source of local antibiotic release at levels which may be useful for bypass grafting in contaminated wounds or for in situ replacement of grafts infected by the central nervous system (CNS) [102].

Infection is a major complication in vascular stents. Stents impregnated with gelatin and dipped in Rifampicin have been shown to resist methicillin-resistant *Staphylococcus aureus* in both animal experiments and in man [103].

TYRX's AIGISRx, a commercially available antibacterial envelope for use with cardiac rhythm management devices (CRMD), and PIVITAB™, a new surgical hernia mesh licensed to C. R. Bard, both elute the powerful antibiotic combination of minocycline and rifampin [104].

5.4 Limitations of antibacterial materials

Antibacterial materials have been widely used clinically as medical devices, in which the active substances, such as antibacterial molecules, are present on or in the matrix of the surface of the devices, such as topical dressings for the management of wounds, including surgical, acute and chronic wounds, and burns, and implants, including long-term implants such as artificial joints, fixation devices, sutures, pins or screws, catheters, stents, and drains. Significant progress has been made in terms of the development of suitable biomaterials as carriers, the control of the release profile of the active substances, the antibacterial surface interaction with the system of the biological medium, the clinical efficacy, and, of course, the control of the manufacturing process of the final integrated device–medicine hybrids together with the regulatory issues for marketing biocides/devices, but there are still many limitations for development and application of antibacterial materials.

One limitation is the selection of the antibacterial substance, which can potentially lead to bacterial resistance. For example, the use of silver is increasing rapidly in the field of wound care, and a wide variety of silver-containing dressings are now commonplace, as reported in section 5.3.1 (wound care). However, concerns associated with the overuse of silver and the consequent emergence of bacterial resistances are being raised. In a review by Percival *et al.* [105], it is stated that although resistance to heavy metals, such as Ag^+, has been studied and reported, exact mechanisms are not known and there is little current evidence of emerging microbial resistance to silver. Unlike in the case of antibiotics, resistance to antiseptics, such as Ag^+, is rare and sporadic. Certainly, with widespread use of Ag^+ in wound care, more potential pathogens are going to be exposed to this agent. With the knowledge that silver-resistance genes exist sporadically in certain types of bacteria, it would be appropriate for future studies to determine the actual prevalence of these genes within clinical and environmental settings. Currently, knowledge is limited. Therefore, it is advised that the best approach is to keep hygiene emphasized in wound care and use wound

dressings with antibacterial materials targeted towards those applications which have demonstrable benefits [106].

Another limitation is the lack of an ideal controlled release system to minimize the cytotoxicity of antibacterial materials, which could potentially lead to the failure of the wound-healing process and tissue/implant integration, and to maximize the efficacy of the anti-infection property. For example, in a recent published paper reviewing the clinical evidence of use of Acticoat™ dressings in burns, there is evidence to suggest that Acticoat™ has improved bacterial clearance compared to other silver-containing dressings. It is easy to use, and has sustained release of silver, allowing less frequent dressing changes. This combined with its low toxicity levels make it a possible ideal dressing for burn wounds. However, despite the wide use of Acticoat™ in burns, the available evidence regarding its use in burns is weak, with only one study considered to be (level of evidence) LOE 1. More well-designed and properly reported, randomized, controlled trials are essential for informed clinical decision making [107].

According to Wittaya-areekul and Prahsarn [108], the ideal wound dressing should have the following properties: (i) provide a moisturized wound healing environment, (ii) provide thermal insulation, (iii) be removable without causing trauma to the wound, (iv) remove drainage and debris, (v) be free from particulate and toxic product, and (vi) promote tissue reconstruction processes. However, it is still difficult to get all these ideal elements in one single wound dressing, not mentioning the antibacterial function. Therefore, the factors in the material itself also limit the development of advanced antibacterial materials.

Another limitation is the difficulty in obtaining an ideal antibacterial substance, possessing all the following features:

- effectiveness against micro-organisms;
- cost-effectiveness in the end product;
- compatibility with ingredients of the final product;
- does not discolour the final product;
- can withstand high processing temperatures;
- effectiveness over a wide pH range;
- low toxicity to humans;
- extensive supporting documentation;
- high biodegradability

To bring a new antibacterial substance into the market with these features, the cost is very high. Manufacturers of active ingredients estimate that the cost for global registration is $5 million. It takes 2 years to conduct the toxicological testing and another 2–3 years for regional governments throughout the world to grant registration. End users will often want to evaluate the biocide over a 1–5 year test period. As a result of these

requirements and small potential market size, biocide manufacturers are extending product lines by turning to existing active ingredients for new applications instead of developing new molecules [55]. This would certainly limit the development of new antibacterial materials for clinical application.

Impending environmental regulations, both in Europe and elsewhere, present major challenges for suppliers of biocides. The European Union's (EU's) Biocidal Products Directive (BPD) and the flagship REACH chemicals policy will force the rationalization of many product lines, removing a large number of active ingredients from the market and requiring manufacturers to source replacement 'green' actives. In June 2007, the EU's Regulation (EC) No. 1907/2006 on the registration, evaluation, authorization and restriction of chemicals (the so-called 'REACH Regulation') entered into force. The REACH Regulation imposes sweeping requirements on both manufacturers and importers of chemicals and products containing them. In particular, REACH imposes new requirements on producers of medical devices, which are in addition to those of EU-specific medical device legislation. This will certainly add extra burden with respect to the development of antibacterial materials using antibacterial bioactive substance for medical device applications.

Another limitation is the consideration of the longevity of the effect of antibacterial properties. Using antibacterial materials does not mean it is not necessary to follow the general hygiene requirement for cleaning. For example, PVC flooring and wall coverings with an antibacterial additive have been promoted as 'hygienic surfaces' for hospital use. However, it should be stressed that it does not necessarily follow that as a result of antibacterial protection, these surfaces are no longer vulnerable to infection, and that conventional cleaning methods can be compromised, as the contamination is usually associated with the soiling of that surface with dirt, food, or bodily fluids. An 'ideal' in-dwelling medical device surface should have the same surface properties as that of a healthy living body, which would resist bacteria adherence, kill bacterial, and promote the growth of living tissues, but such in-dwelling medical devices are difficult to manufacture. Because the host-maintained immune defence system usually rejects a foreign body intrusion, and bacterial species are constantly changing, there are limitations in the device manufacturing process.

5.5 Future trends

It is expected that there will be a strong growth in the antimicrobial material industry, including plastics, implants, and other medical devices, for the following reasons [55].

- The market demands implants or other medical devices with anti-

infection effect, where the infection or other microbial contamination is a common factor, leading to the failure of implantation in clinics.
- The marketing advantages of value-added antibacterial products, which have generally met with consumer favour, and the need to ensure hygienic conditions in industrial, commercial, medical, and other institutional settings, will support further gains.
- Regulatory pressures on traditional antimicrobials, such as oxybisphenoxarsine (OBPA), 1-(1-phenylcyclohexyl) pipe (PCP), and tributyltinoxide (TBTO) promote the search for new, natural, green antibacterial substance.
- Increasing concerns related to disease transmission will drive demand for surfaces treated with antimicrobials.
- High-end industry growth in particular geographical regions, such as Asia.
- Growing use of antimicrobials as hygiene aids.
- Increasing use of plastics in new applications.

In the future, the development of antibacterial materials will focus on creating a surface that would not trigger the host defence system, possesses excellent biocompatibility, and can resist bacterial adherence or release antibacterial active substance in a controllable way. In addition, the antibacterial materials should be easy to make and possess high antibacterial activity together with a broad spectrum of antibacterial properties, fast recovery capability, and sustained delivery of antimicrobial agents [109].

The design of antibacterial materials will be mainly focused on surface treatments, as there are many advantages of surface treatment to incorporate anti-infective agents onto the surfaces of medical devices. These advantages include: a large variety of anti-infective agents on the surface can be selected; straightforward and inexpensive modification of existing devices is possible without changing the device bulk properties. For example, BIOSAFE® antimicrobial's active ingredient is a quaternary ammonium compound, made usable in plastics through organofunctional silane technology. BIOSAFE® is permanent, non-migrating, and non-toxic at a lower cost than silver-based additives. This could be an example of future antibacterial devices.

Continued efforts in the future will be required to advance the understanding of the pathophysiology of device-related infections and their effects on the functions of human homeostasis, such as the microstructure and chemical structure of the adherence mechanism, receptor sites in compromised tissue, and factors that might effectively block the initial bacterial adherence. The progress in these fundamental understandings will encourage the appearance of new technologies, which

will then provide superior anti-infective devices [110]. In addition, new technologies, including nanomedicine with antibacterial effects and tissue engineering for body repair, will generate great interest in clinical research.

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6

Bioactive materials in orthopaedics

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Abstract: Traditional orthopaedic implants are based on metallic and polymeric materials, which quite often lead to implantation failure, due to the loss of the mechanical properties, or bio-incompatibility between the interface of implant and the bone tissues. Bioactive materials have the capability of forming a strong bond between the tissue and the material, which ensures that the implant remains in place. The design of bioactive materials suitable for this application includes using bioceramics, such as hydroxyapatite (Hap), bioactive glasses, bioceramic–glass, and bioactive material-based polymer composites. Metal-based implants coated with bioactive materials are commercially available. The future trend in this field will focus on the design of new biomimetic materials as synthetic bone tissue substitutes, the surface modification of bioactive materials for improved biocompatibility, the design of controlled porous bioactive materials for improved bone tissue formation, the development of new bioactive material-based composites for bone tissue engineering, and the surface treatment of metal orthopaedic implants for improved bioactivity and biocompatibility.

Key words: bioceramics, bone repair, biomimetic materials.

6.1 Introduction

Bone is a structural natural bioactive material as well as an organic metabolic tissue. Structurally, bone serves as a support for the body against gravity, as a lever system for muscular action, and as a protective shield for internal organs and for the blood-forming marrow within the bones. The primary metabolic function is to serve as a repository for calcium, which is necessary for nerve conduction, muscle contraction, clot formation, and cell secretion. Bone includes cortical bone and trabecular bone. These represent approximately 80% and 20% of the skeletal mass respectively [1]. However, the trabecular bone represents 80% of the bone surface area. The cortical

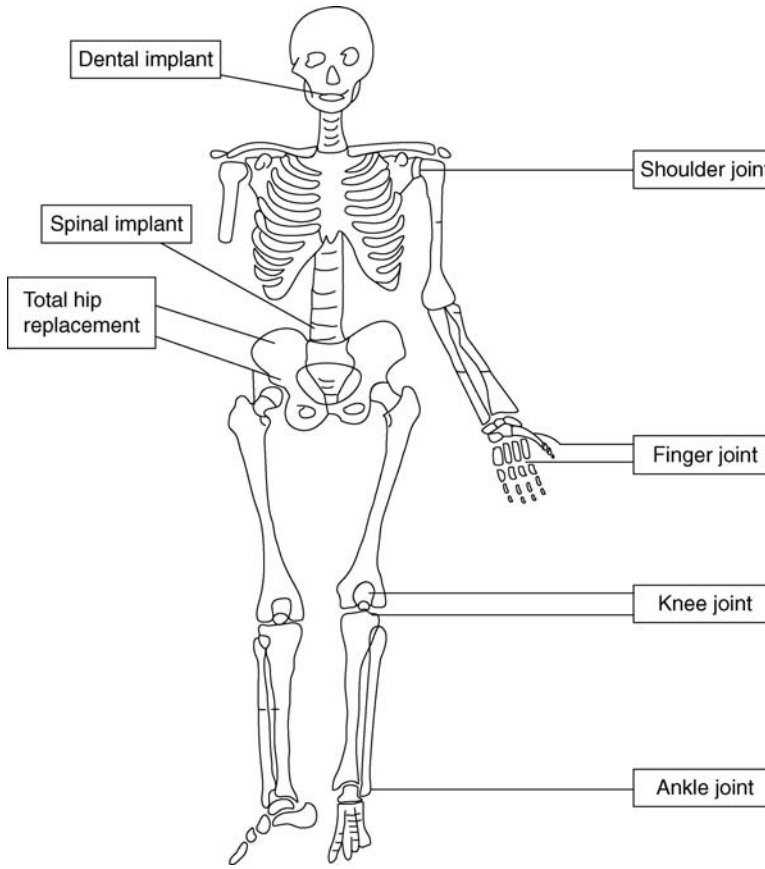
bone is also called compact bone, because it acts like a shell and surrounds the honeycomb-like trabecular bone within. The open cell configuration minimises the mass of bone, while still providing a large bearing area, allowing it to meet its primary mechanical function. Although the material may look spongy, trabecular bone is rigid, and is composed of an interconnected network of rods and plates that are fused together [2]. It is known that changes in trabecular bone volume fraction and architecture are associated with osteoporosis [3].

Cartilage is a type of dense connective tissue, which locates at the articular surface of the bones. Its mechanical properties are intermediate between bone and dense connective tissue, such as tendon. The wearing out of cartilage due to inflammation, ageing, trauma, gender, repeated and over loading, family history, obesity, and diabetes often leads to various types of arthritis.

Osteoporosis and other musculoskeletal diseases, such as rheumatoid arthritis (RA) and osteoarthritis (OA), represent the most common cause of work-limiting health problems, long-standing illness, and sickness absence on a worldwide basis. The medical costs account for nearly 8% of health service and related expenditure [4]. RA is the most common inflammatory arthropathy, with an incidence of 50 per 100 000 per year in adults. The prevalence is 500–600 cases per 100 000 (0.5–1.0%). OA is more benign but remains a major cause of disability and medical consultations, especially among the elderly. It is more common in women than men. In a population of 250 000 there will be 500–600 new cases per year, 10–20% of which will require a specialist opinion. In the over-65s, the incidence is 200–250 per 100 000 per year, with OA accounting for more than 2000 consultations per 10 000 patient per year. Clinical OA of the hips and knees affects 10–20% of people over 65 years [4].

Another area is spinal disease, which is a normal part of ageing. In the spine, osteoarthritis can cause stiffness and pain in the neck or in the lower back. Cervical arthritis (also called cervical spondylosis) affects the upper spine and neck. Lumbar or lumbosacral arthritis affects the lower back and pelvic area. Ankylosing spondylitis is another type of spinal arthritis. It is estimated that as many as 85% of adults experience back pain that interferes with their work and leisure activities, and 25% of people between the ages of 30 and 50 years report lower back pain symptoms [5].

Clinical intervention of the musculoskeletal diseases includes early diagnostics, medication, and surgery. In surgery, orthopaedic implants have been used to repair, replace, and regenerate the damaged or diseased bone tissues in order to restore function (Fig. 6.1). For example, joint prostheses are currently implanted in large numbers. In the 30 member countries of the Organisation for Economic Cooperation and Development (OECD), the rate is around 50–140 hip replacements per year for each 100 000 inhabitants. Given the total population of the OECD countries, this



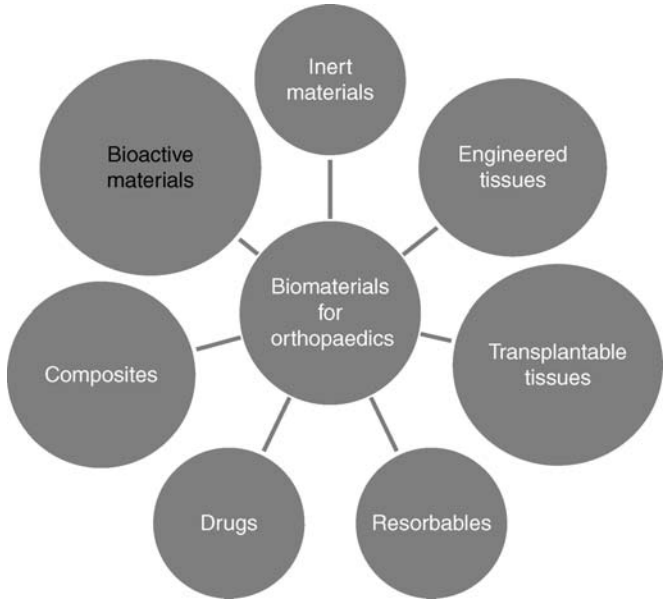
6.1 Implants for repairing bone defects.

means between 500 000 and 1 600 000 hip replacements are implanted in the more developed countries per year. Knee replacement is reaching similar incidence; so more than one million joint prostheses are implanted every year worldwide [6].

Furthermore, there is a trend to extend the treatment options in orthopaedics from traditional metal implants, plates, and screws to biologically-based products for hard and soft tissue regeneration. This new generation of products is fast gaining acceptance and offers the potential to improve a patient's quality of life and reduce health costs, which is reflected in the global orthobiologics market. It was estimated by Espicom that the market was worth US$4.2 billion in 2007 and accounted for 13% of the US$33 billion total orthopaedic market. With an annual growth rate of 17%, orthobiologics is the fastest growing orthopaedic segment. The global orthobiologics market is projected to almost double by 2012, driven by technological advances and an ageing, increasingly active population, giving

Table 6.1 Biomaterials in orthopaedics

Bioactive materials	Inert materials	Composites	Resorbables	Transplantable tissues	Engineered tissues (tissue engineering and regenerative medicine)
Calcium sulfate (plaster of Paris)	Synthetic polymer – PEEK/PP/PS/PET/ UHMPE/ PMMA/ PU	Hap/PHB Hap/PHB-PEG	PLLA/PGA/PCL	Allograft	TiNi foam [8]
Tricalcium phosphate (TCP)		PE/Hap Bioglass/Hap Bioglass/PE Bioglass/PHB Bioglass/PS	Hap	Autograft	DBM
Glass-ceramics Hap Bioactive glass Bioactive materials based composites	Metal or alloy powders such as stainless steel, Ti, Ti.6Al.4V and other Ti alloys, and cobalt. chromium (Co.Cr) alloys (29% Cr, 6% Mo, balance Co by weight)	Collagen/Hap Collagen/nHap Collagen/ Chitosan/nHap	TCP Collagen/chitosan/ biopolymer extracellular biomatrix	DBM (demineralised bone matrix)	Nanocomposites ECM



6.2 Biomaterials for orthopaedics.

rise to more people aged over 60 who are diagnosed with musculoskeletal conditions [6].

From the device design point of view, as legally required, a medical device (orthopaedic implant) needs to follow a design control process, together with risk management, in which the selection, synthesis, processing and characterisation of the biomaterials used for producing the implant must be well investigated and documented. Therefore, it is necessary to review the chemical and physical nature of biomaterials in orthopaedics in order to achieve an improved design and utilisation of bioactive materials [7].

6.2 Biomaterials in orthopaedics

A biomaterial used in the field of orthopaedics needs to meet the following essential requirements:

- biocompatible: safe to use, with no adverse reaction and no immunological reaction;
- mechanical compatibility: mechanically strong enough for function; no fragmentation, no generation of particles, suitable for scaffolding, no causing of mechanical damage to the surrounding tissues (including soft and hard tissue);
- bioactive: supporting the biointegration of the implant into the bone or surrounding tissue without separation, loss, or migration;

- controllable biodegradation when resorbable materials are considered;
- good response to the cells and tissues when engineered tissues are used for implantation;
- well-defined structures, including pore structure and surface structure.

Based on implants currently studied and developed, biomaterials used for producing these implants are summarised in Fig. 6.2 and Table 6.1.

6.2.1 Inert materials

As traditional biomaterials, metals have been used to make orthopaedic joint and bone plates for many years. Total joint replacements (hip or knee joints) and bone plate surgeries now number in the millions worldwide annually, with knee joint replacement surgeries in the USA alone reaching in excess of 300 000 annually. The metals used as implants can be engineering alloys, such as cobalt–chromium alloys, stainless steel, and titanium alloy to ensure safety and biocompatibility [9, 10]. However, to ensure less surgical revision, the design of surface properties to obtain novel bioactive materials with an enhanced bioactivity is currently receiving considerable attention.

Other than metals, in order to meet mechanical compatibility requirements, synthetic polymers have been assessed as bone replacements. Commonly used polymers include polyethylene (PE), polypropylene (PP), polyurethane (PU), polytetrafluoroethylene (PTFE), poly(vinyl chloride) (PVC), polyamides (PA), poly(methyl methacrylate) (PMMA), polyacetal, polycarbonate (PC), poly(ethylene terephthalate) (PET), polyetheretherketone (PEEK), and polysulfone (PSU), which can be selected and processed for different clinical indications according to product specifications.

6.2.2 Bioactive materials

Over the last three decades, many different types of bioactive material have been developed. Among these, the main bioactive materials used clinically are bioactive glasss in the Na_2O–CaO–SiO_2–P_2O_5 system [11], sintered hydroxyapatite (Hap) ($Ca_{10}(PO_4)_6(OH)_2$ [12, 13], sintered b-tricalcium phosphate (TCP) ($Ca_3(PO_4)_2$ [14], Hap/TCP bi-phase ceramic [15], glass-ceramic apatite–wollastonite (A–W) containing crystalline oxyfluoroapatite ($Ca_{10}(PO_4)_6(OF_2)$), and b-wollastonite (CaO–SiO_2) in an MgO–CaO– SiO_2 glassy matrix [16,17].

In the introduction to this book, the definition of bioactive materials provided indicates that strong material/tissue bonding is the key to ensure a high success rate for an implantation. In this respect, the most commonly synthetic bioactive materials are described below.

Hydroxyapatite (Hap)

Chemically, Hap is a calcium phosphate-based apatite (M10(XO4)6Z2), with the chemical formula (Ca_{10}(PO4)6(OH)2), which is also called penta-calcium phosphate. Stoichiometric Hap has a calcium to phosphate molar ratio of 1.67 and as such consists of 39.9% calcium, 18.5% phosphorus, and 3.4% hydroxyl (weight percentages)

Tri-calcium phosphate (TCP)

Tri-calcium phosphate (Ca_3 $(PO_4)_2$) is another biomedically relevant calcium phosphate, with a Ca/P mol ratio of 1.5 versus 1.67 for Hap. Depending on crystal structure, two tricalcium phosphate phases (α-rhombohedric, β-monoclinic, phase change between 1100°C and 1350°C) are distinguished, which are called α-TCP and β-TCP, with β-TCP being the more stable and more widely used crystal structure.

Biphasic Hap/TCP

As the degradation rate and mechanical properties are different between Hap and TCP, the combination of these two components to form a composite can be used to tailor the properties of implants. The most commonly used ratio for biphasic Hap/TCP is 80/20, as shown in Table 1.1 in Chapter 1.

Bioactive glass

The first bioactive glass was BioGlass®, which was invented by Larry Hench in 1971 as a four-component glass system, containing SiO_2, CaO, Na_2O, and P_2O_5. Since then, bioactive glasses with many different compositions have been developed. Chemical composition, chemical structure, and textural properties (pore size, pore volume, pore structure) of biomaterials may have complex influences on the development of the hydroxy carbonate apatite (HCA) layer as the index for evaluation of the bioactivity to bond with tissues [18].

Bioactive glasses can be produced by both melting and a sol–gel process. Melting-derived bioglasses are dense and have no texture properties, with little degradation, while sol–gel-derived bioactive glasses can be tailored to have a controlled pore size with an improved biodegradation rate. Increasing the pore size and surface area leads to higher bioactivity [19, 20].

BioGlass 45S5 is the most commonly used bioactive glass clinically, in the form of commercial products named PerioGlas®, NovaBone®, and

NovaBone-C/M®. By reducing the particle size, the BioGlass can be bioresorbable over a period of 6–18 months [21, 22].

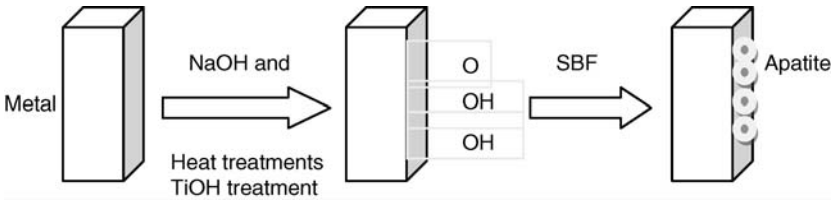
Sol–gel derived bioactive glass has also been developed on the basis of three-component and two-component systems. These materials have been assessed for utilisation in numerous medical applications, including bone tissue repair, soft tissue repair, and drug delivery [23, 24].

Glass-ceramics

Glass-ceramics are multiphase materials, consisting of at least one glass phase and one crystalline phase. Glass-ceramics are manufactured from base glasses, using the mechanisms of controlled nucleation and crystallisation [25, 26]. Moreover, glass-ceramics exhibit special properties that are characteristic of both glass and ceramic materials. Consequently, special combinations of properties can be achieved in this group of materials. Furthermore, materials with novel properties, known neither in glass nor in ceramic materials, can be designed. A typical glass-ceramic is apatite and wollastonite (A–W), which has the ability to form tight chemical bonds with living tissues when implanted in the body. A–W glass-ceramics contain crystalline apatite, Ca_{10} $(PO_4)_6(OH)_2$, and wollastonite, $CaO–SiO_2$, in an $MgO–CaO–SiO_2$ glassy matrix and exhibit greater strength than bioglass and sintered Hap, as demonstrated by Kokubo and co-workers [16, 17, 27].

6.2.3 Composites

The goal of developing composites is to take advantage of different components' strengths and build a material to meet specific clinical needs. For example, synthetic polymers typically have less rigidity than metals. By incorporation of inorganic biomaterials, the rigidity can be enhanced. Based on this concept, in the past few years, many composites have been developed for hard tissue applications, where the synthetic non-biodegradable polymeric component or biodegradable polymers can be used. The non-biodegradable polymers are mainly ultra-high molecular weight polyethylene (UHMWPE), PEEK, and PMMA [28], while the biodegradable polymers are poly (lactic acid) (PLA), poly(glycolic acid) (PGA), poly(e-caprolactone) (PCL), polyhydroxybutyrate (PHB), polyglycolic acid (PGA), poly(hydroxyl valeric acid) (PHV), and their copolymers [29–35]. The inorganic components include Hap micron or nano powder, TCP micron or nano powder, bioactive glass granules, and glass-ceramic granules. The composite is in the form of a thin membrane or a porous scaffold or a microparticulate for specific applications. Nanocomposites are also in this category, as discussed in Chapter 3. Nanofibres have been used for forming composites for bone tissue repair.

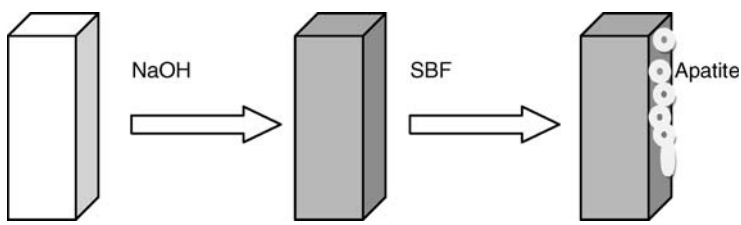


6.3 Induction of a bioactive surface on metals.

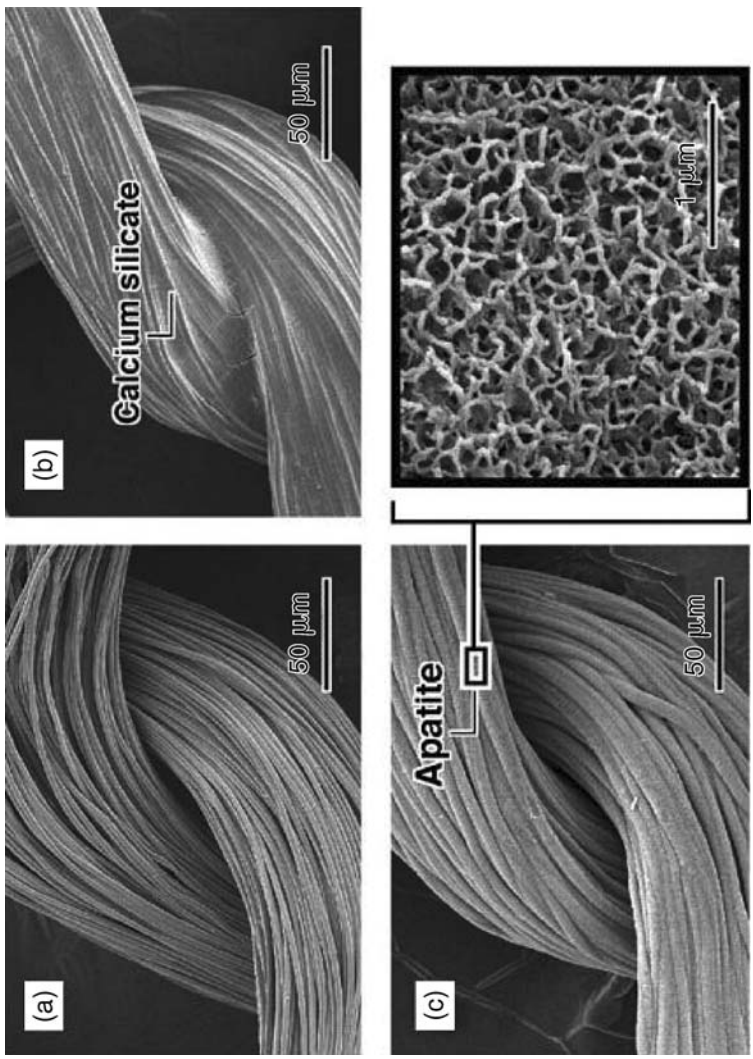
6.2.4 Novel bioactive materials

Bioactive ceramics have been known to integrate spontaneously with living bone by promptly forming bone-like apatite on their surfaces. The formation of bone-like apatite is induced by functional groups on the materials, such as Si–OH, Ti–OH, Zr–OH, Nb–OH, Ta–OH, –COOH, and PO_4H_2 that have a specific arrangement [36–38]. In the body environment, these functional groups assume a negative charge, and induce apatite formation, via the formation of amorphous calcium compound, and the subsequent formation of an amorphous calcium phosphate that finally transforms into bone-mineral-like apatite. Based on this mechanism for apatite formation, many novel bioactive materials have been designed and prepared, based on the surface treatment of existing biomaterials, such as tough bioactive metals and ceramics. In addition, some other soft bioactive inorganic–organic hybrids and bioactive inorganic–organic three-dimensional composites, with a bone-like structure and mechanical properties, can also be produced. These new types of bioactive materials are expected to play an important role in bone repair in the near future. For example, the treatment of titanium metal with sodium hydroxide, heat or sol–gel coating with Ti–OH will create a surface that can induce apatite formation at the metal surface (Fig. 6.3).

Similarly, the treatment of polymers, such as PEEK, high-density polyethylene (HDPE), and UHMWPE film, using sodium hydroxide (NaOH) has a similar effect to the metal surface treatment, which leads to the formation of apatite when the treated materials are soaked in simulated buffered fluid (SBF) [39] (Fig. 6.4). Sodium hydroxide pretreatment



6.4 Induction of a bioactive surface on polymers.



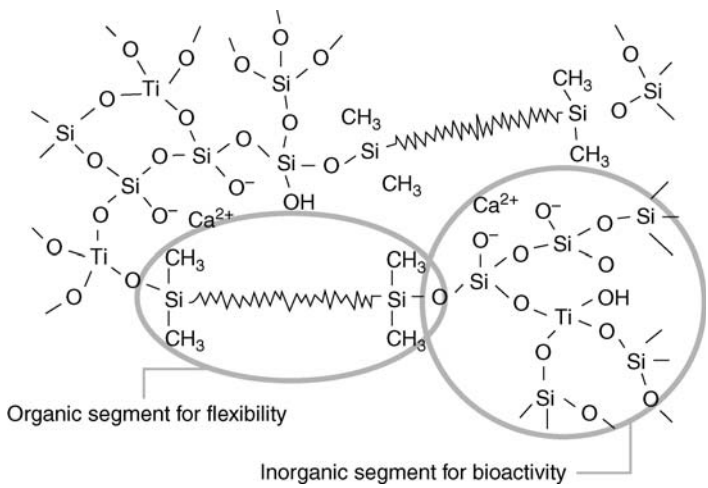
6.5 SEM photographs of the surface of a woven yarn of EVOH fibres before (a), and after (b) calcium silicate coating, and after subsequent soaking in an SBF for 2 days (c) [43].

provides favourable sites for nucleation and growth of apatite. In fact, on all NaOH pretreated samples, an apatite layer formed over time, in strong contrast to polymer films that were simply immersed in 1.5 SBF without exposure to NaOH; no apatite growth was observed on such samples [40–42].

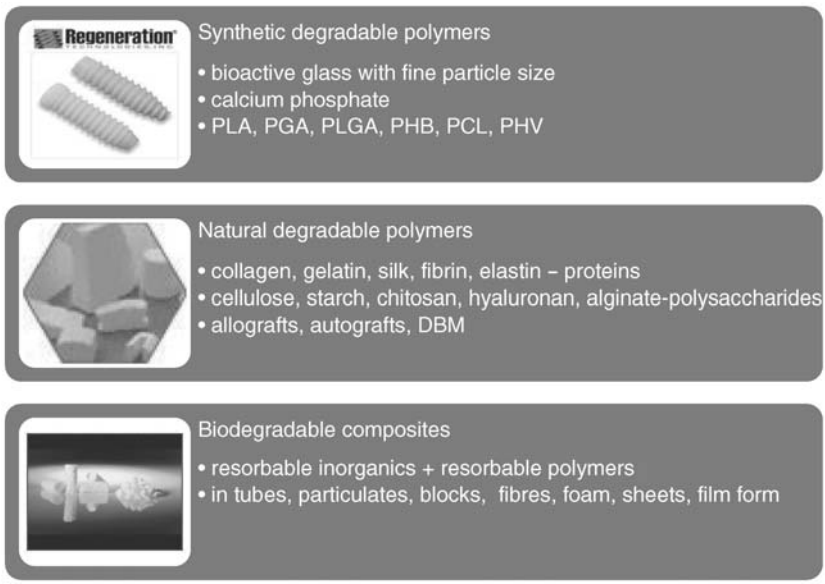
Similarly, by binding silicates on fibres, the fibres will demonstrate apatite formation which will be essential for tissue bonding [43]. Clearly, the surface-treated fibres can form uniform bioactive apatite, as shown in Fig. 6.5.

6.2.5 Soft bioactive materials

In some medical applications requiring the materials to possess both flexibility and tissue bonding activity, the hybrids based on soft segments and hard segments will be a good fit. This is a different approach to the use of composites, which normally involves a mixing process. In contrast, the hybrids are constructed by polymerisation, using a sol–gel process. For example, polysiloxane as the soft segments can form a network with silicates using a sol–gel process to form the network, as shown in Fig. 6.6. In addition, bioactivity can be enhanced when Ti–OH functional groups are introduced [44–50].



6.6 Schematic drawing of the structure of a bioactive inorganic–organic hybrid [44].



6.7 Resorbable bioactive materials in orthopaedics.

6.2.6 Resorbables

Resorbables are those implants which can be resorbed by the body during the course of implantation, with no need for surgical removal once the new bone tissue is formed. They can be divided into synthetic degradable polymers, natural degradable polymers, and degradable composites, as shown in Fig. 6.7.

Biodegradable thermoplastic poly(L-lactic acid) (PLLA) and polycaprolactone (PCL) [51] have been widely used as biomaterials for resorbable bone fixation implants in orthopaedic and oral surgeries. By adjustment of the molecular weight and the copolymerisation with polyglycolic acid (PGA), the biodegradability can be controlled. Many commercial products have been developed based on these thermoplastics.

The most commonly used natural biopolymer for bone tissue repair is collagen. With collagen itself or composites with other biomaterials, biomimetic extracellular matrix (ECM) biomatrix can be formed to be oestrogenic, thereby enhancing bone tissue formation.

In order to simulate the nanostructure of bone tissue, biomimetic approaches have been adopted to produce composites for bone tissue repair. One of the most commonly used synthetic bone grafts is the combination of β-TCP and Hap. By controlling the proportion of the components, the materials exhibit both strength and biodegradability. Other popular bioresorbable composite bone substitutes are based on Hap/PLLA mixtures

for improving the modulus, bioactivity, and degradation rate of PLLA implants, in which the size and shape of Hap particles play a critical role in the fracture behaviour of Hap/PLLA. The addition of Hap improves the biocompatibility of bioabsorbable polymeric implants but reduces their ability of fracture resistance [52–54].

6.2.7 Transplantables/bone graft

Bone grafts are bone that is transplanted from one area of the skeleton to another in order to aid in healing, strengthening, or improving function. Bone or bone-like materials used in bone grafts may come from the patients themselves, from a donor, or from a man-made source. In many cases, they are used to fill in the bone defects that may have been created in or between the bones of the spine by disease, injury, deformity, or during a surgical procedure, such as spinal fusion.

There are three types of graft: autografts, allografts, and xenografts. Autologous (autograft) refers to tissues that are re-implanted in the same individual they have come from. Many tissues, like bone and tendon, can be used in this way. The advantages of autograft are the reduction of cross infection and allergen risks. The disadvantage of allograft is that for bone the surgeon could be 'robbing from Peter to pay Paul'. In other words, there is a risk associated with removing healthy tissue from one part of the body to place it as a graft of dead tissue in another. The commonest site from which to harvest bone is the rim of the pelvic bone, near the hip. However, if too much is taken, there is a risk of breaking the pelvic bone that is weakened by removing the normal bone. It can also be very painful to have bone removed from the rim of the pelvis because it is attached to the abdominal muscles.

Bone allograft comes from femoral heads donated by patients undergoing hip replacement surgery. The benefit of fresh frozen allograft is the volume available to the surgeon. A risk is that of infection from bacteria. The risk of bacterial infection can be reduced if the bone graft is irradiated. However, this affects its strength. Additionally, there is a risk of viral infection, although this is low because of stringent tests to exclude hepatitis B, hepatitis C, and HIV in the live donors. Another risk of the fresh frozen bone graft is that it often comes from elderly patients and so the potential for this bone to stimulate the formations of new bone by the patient receiving the implant can be poor (poor osteoconductivity).

In summary, there is a constant need for bone graft material to provide support, fill voids, and enhance biological repair of skeletal defects. Of the more than three million musculoskeletal procedures performed annually in the USA, about half involve bone grafting, with either an autograft or an allograft. Worldwide, autografts and allografts are used in approximately

2.2 million orthopaedic procedures annually [6]. However, all these techniques have certain limitations due to limited donor supply, donor site morbidity, infection, pain, and genetic differences, together with anatomical and structural differences and high levels of resorption during healing [55].

6.2.8 Engineered bone tissue

Demineralised bone matrix (DBM)

DBM, the most commonly used engineered bone tissue, is derived from processed allograft bone. DMB contains collagen, proteins, and growth factors that are extracted from the allograft bone. It is available in the form of a powder, crushed granules, putty, chips, or as a gel that can be injected through a syringe. DBM is extensively processed and, therefore, has little risk of disease transmission. However, because of the form it takes, it does not provide strength to the surgical site.

ECM for bone tissue engineering

Tissue engineering has emerged as a promising approach that essentially develops viable substitutes capable of repairing or regenerating the functions of damaged tissue [30]. Most tissue engineering, in particular bone tissue engineering, requires a scaffolding system (also called synthetic ECM), in order to support the cells temporarily, so as to accommodate and direct their growth into corresponding tissue [56]. Since bone is a nanocomposite of minerals and proteins, the preference is to consider a nanocomposite that mimics natural bone.

The ECM of natural bone is collagen, glycosaminoglycans, proteoglycans, and glycoprotein, and the inorganic constituent is Hap. A composite formed by combining electrospun n-Hap with collagen fibre possesses high surface area, high porosity, and a well-interconnected open pore network, similar to native bone ECM. These structural characteristics are necessary to enhance the osteogenic cell attachment and to expedite the tissue ingrowth both *in vitro* and *in vivo*. Therefore, the electrospun scaffolding system is of particular interest in bone tissue regenerative applications.

Nano Hap/collagen (nHAC)-based composite structures, through self-assembly or co-precipitation, are good examples as ECM for bone tissue growth [57–60].

In addition to Hap, bioactive inorganics, including tricalcium phosphate and bioactive glasses/glass ceramics, are widely used synthetic scaffold materials in combination with degradable synthetic polymers, including PLA, PGA, PCL, PHB, PHV, and their copolymers, to form composites,

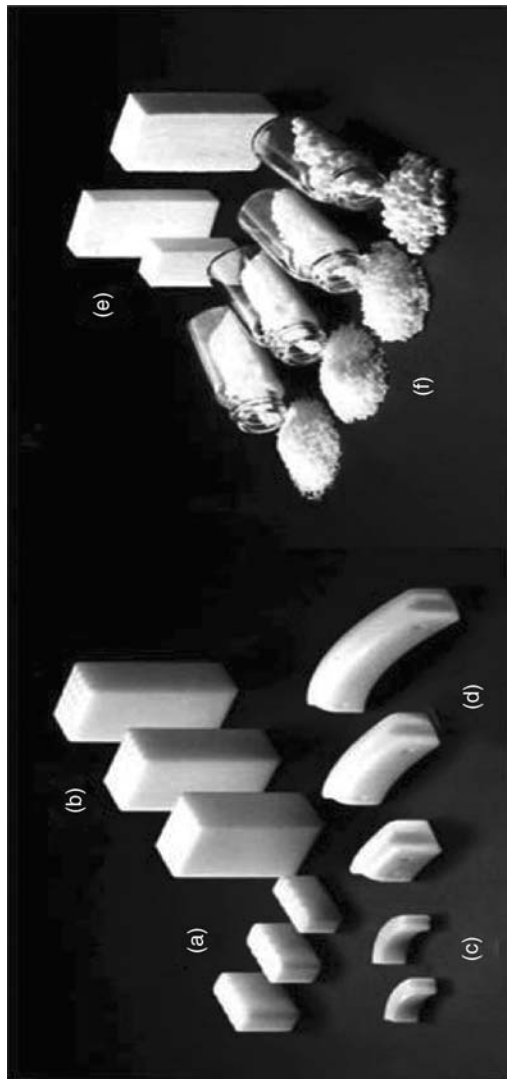
which have been studied extensively for application in implantable bone devices, because of their improved cell affinity, osteoconductivity, and enhanced bone formation [61–68]. Nanofibrous bioactive glass obtained by electrospinning was introduced into a degradable polymer, PCL, to form a nanocomposite, with a thin membrane form. PCL has shown good osteoblastic response *in vitro* and tissue reaction *in vivo* [69–71].

6.3 Clinical applications of bioactive materials in orthopaedics

According to Yaszemski *et al.* [72], to achieve a successful clinical application an orthopaedic implant needs to be:

(a) sterilisable without loss of mechanical and biological properties;
(b) available at short notice to the surgeon in the sterile operating field;
(c) mouldable such that it can fill irregularly-shaped defects;
(d) able to harden over a time span of 10–15 min;
(e) able to provide the reconstructed skeletal region with mechanical properties of the same order of magnitude as those of the bone it replaces (a minimum compressive strength of 5 MPa and a minimum compressive modulus of 50 MPa);
(f) able to degrade over a period of weeks to months in a controlled fashion;
(g) capable of being replaced by new bone;
(h) able to maintain a specified minimum mechanical strength during the period of degradation and new bone growth.

In the past three decades, many bioactive materials have been developed in the form of bulks and particulates with dense and porous structures in order to meet those goals. For example, bioglass in the form of particulates has gained over a million successes in periodontal bone repair (for example, see reference [73]). Hap, in bulk and granular forms with dense and porous structures, is popularly used as bone spacers and fillers, and a number of clinical successes have been documented (for example, see reference [74]). Glass-ceramic A–W, owing to its superior mechanical strength and excellent bone-bonding ability, has been applied, not only as bone spacers and fillers in the bulk and granular forms with dense and porous structures, but also as artificial vertebrae, intervertebral discs, and iliac crests in dense bulk form (for example, see reference [75]). These products are shown in Fig. 6.8 and also listed in Table 6.2, based on a review of regulatory approved medical implants.



6.8 Glass-ceramic A–W in clinical use: (a) intervertebral discs, (b) artificial vertebrae, (c) spinal spacer, (d) iliac crests, (e) porous spacer, and (f) bone filler [76].

Table 6.2 Clinical applications of bioactive materials in orthopaedics

No.	Name of device	Materials	Indications (medical applications)	Reference	Manufacturer
1	NovaBone Putty – bioactive synthetic bone graft, osteoconductive bone void filler, synthetic resorbable bone graft material, bone void filler	NovaBone Putty is an osteoconductive, bioactive, bone void filler device. It is composed of a calcium-phosphorus-sodium-silicate (bioglass) particulate mixed with a synthetic binder that acts as a temporary binding agent for the particulate. The particulate and binder are provided premixed as a pliable cohesive material. The mixed device is supplied sterile, packaged in a PET-G tray or in a disposable plastic syringe. On implantation, the binder is absorbed to permit tissue infiltration between the bioglass particles. The particles are then slowly absorbed and replaced by new bone tissue during the healing process.	NovaBone Putty is indicated only for bony voids or gaps that are not intrinsic to the stability of the bony structure. NovaBone Putty is indicated to be gently packed into bony voids or gaps of the skeletal system (i.e. the extremities, spine, and pelvis). These defects may be surgically created osseous defects or osseous defects created from traumatic injury to the bone. The product provides bone void filler that resorbs and is replaced with bone during the healing process. NovaBone Putty is not indicated for use in load-bearing applications. It does not possess sufficient mechanical strength to support load-bearing defects prior to hard tissue ingrowth. It should not be used for vertebroplasty or kyphoplasty procedures.	www.fda.gov/cdrh/pdf8/K082672.pdf - 01-07-2009	Novabone Inc.
2	Synthetic bone graft substitute Fortoss® Vital	Fortoss® Vital bone graft substitute is a calcium salt based pre-measured powder and liquid component. The two components are designed to be mixed intraoperatively to produce a homogeneous paste, which can then be applied to osseous defects.	Fortoss® Vital is for placement in osseous defects to provide a mouldable, resorbable graft in periodontal, maxillofacial, and dental implant surgery	www.fda.gov/cdrh/pdf8/K082383.pdf - 11-07-2008	Bio-composites Ltd, England ST5 5NL
3	CaP Plus, bone void filler, bone graft material, bone substitute material, resorbable calcium salt bone void filler device	CaP Plus is a biocompatible bone graft substitute material consisting of synthetic calcium phosphate, an inert carrier, carboxymethyl cellulose (CMC), and human demineralised bone matrix (DBM).	Filling bone voids or defects of the skeletal system (such as the extremities and pelvis) that are not intrinsic to the stability of the bony structure. These defects may be surgically created osseous defects or osseous defects created from traumatic injury to the bone. It is a bone graft substitute that resorbs and is replaced with new bone during the healing process.	www.fda.gov/cdrh/pdf6/K063050.pdf - 12-07-2007	ETEX Corporation

4	OssiPro Bone Substitute Material, resorbable calcium salt bone void filler device	OssiPro Bone Substitute Material is an injectable synthetic, biocompatible bone graft substitute material. It is intended for use in bone void filler applications in the spine, pelvis, and extremities. At the time of use, OssiPro is combined with the hydration solution and is mixed to a smooth consistency. The material can be delivered to the defect site by injection with provided syringe or with desired needle/cannula (not provided). After delivering the paste to the treatment site, it forms pores while hardening at body temperature and converts to a macro-porous, poorly crystalline hydroxyapatite (PCHA) scaffold. The end product has a similar chemical identity and crystalline structure to that of natural bone. OssiPro Bone Substitute Material is an osteoconductive bone graft substitute that resorbs and is replaced with new bone over time.	OssiPro Bone Substitute Material is intended for use in filling bone voids or defects of the skeletal system (i.e. the extremities, spine, and the pelvis) that are not intrinsic to the stability of the bony structure. These defects may be surgically created osseous defects or osseous defects created from traumatic injury to the bone. OssiPro is a bone graft substitute that resorbs and is replaced with new bone during the healing process.	Similar to: a-BSM® Bone Substitute Material, and JAXTM Granules Bone Void Filler	ETEX Corporation
5	ApaceraM™ Bone Void Filler, synthetic, porous hydroxylapatite	ApaceraM™ is a hydroxyapatite osteoconductive bone void filler. It is available in four types: AX, B, G, and R, which vary in porosity, shape, and sizes. ApaceraM™ is provided sterile for single patient use.	ApaceraM™ bone graft substitute is a synthetic hydroxyapatite. Use: provided in several particulate and shaped sizes. It is intended for use as a bone void filler for bony voids, gaps, or defects that are not intrinsic to the stability of the bony structure. ApaceraM™ is intended to be placed into bony voids or gaps of the skeletal system (i.e. extremities, spine, or pelvis) caused by degeneration, trauma, or surgery. It also can be used with autograft as a bone graft extender. ApaceraM™ is resorbed and replaced with bone during the healing process.	K033722, ApaPore®D Bone Graft Substitute, ApaTech Ltd., K051774, MBC PTM , Biomnatlante. www.fda.gov/cdrh/pdf7/K071912.pdf - 11-08-2007	Pentax Corporation
6	TheriRidge™ B lock, bone graft substitute	The TheriRidge™ B lock device consists of hydroxylapatite material, the primary mineral content of human bone, with porosity and	TheriRidge™ B lock bone graft substitute is indicated and intended for the augmentation of deficient maxillary and	www.fda.gov/cdrh/pdf2/k023998.pdf - 04-14-2003	Therics, Inc.

Table 6.2 (cont.)

No.	Name of device	Materials	Indications (medical applications)	Reference	Manufacturer
		geometric features that encourage tissue in-growth. The device is available in three (3) basic sizes: small (approximately 10.4 mm x 5.6 x 5.0), medium (approximately 10.4 mm x 10.0 x 5.0), and large (approximately 20.0 mm x 10.0 x 5.0).	mandibular alveolar ridges.		
7	Zuma™ interbody fusion device, vertebral body replacement device, intervertebral fusion device with bone graft, lumbar 21 CFR 888.3080, Product Code MAX, Class II spinal intervertebral body fixation orthosis 21 CFR 888.3060, Product Code MQP, Class II	Zuma is an implantable spinal device made from polyetheretherketone (PEEK) and titanium with markers for radiographic visualisation; it is secured to vertebral bodies with bone screws. The device has an open central area for receiving bone graft material and is offered in a variety of sizes and geometries to accommodate variations in pathology and patient anatomy.	Spinal fusion procedures at one or two contiguous levels (L2-S1) in skeletally mature patients with degenerative disc disease (DDD). DDD is defined as back pain of discogenic origin with degeneration of the disc confirmed by history and radiographic studies. DDD patients may also have up to Grade 1 spondylolisthesis or retrolisthesis at the involved level(s) thoracolumbar spine (T1 to L5) to replace collapsed, diseased, damaged, or unstable complete or partial vertebral body due to tumour or trauma/fracture, to achieve anterior decompression of the spinal cord and neural tissues, and to restore the height of a collapsed vertebral body. The Zuma system is designed to restore the biomechanical integrity of the anterior, middle and posterior spinal column, even in the absence of fusion for a prolonged period. Additionally, Zuma is intended for use with bone graft.	www.fda.gov/cdrh/pdf8/K082926.pdf - 01-07-2009	SeaSpine, Inc.
8	Isoelastic U™ Wrist joint ulnar (hemi–wrist) prosthesis	Metallic and silicone elastomer distal ulnar head implants.	The Isoelastic U™ is indicated for joint replacement arthroplasty of the ulnar head at the distal radio-ulnar joint (DRUJ) for the following indications: – rheumatoid arthritis with or without tendon ruptures – degenerative arthritis or post traumatic arthritis – arthrofibrosis of the DRUJ	www.fda.gov/cdrh/pdf8/K081025.pdf - 12-05-200	REMI Sciences Inc.

			– reconstruction of the distal ulna post tumor resection – failed ulnar head resection – distal ulna instability with X-ray or bone scan evidence of arthritic or inflammatory changes – revision following failed ulnar head arthroplasty Ulna stems are intended for uncemented use		
9	Biolox® ceramic femoral head hip joint metal/ ceramic/polymer semi-constrained cemented or non-porous uncemented prosthesis per 21 CFR 888.3353	New material used in the manufacture of the Biolox® ceramic femoral heads. The femoral heads, manufactured from Biolox® delta* material, are fabricated from an alumina matrix composite. The standard femoral head will mate with a femoral stem through a taper fit. The option femoral head includes a sleeve that is inserted into the head and attached to the femoral stem through a taper fit. The heads are available in sizes 22, 28, 32, 36, 40, and 44 mm.	Joint replacement is indicated for patients suffering from disability due to: – non-inflammatory degenerative joint disease including osteoarthritis and avascular necrosis of the natural femoral head; – rheumatoid arthritis; – correction of functional deformity; – femoral fracture. DJO Surgical hip devices are intended for treatment of patients who are candidates for total hip arthroplasty as per the indications for use. While hip replacements are not intended to withstand activity levels and loads of normal healthy bone, they are a means of restoring mobility and reducing pain for many patients.	– Zimmer Biolox® delta ceramic femoral head – K071535, cleared 19 November 2007 – Biomet Biolox delta ceramic head – K04209 1, cleared 25 March 2005, K05141 1, cleared 29 June 2005, K0613 12, cleared 6 June 2006 – DePuy delta ceramic femoral head – K062748, cleared 30 November 2006 – Stryker Howmedica Osteonics V4OTm Biolox delta ceramic femoral head – K05278 1, cleared 27 October 2005 – Stryker	Encore Medical, L.P.

Table 6.2 (cont.)

No.	Name of device	Materials	Indications (medical applications)	Reference	Manufacturer
				Howmedica Osteonics V40Trm/ C-Taper adapter sleeve – K003379, cleared 30 November 2000 www.fda.gov/cdrh/ pdf8/K082844.pdf - 12-05-20	
10	Total knee replacement prosthesis 21 CFR 888.3560: knee joint patello-femorotibial, polymer/metal/ polymer semi-constrained, cemented prosthesis, Class II	Co–Cr–Mo femoral component with an asymmetric trochlear groove, available in sizes 1.5–6, in right and left versions. The fixation surface is textured. It incorporates two pegs to provide additional stability and recessed cement pockets for enhanced cement fixation.	Total knee replacement is intended to provide increased patient mobility and reduced pain by replacing the damaged knee joint articulation in patients where there is evidence of sufficient sound bone to seat and support the components.	www.fda.gov/cdrh/ pdf8/K082500.pdf - 12-05-2008	DePuy Ortho-paedics, Inc.
11	Foundation® porous-coated (FMPTM) acetabular shells hip joint, metal/ polymer/metal semi-constrained, porous-coated, uncemented prosthesis	Hip joint metal/polymer/metal semi-constrained porous-coated uncemented prosthesis.	The modification consists of an additional method of porous coating currently conducted on the hip devices listed above. Joint replacement is indicated for patients suffering from disability due to: – noninflammatory degenerative joint disease including osteoarthiritis and avascular necrosis of the natural femoral head; – rheumatoid arthritis; – correction of functional deformity; – femoral fracture.	www.fda.gov/cdrh/ pdf7/K072888.pdf - 02-05-2008	Encore Medical, L.P.
12	DUOWEDGE	Tri-calcium phosphate (TCP) 40%, hydroxyapatite (HA) 60%. Average porosity of the porous section	Valgisation medial opening wedge osteotomy: – for medial femorotibial gonarthrosis with genu		

		is approximately 60%, with pore size ranging from 400 µm to 700 µm. The porous network is totally interconnected. It is completely tridimensional and there are no blind pores.	varum – for open osteotomy with chronic laxity – for open osteotomy to correct genu recurvatum.	
13	KAGE and KG Bone	KG Bone is a synthetic bone substitute made of HA (60%) and TCP (40%). Mean porosity is 60%, fully interconnected, with 600 µm pore size.		
14	JectOS	JectOS is an injectable synthetic bone substitute made of 99% calcium phosphate. Phosphate: – 55% DCPD (dicalcium phosphate dihydrated) – 45% TCP (tricalcium phosphate) JectOS is a product easy to set and easy to use by surgeons thanks to its long working time (4 to 5 min). JectOS is biocompatible, bioresorbable, and osseoconductive. This will ensure proper osseointegration in human bone. JectOS is delivered in two vials (one vial containing liquid, the other containing powder) and the ancillary accessories necessary for mixing and injecting.	JectOS is indicated for the filling of cancellous bone defects. JectOS is very useful for bone void filling in trauma cases such as radius, tibial, and calcaneum fractures.	http://www.kasios.com/
15	Kasios TCP	βTCP (99.9%) βTCP is a calcium phosphate molecule similar to the mineral phase of the natural bone. Available in granules, rods, blocks, and wedges.	Kasios TCP is indicated for filling bone voids or defects of the skeletal system (such as the extremities, spine, and the pelvis) that are not intrinsic to the stability of the bony structure. These defects may be surgically created osseous defects or osseous defects created from traumatic injury to the bone. Kasios TCP is a bone graft substitute that resorbs and is replaced with bone during the healing process.	http://www.kasios.com/

Table 6.2 (cont.)

No.	Name of device	Materials	Indications (medical applications)	Reference	Manufacturer
16	TCH®	Biphasic ceramic made of hydroxyapatite (75%) and tricalcium phosphate (25%).	TCH® has been clinically successful in the following indications: – for both epiphyseal and diaphyseal simple and complex fractures – filling after removal of osteosynthesis materials and after benign synovioma curettage – non-union or pseudarthrosis, arthrodesis, and osteotomies – prosthesis revision surgery – spinal fusion.	http://www.kasios.com/	

Table 6.3 Limitations of bioactive materials in orthopaedic applications

Bioactive materials in orthopaedics	Limitations
Ceramics	• Possibility of inflammation • No presence of natural growth factors to promote the bone growth • Protein adsorption can be limited due to not well-defined surface and bulk pore structures • Unsuitable mechanical properties for structural bone replacement or flexible tissue replacement • Difficulty in controlling biodegradability
Bioactive glass	• Unsuitable mechanical properties for structural bone replacement or flexible tissue replacement • Difficulty in controlling biodegradability • Alkaline nature limits medical applications
Bioactive glass ceramics	• Difficulty in controlling biodegradability • Unsuitable mechanical properties for certain structural bone replacement or flexible tissue replacement
Demineralised bone matrix (DBM) DBM+ bone marrow cells	• Possibility of disease transmission • Unsuitable mechanical properties for certain structural bone replacement or flexible tissue replacement • Batch variability in production
Composites (collagen + inorganic bioactive molecules + bone growth factors etc.)	• Unsuitable mechanical properties for certain structural bone replacement or flexible tissue replacement • Batch variability in production
Biomaterials + BMPs as a delivery system	• Further study into clinical effects on osteoconductive, osteogenic, and osteoinductive properties • Production costs
Biodegradable polymers	• Lower stiffness than natural bone or metal • Degradation byproducts/abrupt mechanical failure • Poor bioactivity
Nanocomposites	• Further requirement of fundamental understanding of cell–material interaction • Limited production scalability • Consideration of nanotoxicity
Tissue engineered products	• Difficult to reproduce scaffold in production line leading to batch variation • Regulatory obstacles to overcome • Difficulty in achieving mechanically strong formed bone tissue
Bone grafts	• Limited resources • Possibilities of disease transmission • Possibility of infection • Patient surgical suffering

6.4 Limitations of bioactive materials in orthopaedics

Despite the many advances in bone graft substitutes, orthopaedic prostheses, and orthobiologics to date, no ideal bone substitute has been found and there remains a real need for an alternative with optimal bone regeneration properties. Limitations of bioactive materials used clinically are listed in Table 6.3. This table clearly demonstrates the limitations of current therapies in orthopaedics. For instance, the increase in stiffness of biodegradable synthetic polymeric implants is always required from the

clinical point of view because it is usually much lower than that of natural bone and the metal-based orthopaedic prostheses. Low stiffness of bone fixation implants results in easy deformation of the implants during *in vivo* use, while higher stiffness such as that with metal implants, possibly causes stress shielding, and therefore bone resorption in that region. Thus, how to increase the modulus of PLLA through Hap filling, without sacrificing its fracture properties, is an important issue [77]. Future approaches involving the use of stem cells and gene therapy could be a solution to some of the above limitations.

6.5 Future trends

6.5.1 Mechanically compatible bioactive material-based implants

In considering their role as the replacement of mechanically strong bone tissues, the mechanical properties of the implants is always a priority. Niinomi [78] coined the term 'mechanical biocompatibility' to express the idea that the mechanical properties of implant materials must be chosen in such a way as to be compatible with those of the natural materials around them: stiffness matching is the most obvious example, but the concept has other, more subtle, implications. Other papers have revealed synergies between mechanical and biological factors in experimental studies: for example Nychka *et al*. [79] found that residual stress affected the bioactivity of a bioactive glass, while Adachi *et al*. [80] linked calcium signalling to mechanical deformation at the cellular level. The future trend will be focused on the identification of a mechanically matched bone tissue replacement while having good biocompatibility based on novel composites or surface modification.

6.5.2 Orthobiologics

There is a growing preference to use synthetic bone grafts or their combination with bone growth factors rather than the current gold-standard autografts and allografts in the field of orthobiologics, in order to reduce the limitations associated with the tissue grafts procedures. The synthetic and biodegradable, polymer/inorganic bioactive material composites are particularly attractive as tissue engineering scaffolds, owing to ease of shaping, bioactive behaviour, and adjustable biodegradation kinetics with a porous and interconnected three-dimensional polymer network. From the materials science perspective, the present challenge in tissue engineering is to design and fabricate reproducible bioactive and bioresorbable three-dimensional scaffolds of tailored porosity and pore structure, which are able to maintain

their structure and integrity for predictable times, even under load-bearing conditions.

The incorporation of biomolecules, such as growth factors, with the aim of accelerating local bone healing is promising and currently under extensive research. However, incorporating biomolecules during scaffold processing is not straightforward, as biomolecules are sensitive to elevated temperatures and extreme chemical conditions. A promising strategy is the immobilisation of proteins and growth factors in the post-processing phase via surface functionalisation of the scaffold [81].

6.5.3 Stem cells and ideal biomaterials

Stem-cell therapies are forecast to be the fastest-growing category, with a compound annual growth rate (CAGR) of 53.9% over the next five years [6]. Stem cell therapy is also showing huge potential as an alternative to autograft. Mesenchymal stem cells (MSCs), found in bone marrow, can form a variety of cells in the laboratory, including fat cells, cartilage, bone, tendon and ligaments, muscles cells, skin cells, and even nerve cells. Unlike most other human adult stem cells, MSCs can be obtained in quantities appropriate for clinical application, making them good candidates for use in tissue repair. They also offer the advantage of low morbidity and cost.

While current research is still focused on the interaction between stromal cells and biomaterials, essential goals for biomaterials seem to originate from introducing stem cells. Scaffolds seeded with stem cells allow local cell function adaptation by differentiation of stem cells, as demonstrated by Levenberg *et al*. in 2003 [82]. This new approach enables the scaffold surface to mimic complex local biological functions and may lead, in the near future, to *in vitro* and *in vivo* growth of tissues and organs.

6.5.4 Gene therapy

Gene therapy has the potential to provide the ideal solution for treating a variety of musculoskeletal disorders. The promise of gene therapy is the ability to transfer efficiently new genetic material into patient cells, either to replace defective genes or introduce therapeutic genes, in order to promote new bone tissue formation to treat the disease. To date, much of the research in gene therapy for the enhancement of spine fusion has centred on the transfer of genes-encoding bone morphogenetic proteins (BMPs) and related proteins. Recombinant, osteogenic growth factors are now available to enhance bone repair, particularly in those applications related to the treatment of fracture non-unions and the enhancement of fusion of the spine [6, 55].

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7.1.2 Cardiac electrophysiology devices

Cardiac electrophysiology devices include pacemakers, defibrillators, electrophysiology diagnostic and ablation catheters, and surgical ablation devices, which are used for diagnosing and treating abnormal electrical activities in the heart (arrhythmias) to achieve good cardiac rhythm management (CRM).

7.1.3 Interventional cardiology devices

Treatment of coronary artery diseases is now being performed using diagnostic procedures, such as angiography, and interventional procedures such as stenting and angioplasty, through catheter-based technologies. When coronary arteries become narrowed and blocked, the blockages are detected and treated by inserting catheter-based devices through a small incision in the groin or other arterial access point.

7.1.4 Peripheral vascular devices

Peripheral vascular devices are used in the treatment of diseased blood vessels outside the brain and heart. The most common form of peripheral vascular disease involves fat and plaque build up, which leads to the narrowing of arteries and insufficient blood flow, termed atherosclerosis [8]. Treatment of peripheral vascular disease can be performed surgically to replace the blocked blood vessel with vascular grafts or via minimally invasive catheter-based techniques. The devices include vascular prostheses and stents.

7.1.5 Transcatheter embolisation and occlusion devices

These devices were developed with the minimally invasive catheter-based techniques pioneered by Charles Dotter in the 1960s [9]. The delivery of agents and devices via catheters has been used to occlude haemorrhaging vessels and vessels at risk of haemorrhage, and to embolise the vessels supplying tumours and vascular malformations. These devices have been used in gynaecological neoplasms [10] and oncology, for example the treatment of liver cancer with radioembolisation spheres [11].

There is a great need to develop devices for the circulatory system. According to various market research carried out by Millennium Research Group, the interventional cardiology (IC) devices market, including the products of coronary stents, percutaneous transluminal coronary angioplasty (PTCA) balloon catheters, and IC accessory devices, was valued at nearly $3.5 billion in 2009 and will expand through 2014 in the Asia-Pacific

market alone [12]. The US market for other products, such as heart valves, vascular access devices, namely short peripheral intravenous catheters (PIVCs), central venous catheters (CVCs), implantable ports, dialysis catheters, and peripherally inserted central catheters (PICCs), will experience steady growth through 2013 [13, 14]. In these applications, bioactive materials play very important roles.

7.2 Applications of bioactive materials in devices for the circulatory system

Various bioactive materials have been utilised in devices for the circulatory system. These bioactive materials have the functions of antithrombogenicity, which prevents or inhibits blood clotting, while the devices contact blood in the circulatory system [15]; an antibacterial or anti-infection action of implants used as cardiovascular devices, such as blood vessels and heart valves [16]; anti-tumour functionality, such as radioactivity [11], heat-generating properties [17], drug-eluting stents to prevent restenosis [18], and other controlled drug release devices used in the cardiovascular system [19].

Stem cells, tissue engineering, and gene therapy technology have made it possible to create blood vessels in which the bioactive materials act as a biomatrix scaffold to generate new vascular tissues, such as heart valves and small-calibre vessels. The use of autologous endothelial (precursor) cells may be the optimal means of seeding a biological or artificial scaffold [20, 21].

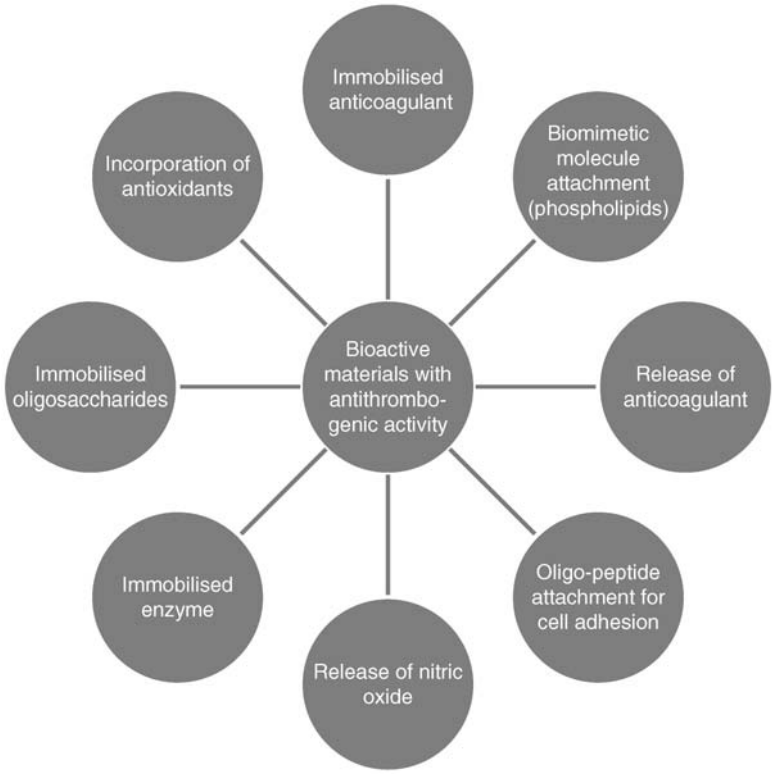
7.2.1 Non-thrombogenic bioactive materials

Blood compatibility is the key consideration factor when biomaterials are designed for blood-contacting applications. Blood tends to coagulate in contact with a foreign surface and the surface properties are critical for maintaining the non-thrombogenicity of the surface [22].

Molecular design of bioactive materials to resist blood coagulation can be achieved by various approaches to the material production, as shown in Fig. 7.1. The bioactive material exhibits a surface which is able to simulate or mimic the function of a blood vessel surface, in order to secrete bioactive substances, function metabolically, and respond to physiological effects.

Heparinised surfaces

The endothelial surface of vascular vessels has been the inspiration for the development of non-thromobogenic surfaces. It has been demonstrated that the blood vessel wall contains substances that are structurally and functionally related to heparin and this promoted acceptance of the concept



of immobilisation of the heparin anticoagulant activity [23]. In fact, heparinised surfaces are the most commonly applied non-thrombogenic surfaces for current blood-contacting medical devices, such as stents, CPB tubing, catheters, and valves. The design of such a surface can be achieved by covalent bonding, ionic bonding, blending, and coating techniques [24].

Other anticoagulant or antiplatelet agent modified surfaces

Surface immobilisation or incorporation of other bioactive substances with antithrombogenic activities is another approach. Active substances, such as the enzymes urokinase [25] and lumbrokinase [26], hirudin [27], human thrombomodulin [28], or inhibitors for activation and aggregation of platelets, such as prostacyclin [29], have been used to modify polymeric surfaces. The bioactive substances are either tightly bound to the surface or simply blended into the polymer system for controlled release. The local release of NO plays a critical role in controlling the function of the human cardiovascular system by regulating vascular cell homeostasis [30, 31].

Several nitric oxide-releasing bioactive materials, such as nanofibres, have been developed to coat medical devices and deliver nitric oxide *in vivo* to treatment sites [32, 33].

Surfaces containing phosopholipid

Another biomimetic approach is to modify polymeric surfaces by introducing phosopholipid polar groups to produce a blood compatible surface. Phosphorylcholine (PC) modified surfaces are the most commonly used in creating biocompatible surfaces. They have been studied for coating stents [34], vascular grafts [35], and dialysis membranes [36]. The bioactive materials containing PC include poly (MPC-CO-BMA) (2-methacryloyloxyethyl phosphorylcholine-co-butyl methacrylate) [37], MPC-co-cyclohexyl methacrylate and MPC-co-2-ethylhexyl methacrylate [38, 39], and cross-linkable PC polymer [40].

Endothelialisation

Endothelial cell seeding of small-calibre vascular prostheses has been shown to reduce long-term platelet deposition, thrombus formation, and thus graft failure [41]. Numerous approaches have been explored to facilitate the achievement of endothelialisation. An effective method of promoting the integration and adhesion of the cells onto the device is to immobilise agents, such as extracellular matrix (ECM) protein and oligopeptides, e.g. arginine–glycine–aspastic acid (RGDs) [42], tropoelastin [43], human elastin [44], and vascular endothelial growth factor (VEGF) [45], directly onto the device surfaces.

7.2.2 Antibacterial and anti-infective bioactive materials

Medical devices used in the cardiovascular system, such as vascular grafts, prosthetic heart valves or central venous catheters, are subject to the risk of microbial infection. For example, vascular graft infections are associated with significant morbidity and mortality and exist as a dreaded complication of vascular surgery [46]. Infection can lead to prosthetic valve endocarditis, with rates ranging between 0.5% and 4% [47]. Catheter-related bloodstream infections have been reported to occur in 3–8% of inserted catheters and are the first cause of nosocomial bloodstream infection in intensive care units (ICUs) [48].

Many approaches have been developed to resolve this issue, as discussed in the Chapter 5 subsection on cardiovascular materials. In brief, the most commonly used approaches are via covalent bonding, coating, and controlled release systems. For example, an antibiotic substance, gentamicin

sulphate, has been immobilised on a polyethylene terephthalate prosthesis sealed with gelatin. Antibacterial activity was assayed in Luria-Bertani medium against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* strains. Prosthesis endothelialisation was performed using bovine aorta endothelial cells (BAECs). It was concluded that covalent gentamicin immobilisation resulted in effective antibacterial protection of vascular prostheses against clinical and reference strains of *S. aureus*, *E. coli*, and *P. aeruginosa* and allowed for a strong adherence of endothelial cells to antibiotic-modified prostheses [49]. Olanof *et al.* [50] dispersed gentamicin into silicone rubber rings, which were then incorporated in a periannular configuration surrounding the sewing cushion of a mechanical heart valve prosthesis to prevent bacteraemia. This particular approach was claimed to be the most suitable approach for cardiac valve replacement in the setting of valvular bacterial endocarditis.

Peptides with antibacterial properties, such as RNAIII inhibiting peptide (RIP) and temporins, have been attached to vascular grafts to prevent biofilm formation on the grafts. RIP is a novel antibiotic and, instead of killing bacteria, it inhibits cell–cell communication, leading to prevention of the biofilm formation. Temporin is a basic, highly hydrophobic, antimicrobial peptide amide, which has variable antibiotic activity against a broad spectrum of microorganisms. The combinations of temporin A and RIP exerted the strongest antistaphylococcal efficacy, eliminating infection by 100% [51].

7.2.3 Bioactive materials used for embolisation and brachytherapy

The embolisation technique has been developed to deliver materials to the tumour area by intra-arterial infusion via percutaneous catheterisation to stop blood flow to the tumour cells or act as local reservoirs for drugs to be diffused to the surrounding tumour tissue [52–54]. It can also be used to treat numerous vascular lesions, including haemorrhages and arteriovenous malformations of different origin [55]. Various bioactive embolic materials, including short-term, long-term, and permanent embolisation materials, have been developed into commercial applications. The short-term embolic materials include autologous blood clot and hypertonic glucose, such as starch microspheres [56]. The long-term materials include gelatin sponges and microspheres [57]. Permanent embolisation materials include metallic coil, NBCA, and PVA, which are not degradable [58].

Another embolisation technique combined with radioactive materials is called vascular brachytherapy, which can be used for the treatment of stent

restenosis [59, 60]. Vascular brachytherapy has demonstrated its efficacy in limiting recurrence of existing in-stent restenosis [61].

Biocompatible and biodegradable materials based on superparamagnetic nanoparticles have been used for hyperthermia cancer therapy *in vivo* or *in vitro*. By the delivery of the commercial nanoparticles and temperature-sensitive hydrogel into the tumour sites, with a high-frequency magnetic field, a temperature increase is induced in tumour cells to repress significantly the growth of liver tumours [62, 63].

7.2.4 Drug-eluting stents

Drug-eluting stents (DES), which are stents coated with bioactive materials consisting of drugs and polymers, have become increasingly popular as standard therapy for coronary interventions to enlarge blood vessels [64]. However, after implantation, the blood vessels occasionally reocclude at the site of treatment, in a process called restenosis, due to vascular smooth muscle cell (VSMC) proliferation and intracellular matrix synthesis in response to stent-induced inflammatory reactions [65]. In-stent restenosis (ISR) is a challenging syndrome that affects both drug-eluting stents and bare-metal stents [66]. DES have been developed to release drugs, such as rapamycin and paclitaxel, over a period of several weeks or months, directly to the wall of the blood vessel to reduce the chance of restenosis. Sirolimus-eluting coronary stents have a long history of treating restenosis [67]. The process to produce this stent is as follows: 316L stainless steel stents are initially coated with parylene C. The drug-eluting coating is a blend of poly (ethylene-co-vinyl acetate) (PEVA), poly(n-butyl methacrylate) (PBMA), and sirolimus (rapamycin). Solutions are sprayed on the stent in such a way that the CYPHER1 stent coating has all three components present throughout the entire drug-eluting coating. The spray-coating process distributes the polymer and drug system on both the outer and inner surfaces of the stent before they are crimped on the delivery system [68].

Coating materials include synthetic polymers, bioabsorbable polymers, and inorganic materials. Commonly used coating materials include poly-n-butyl methacrylate and polyethylene-vinyl acetate used for making Cypher stents [68], as well as other materials, such as temperature-sensitive material N-isopropylacrylamide (NiPAAm)/N-tert-butylacrylamide (NtBAAm)-derived copolymers [69], biomimetic phosphorylcholine (PC)-containing materials [70, 71], natural biopolymer hyaluronan-based coating [72], and other synthetic polymers [73].

Biocompatible and bioresorbable polymers used as stent coating materials include PLGA [74, 75], poly (lactide-co-S-caprolactone) copolymer and polyester amide [76]. Fully bioresorbable stents, based on PLA, have been developed in order to promote the natural remodelling of an injured artery

after angioplasty [77, 78]. In addition to these coatings, inorganic-based coating materials, such as nanoporous alumina coating [79] and Hap ceramic [80], have found favour as biocompatible matrices for DES development.

7.2.5 Regenerative medicine and tissue-engineered cardiovascular devices

It has been found that the lack of an endothelium-mimetic surface on implants leads to possible complications and even failure of cardiovascular devices after implantation. In order to overcome this limitation, as reported previously in this chapter, numerous approaches have been developed, including surface modification, drug–device combination, and selection of different strategies. Tissue engineering and regenerative medicine have opened a new era in the treatment of cardiovascular disease.

Bioactive materials play very important roles in tissue engineering, as a biomatrix for holding cells and as a scaffold for cell attachment, proliferation, and vascularisation. After the new tissue formation, the materials need to be degraded. The development of a tissue-engineered blood vessel substitute has motivated much of the research in the area of cardiovascular tissue engineering over the past 20 years. Vascular tissue engineering includes strategies using different biomatrices, such as cell-seeded collagen gels, cell-seeded biodegradable synthetic polymer scaffolds, cell self-assembly, and acellular techniques, for the development of tissue-engineered cardiovascular tissue [81]. Based on the vascular tissue-engineering approach, the biomatrix can also be used for the treatment of cardiovascular devices in order to improve the device biocompatibility [82].

Tissue-engineered heart valve

Heart valve substitutes are of two principal types: mechanical prosthetic valves with components manufactured of non-biological material (e.g. polymer, metal, carbon), or tissue valves, which are constructed, at least in part, of either human or animal tissue [83–85]. There are many complications for heart valve substitutes, including calcification, infection, and thromboembolism, which lead to the failure of the implants. Tissue engineering provides the possibility of creating an ideal heart valve without the above potential complications [86, 87]. Different factors need to be considered when developing a tissue-engineered heart valve, including cells, scaffold, biological signals, and construct [85].

Endothelial progenitor cells (EPCs) are bone marrow-derived haematopoietic stem cells capable of differentiating into the endothelial cells that line the blood vessels and cardiac valves [86]. By seeding EPCs on a PGA-PLLA

biodegradable scaffold, the endothelial phenotype can be maintained during the period of implantation time [88]. One of the tissue-engineered heart valves was produced *in vitro* and then implanted *in vivo*. The scaffold consisted of PGA and the strong, flexible poly-4-hydroxybutyrate (P4HB), in order to achieve good mechanical properties. By seeding with the differentiated autologous vessel-derived ovine endothelial cells and smooth muscle cells, the *in vitro* bioprocess was carried out for 14 days to generate heart valve tissue, and then the construct was implanted *in vivo,* as a pulmonary valve replacement in an ovine animal model. After 20 weeks *in vivo*, the scaffold material had been degraded and replaced by a partially endothelialised uniform, layered tissue, with layer-specific ECM predominance, similar to that of the native valve, including a layer containing elastin near the inflow surface, glycosaminoglycans centrally, and a fibrous layer with abundant collagen near the outflow surface. Mechanical properties were comparable to those of native tissue at 20 weeks [89]. One study used a decellularised pulmonary allograft seeded with autologous endothelial cells and conditioned in a bioreactor to reconstruct the right ventricular outflow tract of adults undergoing the Ross procedure. It was reported with a one-year follow-up, seeded endothelial cells remained on the construct and were fully functional and the construct mechanical strength was maintained. There was no calcification and/or thrombogenesis. However, whether the seeded cells contributed to valve function remains uncertain [90].

Tissue-engineered cardiovascular grafts

Tissue-engineered cardiovascular grafts, based on endothelial cells, smooth muscle cells and neonatal cardiomyocytes, could improve cardiac function and prevent cardiac congestive failure [91, 92]. Endothelial progenitor cells (EPCs), umbilical cord cells, and bone marrow cells (BMCs) have been seeded onto a variety of graft materials prior to implantation. In one study, the EPCs seeded vascular grafts remained functioning for 130 days as a carotid graft, while the unseeded grafts occluded within 15 days [93]. Tissue-engineered vascular autografts seeded with BMCs have been used to treat children with congenital heart defects. The mean follow-up after surgery was 490 ± 276 days and no complications, such as thrombosis, stenosis, or obstruction of the tissue-engineered autografts, were found. All tube grafts were patent, and the diameter of the tube graft increased with time [94].

The design and production of small-calibre blood vessel grafts is a bioengineering challenge, owing to the difficulty in maintaining the non-thrombogenicity of the surface. Therefore, there is a great clinical need for tissue-engineered small-diameter grafts. The tissue-engineered small-diameter graft consists of two main components: one endothelium-lined surface supported with the second elastic scaffold grafts. This can be partially

achieved via a cell-engineering technology, although considerable effort is still required to overcome some of the complications [95, 96].

Another approach to creating cardiovascular grafts is via cell-sheet technology [97]. The concept is to harvest a stable cell sheet of single-cell thickness, without losing the intercellular connection, and then the single-cell-sheet can be transferred to a second and third cell sheet. This cell matrix/grafts combination can then be implanted to function in the circulatory system in order to repair injured heart and blood vessel [98, 99].

7.2.6 Bioactive materials in commercially available cardiovascular devices

Previous sections have clearly demonstrated the importance of bioactive materials in the design and production of cardiovascular devices for clinical application. In the cardiovascular industry, there are currently numerous manufacturers worldwide, who dedicate their resources to developing and marketing cardiovascular devices in order to meet an increasing demand. Table 7.2 lists the main cardiovascular companies, with their key products, to highlight the importance of bioactive materials in this field.

Table 7.2 Commercially available cardiovascular products

Products	Bioactive materials used	Company
XIENCE V drug-eluting stents; MitraClip system to treat mitral regurgitation (MR)	Biocompatible fluorinated copolymer + everolimus; metal coated with polyester	Abbott Lab
AbioCor artifical heart system	Titanium and Angioflex™, ABIOMED's proprietary polyether-based polyurethane plastic	ABIOMED
Heart valve/catheters	Elast-Eon™ and ECSil's™ biomaterials	AorTech international
Bioresorbable peripheral and coronary polymer stents	Bioresorbable L,D-PLA	Arterial Remodeling Technologies
ClearWay™ RX -OCI therapeutics (occlusion, containment, infusion) for local undiluted drug IV delivery	Atraumatic microporous PTFE balloon delivery system	Atrium Medical
ATS heart valves	Tubular design of ATS 3f® aortic bioprosthesis	ATS Medical [100]
INCOR® LVAD; EXCOR® Pediatric	Silicone and polyester cannulas	Berlin Heart
Stem cell regenerative medicine: MyoCell®	Muscle stem cells and related devices for congestive heart failure (CHF)	Bioheart Inc.

Table 7.2 (cont.)

Products	Bioactive materials used	Company
BioMatrix Flex™ and BioMatrix™ DES	Abluminal biodegradable polymer contain	Biosensors International Group [102]
PROMUS® everolimus-eluting coronary stent; TAXUS® Liberté® paclitaxel-eluting coronary stent;	Fluorinated copolymer matrix	Boston Scientific
Silver Graft, Uni-Graft® W / Uni-Graft® W aortic arch; Vascular-Patch	Silver-containing polyester/ polyester graft/polyester urethan	B Braun
TMR (transmyocardial revascularisation)	Laser fibre to stimulate the revascularisation of heart musle	Cardiogenesis [103]
Intrepide DES	PLC coating material containing Trapidil	Clearstream Technologies Group plc
CYPHER® sirolimus-eluting stent	Synthetic polymer containing sirolimus as coating material	Cordis Corporation
Pericardial heart valve	Tissue-based aortic heart valve	Edwards Lifesiences
Onyx® LES for the pre-surgical embolisation of brain arteriovenous malformations (bAVM).	EVOH (ethylene vinyl alcohol) copolymer dissolved in DMSO (dimethyl sulphoxide)	EV3
CROSSER® Coronary chronic total occlusions (CTO) recanalisation system	Polymeric catheters	FlowCardia [104]
Coronary bio active stent (BAS)	Titanium–nitride oxide (NO) coating	Hexacath
Mguard technology	PET fibre net to wrap stents acting as an efficient drug delivery platform together with blocking embolic showers and plaque detachment from the arterial wall	InspireMD
FiberNet EPS	Capture and remove embolic material produced while performing percutaneous transluminal interventional procedures in carotid arteries	Lumen Biomedical [105]
CABG grafts/CPB system	Grafts/polymeric catheters / tubings	Medtronic
BioSTAR® bioabsorbable septal repair implant	Acellular collagen matrix and a heparin coating	NMT Medical Inc. [106]

Table 7.2 (cont.)

Products	Bioactive materials used	Company
Genous bioengineered stents	Antibodies immobilised on the stent that capture circulating endothelial progenitor cells (EPCs) for accelerated natural healing	OrbusNeich [107]
Mini-bypass system/ extracorporeal circulation	General medical polymer used in circulation system	Sorin Group
ARROWg + ard® central venous access catheters	Catheters containing anti-infection substances	Teleflex Medical [108]
Thoratec PVAD™, Thoratec IVAD™, HeartMate® XVE left ventricular assist device; HeartMate II® left ventricular assist system	Pumping /cannulas	Thoratec [109]
D-Stat products for stopping bleeding/catheters	Thrombin	Vascular Solutions, Inc.
XTENT Custom NX peripheral (NXP) stent system; XTENT custom NX bioabsorbable stent, enable the treatment of single, multiple, and long lesions with one device	Biolimus A9 and PLA	XTENT [110–112]

7.3 Limitations of bioactive materials in devices for the circulatory system

The global cardiovascular devices market, driven primarily by an ever-expanding patient base undergoing cardiac procedures, has emerged as the largest and one of the fastest-growing markets in the medical equipment industry [113]. The introduction of coronary stents marked a major turning point in the practice of interventional cardiology. A report by Global Markets Direct, '*The future of the cardiovascular devices market to 2012*,' concludes that the global drug-eluting stents market, valued at $5.75 billion in 2007, is expected to grow 7.2% by 2012, reaching $8.15 billion [113].

However, there are still many limitations to the control of the in-stent restenosis, in spite of technological advances and an improved understanding of the restenotic process. The limitations include the complexity in the patient base of the 'real-world' application of DES, the clinical placement of a drug-eluting stent in the patient, and the less defined therapeutic approach to DES restenosis [114, 115]. According to this report, the molecular mechanisms of arterial remodelling are not well understood and insight into the mechanisms of DES failure is still limited [115].

In the field of bioactive materials for blood-contacting and tissue replacement applications, understanding the interface between biomaterials and the physiological environment in the clinical situation remains a challenge. Although significant progress has been made in the design of blood compatible (non-thrombogenic) polymers, there is still a challenge to create a surface comparable to that of natural endothelium for application in the cardiovascular system. Before biomaterials can truly be viewed by the host as 'native' material, many unanswered questions, issues, and obstacles must be addressed, including integrity of the surface, stability of the bioactive molecules, spacer effects, interaction with biological medium, duration of the release, flow condition, and dose effect [116].

In particular, the inflammatory response of materials *in vivo* has led to numerous biomimetic approaches to the development of cardiovascular devices, which require the devices to be less invasive, anti-infectious, anti-inflammatory, biocompatible, and even biodegradable, when tissue-engineered devices are used. For example, paediatric cardiac surgery often requires cardiopulmonary bypass (CPB) during the surgical intervention. CPB is known to elicit a systemic inflammatory response, with activation of the complement and coagulation systems, stimulation of cytokine production, cellular entrapment in organs, neutrophil activation with degranulation, platelet activation, and endothelial dysfunction. An improved understanding of the pathological events is critical for the future development of new CPB devices [117] and the contribution of CPB and cardioplegic arrest to morbidity and mortality following cardiac surgery remains unclear [118]. The recently developed miniaturised cardiopulmonary bypass (M-CPB) for beating-heart coronary artery bypass grafting (CABG) has demonstrated minimal pump-related inflammatory response and organ injury, with significantly more complete coronary revascularisation [119]. However, more clinical studies are still required.

In another emerging field of tissue engineering and regenerative medicine, stem-cell related technology for the treatment of cardiovascular diseases remains in an early but very exciting stage. Before stem cells can be applied to human medical problems, substantial advances in basic cell biology and clinical techniques are necessary. In addition, very challenging regulatory decisions will be required on the individually created tissue-based therapies resulting from stem cell research. Technical issues include developing the ability to control the differentiation of stem cells into a desired cell type (like a heart or nerve cell) and to ensure that uncontrolled development, such as a cancerous tumour, does not occur. Immune rejection must also be overcome when transplantation is carried out and the production of an ample amount of the desired cell type, which can afterwards effectively contribute to recover the impaired tissue. At present few reliable human-cell-based *in vitro* models for cardiovascular disease exist [120, 121].

When tissue-engineered cardiovascular devices are considered, five factors need to be taken into account: cells, scaffold, construct, bioprocessing (signals), and implantation through the whole damaged tissue repairing process [85]. For example, the most challenging goal in the field of cardiovascular tissue engineering is the creation/regeneration of an engineered heart muscle, as heart valve and blood vessels can have substitutes. One of the challenges includes the design of bioactive scaffolds, which allow composition variation to accommodate divergence in the evolving myocardial structure and to promote vascularisation and/or innervations within engineered myocardial tissue. Consideration should be given to multiple design and delivery approaches for *in vitro* myocardial tissue preparation. A multiple assembly approach of different cell types and biomaterials into multi-dimensional structures that mimic the architecture and function of native heart muscle might be required [122].

7.4 Future trends

This chapter has reviewed the current development of bioactive materials applied in cardiovascular devices, but focuses mainly on bioactive materials in cardiovascular implants, where biomaterials play very important roles both mechanically and physiologically. The key applications of bioactive materials have been found in design and manufacturing cardiovascular implants as heart valves, blood vessels, catheters for the circulatory system, interventional cardiac surgery and embolic materials for stopping bleeding, and concentrated local drug delivery for cancer treatment. Surface modification for improved biocompatibility remains an important topic, where anti-inflammation and anti-infection have to be addressed under clinical conditions.

One of the most important cardiovascular devices is the stent. Future stent development will focus on drug-eluting stents, based on fully biodegradable stents, to reduce the possibility of the inflammatory response due to the presence of the coating material and the metal, which could lead to the restenosis [123].

Other technologies for utilisation in future development include non-invasive cardiovascular imaging, sensing/monitoring, percutaneous repair and replacement of heart valves, device-based closure of congenital heart defects, and the use of cellular therapy to treat heart disease and stroke. The shift from traditional implantation of valves to the new technology of transcatheter valves is one of the key events that substantiate this trend. The time-saving, less painful, and minimally invasive characteristics associated with this procedure will continue to bolster the penetration of transcatheter valves [124].

Another area for future development is utilisation of biocompatible and

bioresorbable materials in an innovative way, together with the modification of properties for improved biocompatibility and other bioactivities.

In the fields of regenerative medicine and tissue engineering, considerable research will be necessary on

(a) the progressive evolution of structure;
(b) gene expression, function, and the content of the viable cells;
(c) the composition and organisation of the collagen, elastin, and glycosaminoglycans in a tissue-engineered valve, to validate and maximise the utility of this intellectually appealing and potentially useful approach;
(d) mechanotransduction, the effect of mechanically induced ECM deformation on cellular growth, gene expression, cell–cell interactions, and cell–ECM interaction [85].

In conclusion, bioactive materials have found wide application in the circulatory system. Although many advanced devices have reached the market, to achieve improved design of cardiovascular devices, there is a fundamental need to understand the interaction between the material/biological systems. In future, a non-invasive (engineering design) and biomimetic approach (material science), together with tissue engineering and regenerative medicine (cells and biology), will pave the way for better clinical treatment of cardiovascular disease.

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Bioactive materials in gene therapy

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Abstract: Gene therapy is a new form of molecular medicine to cure the inherited or acquired diseases through delivering therapeutic genes to targeted cells and replacing the disorder genes. Nowadays, large numbers of non-viral gene vectors, including cationic liposomes and polycations, have attracted much attention due to their lower immune response, simpler preparation and greater stability as compared with viral vectors. Based on the concise introduction of cell biology, this chapter reviews the most significant non-viral gene delivery systems, and introduces current strategies for non-viral gene delivery *in vitro* and *in vivo*.

Key words: gene therapy, non-viral gene vector, biomaterial, DNA, tumor target.

8.1 Introduction

8.1.1 Gene therapy and gene vector

In 1953, James Watson and Francis Crick presented the structure of the DNA helix, the molecule that carries genetic information from one generation to the other, which was one of the most famous discoveries of the 20th century. DNA allows all modern living things to function, grow and reproduce. However, it could be damaged by many different sorts of mutagens, which change the DNA sequence. According to recent research, many diseases are caused by defective DNA sequences, such as immune disorders (SCIDs), heritable disorders (adenosine deaminase deficiency, cystic fibrosis, and Gaucher's disease), and cancer. Most inherited diseases are caused by mutation in more than one gene. Accordingly, therapeutic genes should be introduced into these defective DNA sequences in order to repair and reactivate them.

Gene therapy is the insertion of therapeutic genes into an individual's cells

and tissues to treat a disease, such as a hereditary disease, in which a deleterious mutant allele is replaced with a functional one. It has the potential to treat devastating inherited diseases for which there is little hope of finding a conventional cure. Gene therapy has been rapidly developed recently, and it will play an important role in future medical research and clinical applications (Vara *et al*., 2002; Cavazzana-Calvo *et al*., 2004; Kasper *et al*., 2008). This notion has given birth to a wide variety of possibilities for true therapeutic approaches to the treatment of human pathological conditions.

There are two general approaches for delivering genes to cells in the body: *ex vivo* and *in vivo*. During *ex vivo* gene transfer, tissue is removed and the cells are genetically modified extracorporally. The modified cells are then reimplanted. For *in vivo* delivery, vectors are administered directly to the recipient and gene transfer occurs in situ. Each strategy comes with advantages and disadvantages and is further constrained by the nature of the target tissue.

As the delivery of 'naked' DNA to a cell is too inefficient, most gene transfer is carried out using a powerful gene delivery vehicle. Gene transfer strategy requires three essential elements: a vector (gene delivery system), a gene to be delivered (therapeutic gene), and a relevant target cell to which the DNA or RNA is delivered. Generally speaking, vectors proposed for gene delivery fall into two categories: viral and non-viral. They differ primarily in their assembling process. A viral vector is assembled in a cell, whereas a non-viral vector is constructed in a test tube. The in-depth understanding of the biological self-assembly process may provide an idea for constructing synthetic self-assembling systems. Gene transfer using viruses is known as transduction. Gene transfer with non-viral vectors is known as transfection. Significant advances in gene therapy have been made over the past few years, and are attributed to the development of effective gene transfer vectors.

Viruses make obviously essential starting points for gene vector development. In 1976, Paul Berg first harnessed a modified SV40 virus containing DNA from the bacteriophage lambda to infect monkey kidney cells maintained in culture (Goff and Berg, 1976). Since that time, a number of different approaches to cancer gene therapy have been investigated, which mainly use replication-defective viral vectors to deliver anti-angiogenic factors, tumour-suppressor genes, prodrug-activating genes, and immunostimulatory genes (Thomas *et al*., 2003; Phillips *et al*., 2007). Viruses are obligate intracellular parasites and highly evolved biological machines that efficiently gain access to host cells and exploit the cellular machinery to facilitate their replication, so they are very efficient at integrating their own DNA into the host chromatin. By replacing genes that are needed for the replication phase of their life cycle with foreign genes, the recombinant viral vectors can transduce the cell type it would normally

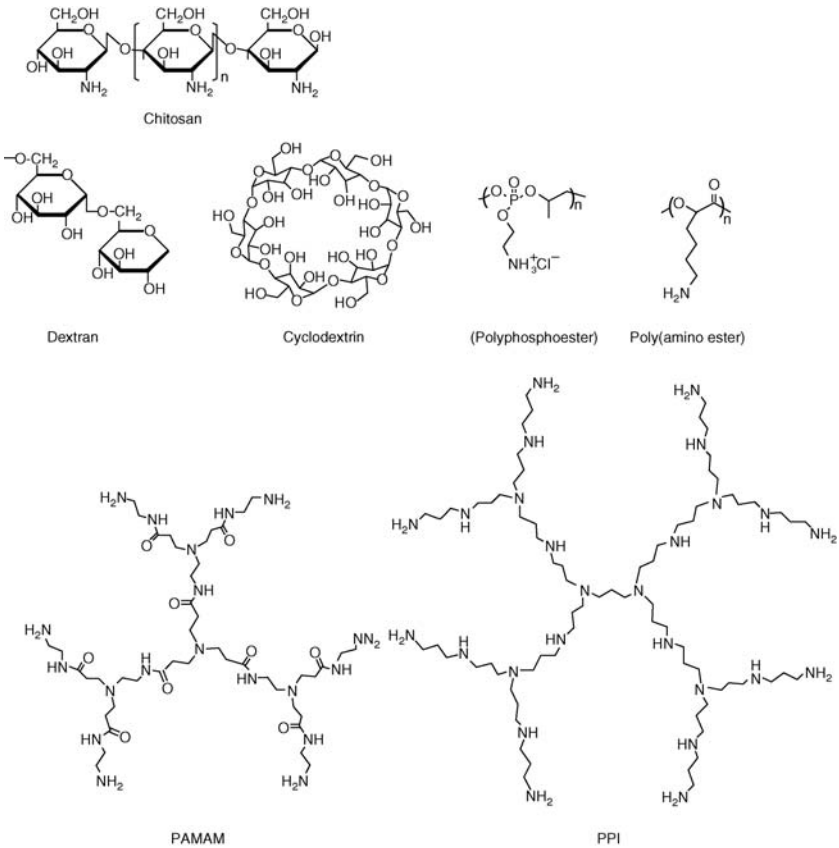
infect. Ideal virus vectors for most gene therapy applications harness the viral infection pathway but avoid the subsequent expression of viral genes that leads to replication and toxicity. Many viral vectors have been widely used in human clinical trials, such as retrovirus, adenovirus, lentivirus, adeno-associated virus, and herpes simplex virus.

Although viral vectors present very high transfection efficacy, safety concerns have been raised. Toxic immunological reactions are the major drawback of viral vectors. A tragic example is the death of a young patient with a rare metabolic disorder of the liver that was caused by the adenovirus vector he received during a gene therapy treatment (Marshall, 1999). Moreover, viral vectors are difficult and expensive to produce.

Consequently, the limitations of viral vectors have motivated the exploration for new synthetic vectors, including lipid-based vectors, chemically modified viruses, inorganic materials, and polymer-based gene delivery systems. These non-viral vectors are considered to be less toxic, less pathogenic and immunogenic, and can also be produced on a large scale. Additionally, non-viral systems can offer remarkable structural and chemical versatility for manipulating physicochemical properties, storage and reconstitution stability, and larger gene capacity. Many natural and synthetic non-viral gene vectors have been investigated, including lipid-based vectors, polymeric vectors (polyethyleneimine (PEI), poly-L-lysine (PLL), polymethacrylate, carbohydrate-based polymers, linear poly(amido-amine) (PAA), chitosan, dextran, β-cyclodextrin, polyphosphoester and poly(amino ester)), dendrimer-based vectors (polyamidoamine dendrimer (PAMAM) and polyporpylneeimine (PPI)), polypeptide vectors, and nanoparticles (Han *et al.*, 2000; Xiang *et al.*, 2003; Morille *et al.*, 2008) (see Fig. 8.1).

8.1.2 Delivery barriers in non-viral gene therapy

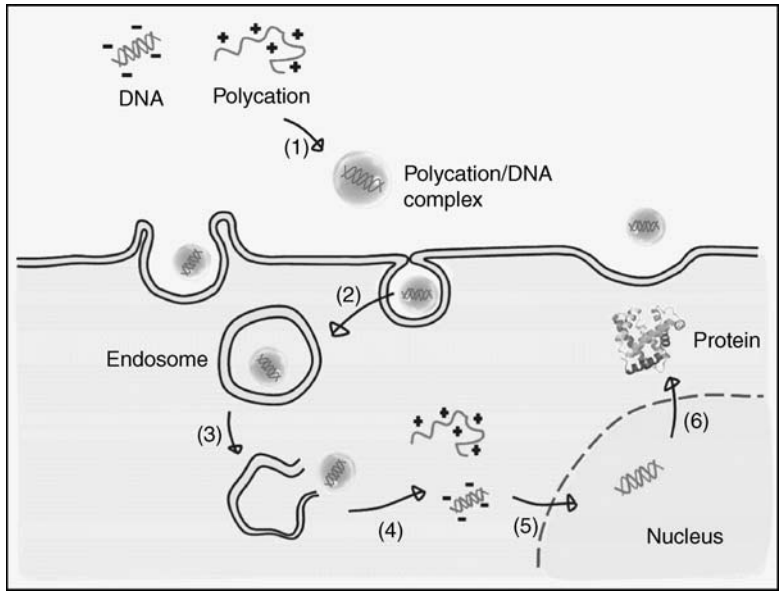
Gene transfer in eukaryotic cells is a multi-step process, including condensation of DNA, cellular uptake, endosomal release, nuclear transport, vector unpacking, and DNA translation (Lucas and Lechardeur, 2002; Merdan *et al.*, 2002; Conner and Schmid, 2003; Kirkham and Parton, 2005; Evans *et al.*, 2006) (Fig. 8.2). Many barriers that appear during gene delivery must be overcome in order to achieve successful gene therapy. Non-viral vector systems offer potential approaches for packaging DNA for gene delivery; however, they are different from viral vectors, which use the ability of viruses to overcome cellular barriers and immune defense mechanisms, so non-viral gene vectors, present significantly reduced transfection efficiency because they are obstructed by numerous extra- and intracellular barriers. Accordingly, a significant amount of research in the past decade has focused on designing cationic compounds



8.1 Structures of some non-viral gene vectors.

that can form complexes with DNA and can avoid both *in vitro* and *in vivo* barriers for gene delivery.

Cellular uptake of 'naked' DNA via permeation from plasma membrane is hindered by the size and negative charge of DNA, so that the initial diffculty in gene delivery is the preparation of the polymer/DNA complex. Binding DNA to a cationic vector is an electrostatic interaction between the negatively charged phosphate backbone of DNA and cationic molecules, which can lead to charge neutralization and form compact structures. However, it also causes further aggregation, some of the complexes are too large to be endocytosed and rapidly removed from blood circulation. This reduces the bioavailability of the transferred gene, which will be expressed in the cells. It has been concluded that the size and potential of the complex formed depend significantly on the physicochemical properties of the cationic polymer (molecular weight and structure) and preparation conditions (concentration of DNA, pH, buffer, and nitrogen/phosphate



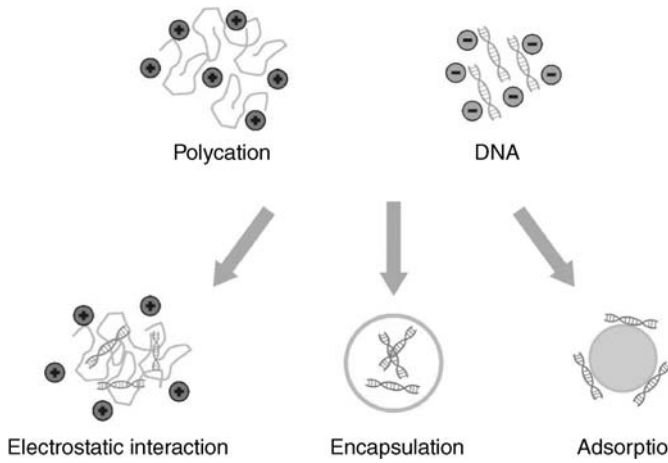
8.2 Barriers to gene delivery. Design requirements for gene delivery systems include the ability to (1) package therapeutic genes; (2) gain entry into cells; (3) escape the endolysosomal pathway; (4) effect DNA/vector release; (5) traffic through the cytoplasm and into the nucleus; and (6) enable gene expression.

(N/P) ratio) (Mellman, 1996; Mislick and Baldeschwieler, 1996; Rejman *et al*., 2004; Khalil *et al*., 2006).

Cellular uptake can be improved by binding DNA with polycationic vectors, such as liposomes, proteins, nanoparticles, or synthetic agents, which mask the strong negative charge of the DNA and facilitate uptake across the negative cell membrane (Fig. 8.3). Physical approaches, including gene gun, electroporation, needle injection, ultrasound, and hydrodynamic delivery, also can be utilized to improve cellular uptake as physical forces were employed to permeate the cell membrane and facilitate intracellular gene transfer.

The presence of the positive-charge-carrying moieties forms the basis for this method of DNA packaging; however, the cationic charges also appear to correlate with the high degree of cytotoxicity typically associated with polycations. Moreover, a strong electrostatic charge, though conducive for DNA packaging and protection, may lead to difficulties of DNA release once the complexes arrive in the cytoplasm.

Before complexes reach the cell surface, several hurdles must be overcome. For example, after intravenous administration, serum inactivation and DNA degradation must be avoided. Non-viral gene delivery is



8.3 The three main strategies employed to package DNA are via (1) electrostatic interaction, (2) encapsulation within, or (3) adsorption onto biodegradable nano- or microspheres.

generally based on whether or not the complex is conjugated to targeting ligands. For the non-targeting case, the complex first binds to the negatively charged cell membrane, owing to its excess positive charge, and subsequently is taken up by endocytosis. The rate of entry into cells varies with cell type and occurs relatively slowly. In order to provide cationic vectors with cell specificity in gene delivery, cell targeting ligands are attached to the vectors, which can be recognized by specific interactions with the target cells and internalized by clathrin-dependent endocytosis (Pouton and Seymour, 1998; Lechardeur *et al*., 2005; Rejman *et al*., 2005; Gersdorff *et al*., 2006). The ligands could be vitamins, carbohydrates, peptides, proteins, or antibodies, and the choice of ligand is the key to extracellular gene delivery, cellular uptake, and intracellular gene expression. The specificity of gene expression could be increased and toxicity might be reduced. Selectivity of target-specific gene delivery can be achieved by using endogenous ligand–receptor interactions, such as transferrin–transferrin receptor. However, the defect of ligand–receptor interactions for targeted gene delivery is the background from soluble free receptors, receptors in non-target tissue, and the presence of circulating ligand. In order to avoid these problems, recent research has introduced a range of novel exogenous ligands, such as antibodies, antibody fragments, and peptides.

After endocytosis, the DNA-containing particles are largely retained in endosomes or lysosomes, which is another major barrier for transfection. ATP-mediated proton accumulation makes the endosomal and lysosomal compartments of cells significantly more acidic (pH 5.0–6.2) than the cytosol or intracellular space (pH 7.0–7.4). Non-viral DNA vectors can utilize the

acidic environment of endosomes and lysosomes to escape degradation and exhibit high transfection efficacy (Wattiaux *et al.*, 2000; Niidome and Huang, 2002; Akinc *et al.*, 2005). Once released into the cytoplasm, complexes must overcome multiple barriers in the cytosol that hamper delivery of the complex into the nucleus of the host cell, possibly due to cytoskeletal elements within the cytoplasm that function as molecular sieves and prevent the diffusion of large molecules. Vectors that compact DNA into small particles should aid in the movement of the DNA to the nucleus. Cationic carriers can also offer protection for DNA from degradation in the cytoplasm.

The ultimate expression of the DNA is still hindered by inefficient transport of DNA into the nucleus, where the transcription machinery resides. To gain access to the nucleus, plasmid DNA must cross the nuclear membrane. Trafficking between the cytoplasm and the nucleus takes place through pore complexes within the nuclear envelope. Passive diffusion through the nuclear pore complex (NPC) generally occurs only for compounds less than 9–11 nm in diameter, which means no complex is allowed inside. Moreover, dividing cells often exhibit higher transfectability than non-mitotic cells, indicating that plasmid DNA can reach the nucleus during nuclear envelope disassembly as cell division occurs.

While cationic DNA carrier systems often exhibit successful gene delivery *in vitro*, systemic delivery is obstructed by complex instability under physiological conditions, which could be considered as *in vivo* barriers for gene delivery. The physiological salt concentration often promotes aggregation of cationic complexes, due to the presence of NaCl, and could weaken the association with DNA by neutralizing the cationic charge on polycations and thereby enlarge the particle size of complexes, which may lead to vascular blockage. Among the barriers to transfection, serum is known to have an inhibitory effect by binding serum albumin to polycation/DNA complexes, which leads to structural reorganization, aggregation, or dissociation of the complexes. Aggregation results in rapid clearance and increased toxicity of polyplexes, and reduced binding of polyplexes to cells.

8.2 Applications of bioactive materials in gene therapy

After over 40 years, gene therapy has made great progress, especially in the non-viral gene delivery system. These non-viral vectors have been successfully used in animal models and are currently being tested in clinical trials to treat maladies, such as cardiovascular disease, cystic fibrosis, Parkinson's disease, and various cancers.

The advantages of the chemically based non-viral systems are obvious. Besides their lesser toxicity and the lower immune responses compared with viral vectors, no integration into the genome occurs. Moreover, the non-viral methods are not limited by the size of the gene to be introduced,

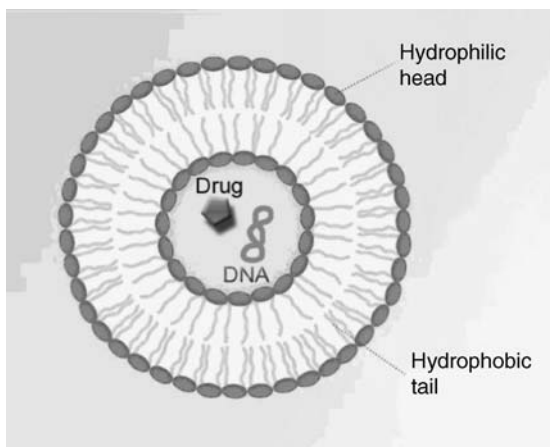
whereas viral constructions are limited to 6–8 kb. Plasmid constructs designed to be delivered by different non-viral vectors are much easier to engineer, verify, and to produce in large, purified quantities.

To overcome different cellular barriers during gene delivery, functional groups have been introduced into non-viral gene vectors design. For example, receptor-mediated cell uptake can quickly deliver ligand-targeted polyplexes into endosomes, membrane active compounds (lipids and peptides) can enhance the release of endocytosed materials, moreover, nuclear localization signal peptides can enhance both the nuclear transport and expression of DNA (Wagner *et al*., 1992; Plank *et al*., 1994; Sebestyen *et al*., 1998; Tachibana *et al*., 1998; Simoes *et al*., 1999). The mechanism of different uptake pathways and their following intracellular transport should be further investigated, as each non-viral gene vector has its own method for internalization. The cell uptake process, intracellular trafficking, and applications of some significant non-viral gene vectors are described below.

8.2.1 Liposome-mediated gene delivery

Liposome-mediated gene transfer was one of the earliest strategies used to introduce exogenous genetic material into host cells. Liposomes were first discovered by British haematologist Alec D. Bangham in 1961 (Bangham and Horne, 1964). A liposome is a tiny vesicle made out of the same material as cell membrane, which can be filled with drugs and genes (Fig. 8.4).

Since 1987, when Felgner *et al*. first reported that a double chain monovalent quaternary ammonium lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N, N-trimethylammonium chloride (DOTMA), effectively binds and delivers DNA to cultured cells, many lipids have been synthesized and become



8.4 Liposome for drug and gene delivery.

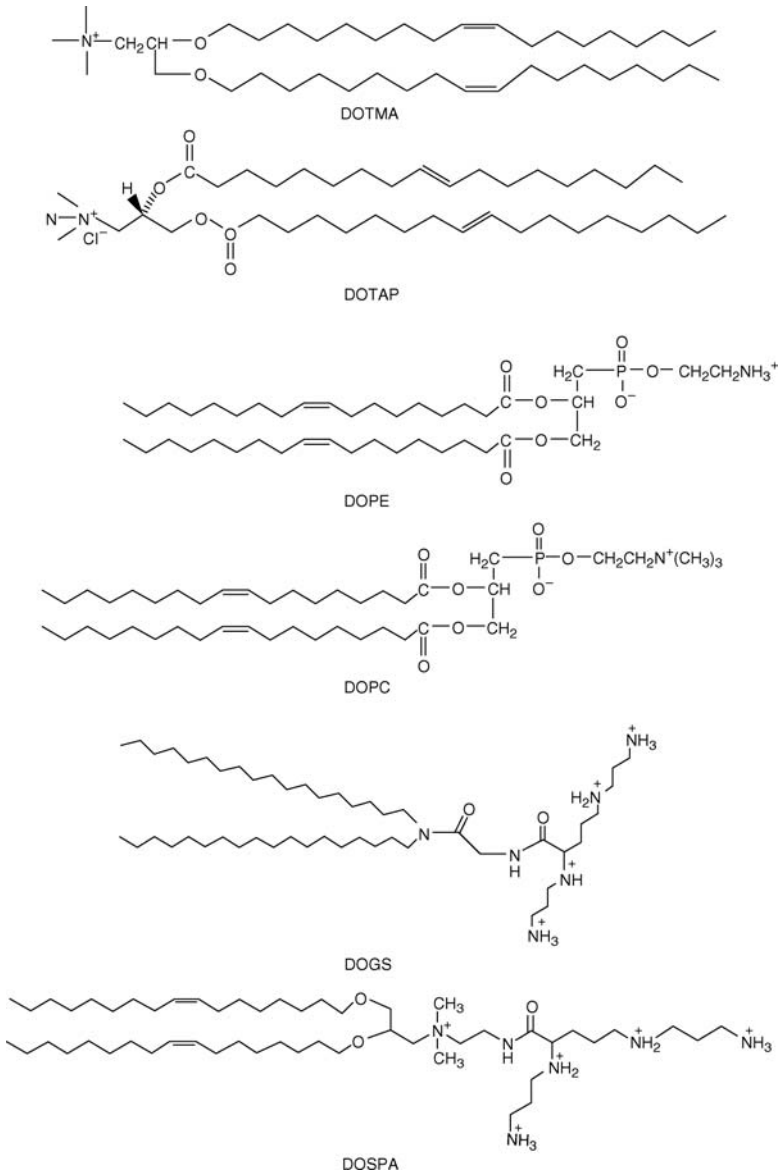
commercially available, including DOTMA, 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), and dioctadecylamido-glycylspermine (DOGS). Cationic liposomes are made of positively charged lipids and are increasingly being studied for use in gene therapy, due to their favourable interactions with negatively charged DNA and cell membranes. Cationic liposomes are also known as cationic lipoplexes. Neutral lipids are often a component for a cationic liposome formulation, in which they play an assistant role, such as dioleyl phosphatidylethanolamine (DOPE), cholesterol and dioleoyl phosphatidyl choline (DOPC). Chemical structures are shown in Fig. 8.5.

Cationic liposomes, as a probable alternative to viral delivery systems, are used for gene transfer, owing to their unique properties and mechanism. Because of the electrostatic interaction, a complex can be easily formed between cationic liposomes (positive) and DNA (negative) (Gershon *et al*., 1993; Sternberg *et al*., 1994; Gustafsson *et al*., 1995). The liposome/DNA complex, or lipoplex, must be sufficiently small to enter the cells. Liposome encapsulates a region of aqueous solution inside a hydrophobic membrane, so that dissolved hydrophilic solutes (such as plasmid DNA) cannot pass through the lipids. After the lipoplex uptake through endocytosis, the lipid bilayer fuses directly with endosomal membrane (reorganization of phospholipids) and opens the door for DNA, resulting in the release of DNA into the cytoplasm. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane), they can be delivered using the lipid bilayer. The possibility of cationic liposomes application for gene delivery, both *in vivo* and *in vitro*, was verified in a series of experiments.

Sato *et al*. (2008) used vitamin A-coupled liposomes to deliver small interfering RNA (siRNA) against gp46, the rat homologue of human heat shock protein 47, to hepatic stellate cells. The siRNA-bearing vitamin A-coupled liposomes resolved liver fibrosis and prolonged survival in rats with otherwise lethal dimethylnitrosamine-induced liver cirrhosis in a dose- and duration-dependent manner.

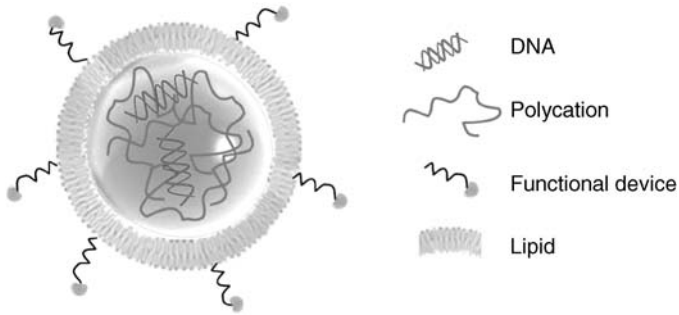
Kim *et al*. (2009) developed a liver-specific siRNA delivery technology using DTC-Apo composed of cationic liposomes (DTC) and apolipoprotein A-I(apo A-I). DTC-Apo nanoparticles could systemically deliver siRNA into mouse hepatocytes expressing hepatitis C virus (HCV) proteins and inhibit their expression efficiently, which means it was a highly potential delivery vehicle to transfer therapeutic siRNA especially targeting HCV to the liver.

Recently, a novel liposome gene delivery system was developed, based on a new packaging concept termed 'programmed packaging', and was named the MEND (shown in Fig. 8.6). The system consists of a condensed DNA core and a lipid envelope structure equipped with various functional devices.



8.5 Chemical structures of several commercially available liposome reagents for gene transfection.

MEND could be prepared in three steps: (i) DNA condensation with polycations; (ii) hydration of the lipid film for electrostatic binding of the condensed DNA; and (iii) sonication to package the condensed DNA with lipids. This packaging mechanism was based on electrostatic interactions between DNA, polycations, and lipids. It was proposed to develop rational



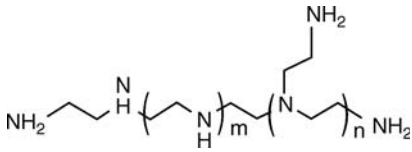
8.6 Structure of MEND.

non-viral gene delivery systems equipped with various functional devices, including ligands for specific receptors, pH-sensitive fusogenic peptides for endosomal escape, and a nuclear localization sequence (NLS) for enhanced nuclear delivery to overcome barriers in the process of gene delivery to the nucleus of target cells.

Akita *et al*. (2009) developed an innovative nanotechnology to construct a tetra-lamellar multi-functional envelopetype nano device (T-MEND), in which a DNA/polycation core was coated with nuclear and endosomal membranes–fusogenic lipid envelopes in a step-wise manner to overcome the intracellular barriers via serial membrane fusion. As a result, the T-MEND achieves dramatic levels of transgene expression in non-dividing cells.

Ko *et al*. (2009) applied PEGylated (PEG: polyethylene glycol) liposome technology for systemic delivery of PEI polyplex of oligodeoxynucleotides (ODN), based on encapsulation of the PEI/ODN polyplexes into PEGylated liposomes. These polyplexes remained stable in the presence of serum. Furthermore, targeting of the PEG-stabilized liposome with antibody specific to the transferrin receptor redirected biodistribution of the entrapped ODN, leading to significant accumulation in the targeted organ, i.e. the brain. Encapsulation of the PEI/ODN polyplexes within a long-circulating liposome provided a promising ODN delivery system for *in vivo* application.

Ewert *et al*. (2006) reported the synthesis of a new multivalent cationic lipid, MVLBG2, with a dendritic headgroup. Hexagonally arranged tubular lipid micelles were surrounded by DNA rods forming a three-dimensionally continuous substructure with honeycomb symmetry. Complexes transfection efficiency reached and surpassed that of commercially available, optimized DOTAP-based complexes. Complexes containing MVLBG2 were significantly more transfectant over the entire composition range in mouse embryonic fibroblasts, a cell line empirically known to be hard to transfect.



8.7 Chemical structure of PEI.

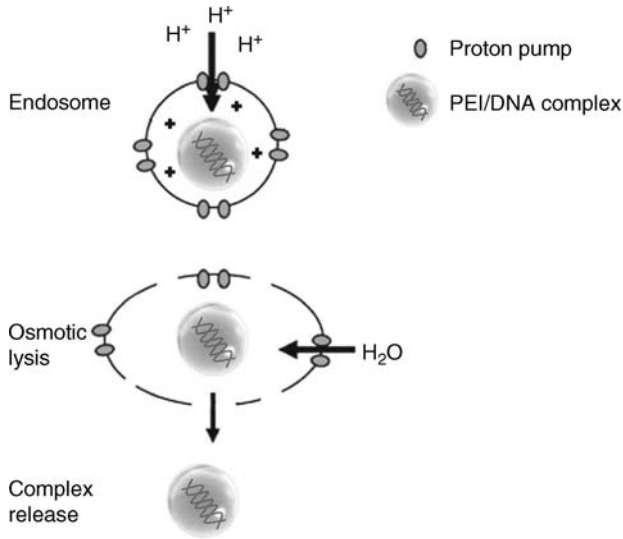
8.2.2 Polyethyleneimine-mediated gene delivery

Polyethyleneimine (PEI), an organic polyamine polymer, is one of the most prominent examples of cationic polymers capable of gene transfection *in vitro* and *in vivo* into various cell lines and tissues (Fig. 8.7). PEI was also applied in different fields from gene therapy and several studies have emphasized the importance of this polymer in medicinal chemistry. Since the first successful PEI-mediated oligonucleotide transfer conducted by the group of Jean-Paul Behr (Boussif *et al.*, in 1995), PEI has been derivatized to improve the physicochemical and biological properties of polyplexes. Several PEI transfection agents have been made commercially available, including ExGen500 and jetPEI.

PEI exists as both a branched and linear structure. Branched PEI (bPEI) is synthesized via acid-catalyzed polymerization of aziridine, whereas the linear structure (lPEI) is synthesized via ring opening polymerization of 2-ethyl-2-oxazoline followed by hydrolysis. PEI-derived vectors have been used to deliver oligonucleotides, plasmid DNA (pDNA), and Epstein-Barr virus (EBV) DNA, as well as RNA and intact ribozymes.

The mechanism of PEI-mediated gene delivery has been illustrated in many literature studies. As a polycation, PEI will spontaneously adhere to and condense DNA to form spherical complexes that are readily endocytosed by cells. These complexes interact with the cell membrane and are endocytosed. PEI that has amine groups with low pKa values has been shown to exhibit 'proton sponge' character. When the complex is endocytosed by endosome, PEI is capable of buffering the endosomal vesicle, which leads to endosomal swelling and lysis, so that DNA can be released into the cytoplasm (Fig. 8.8). Vesicle transport may depend on the cytoskeleton, and PEI/DNA complexes also travel along cytoskeletal tracks in order to contact the nucleus. The polyplex is subsequently translocated into the nucleus, followed by decondensation and separation of the DNA from the polycationic delivery vehicle, either outside or inside the nuclear membrane. The released DNA subsequently undergoes transcription and translation, giving rise to the protein product.

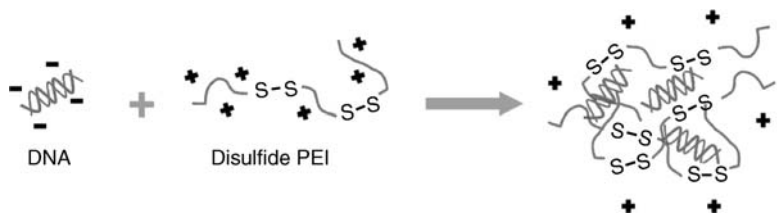
The efficacy of bPEI-derived vectors and their cytotoxicities strongly depend on material characteristics and polyplex properties, such as molecular weight, degree of branching, cationic charge density, buffer



8.8 Proton sponge effect of PEI-mediated gene delivery systems.

capacity, DNA content, particle size, and zeta potential. Moreover, it is considered that transfection efficacy is also influenced by experimental conditions, like the polyplex concentration, the presence or absence of serum during transfection, the incubation time, and the transfection model chosen for the gene delivery experiment.

Godbey *et al.* (1999) reported that the cytotoxicity of PEI derives from two mechanisms. Free PEI can cause cell death prior to cellular internalization by membrane destabilization. Alternatively, 7–9 h after cellular internalization (when DNA has been released from the complex), free PEI can induce cellular stress responses such as endothelial cell activation. In an effort to minimize the latter process, analogues that break down into less toxic, low molecular weight structures after cellular uptake have become appealing. Synthesis of biodegradable PEI compounds involved either the incorporation of reducible disulfide linkages (Fig. 8.9) or ester conjugation. Lee *et al.* (2007) synthesized reducible PEI derivatives by treatment of low molecular weight PEI (800 Da) with either dithiobis



8.9 Disulfide PEI/DNA complex.

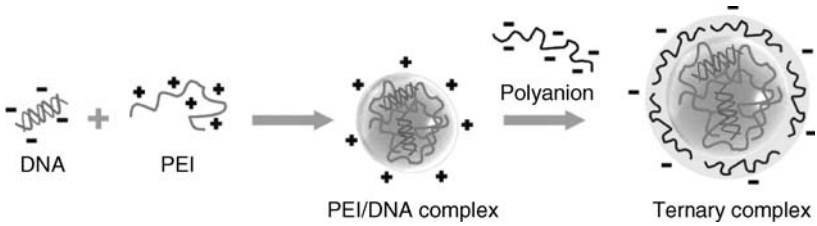
(succinimidylpropionate) or dimethyl-3,3′-dithiobispropionimidate. Sun *et al*. (2008b) synthesized the reducible SS-PEI by Michael addition between cystamine bisacrylamide and low-molecular-weight branched 800 Da PEI. Peng *et al*. (2009) have prepared disulfide crosslinked polyethyleneimines (PEI_X-SS_Y, where X refers to the molecular weight of raw PEI, and Y refers to the thiolation degree) in two steps: first, thiol groups were introduced on a raw polyethyleneimine (PEI) by the amine-induced ring-opening reaction of thiirane. Second, thiol groups were oxidized by dimethyl sulfoxide (DMSO) to form the disulfide cross-links. In addition to disulfide linkages, PEI derivatives with acid-labile ester linkages have been explored by several research groups to create biodegradable gene carriers.

During recent years, an extensive variety of modifications to PEI structure have been employed in order to improve transfection efficiency of the complexes. To suppress non-specific interactions, the cationic surface of PEI has been modified by the covalent or non-covalent attachment of a hydrophilic polymer layer, using polyethylene glycol (PEG), pluronic, polyacrylic acid (PAA), poly(N-(2-hydroxypropyl)mechacrylamide) derived copolymers, dextran, transferrin, human serum albumin, and so on.

Thomas and co-workers (2005) found that removal of the residual N-acyl moieties from commercial linear 25-kDa PEI enhanced its plasmid DNA delivery efficiency 21 times *in vitro*, as well as 10 000 times in mice with a concomitant 1500-fold enhancement in lung specificity. They synthesized linear PEIs by acid-catalyzed hydrolysis of poly(2-ethyl-2-oxazoline), yielding the pure polycations. As a validation of the potential of such linear, fully deacylated PEIs in gene therapy for lung diseases, systemic delivery in mice of the complexes of small interfering RNA (siRNA) against a model gene, firefly luciferase, and PEI25 or PEI87 afforded a 77% and 93% suppression of the gene expression in the lungs, respectively.

Pun *et al*. (2004) prepared β-CD-lPEI and β-CD-bPEI by lPEI and bPEI, respectively, grafted with β-cyclodextrin. The *in vitro* toxicity and transfection efficiency are sensitive to the level of cyclodextrin grafting. The cyclodextrin-containing polycations, when combined with adamantane-poly(ethylene glycol) (AD–PEG) conjugates, form particles that are stable at physiological salt concentrations. PEGylated β-CD-lPEI-based particles give *in vitro* gene expression equal to or greater than lPEI as measured by the percentage of enhanced green fluorescent protein (EGFP) expressing cells. Tail vein injections into mice of 120 μg of plasmid DNA formulated with β-CD-lPEI and AD–PEG do not exhibit observable toxicities, and both nucleic acid accumulation and expression are observed in the liver.

Kurosaki *et al*. (2009) discovered a vector coated by γ-polyglutamic acid (γ-PGA) for effective and safe gene delivery. They prepared several ternary complexes constructed with pDNA, polyethyleneimine (PEI), and various polyanions, such as polyadenylic acid, polyinosinic-polycytidylic acid, α-



8.10 Formation of DNA/PEI/polyanion ternary complexes.

polyaspartic acid, α-polyglutamic acid, and γ-PGA. (Fig. 8.10) Those polyanions changed the positive ξ-potential of pDNA/PEI complex to negative, although they did not affect the size. The pDNA/PEI/γ-PGA complex showed high uptake and gene expression. Most of the pDNA/PEI/γ-PGA complexes were located in the cytoplasm without dissociation and a few complexes were observed in the nuclei. Hypothermia and the addition of γ-PGA significantly inhibited the uptake of pDNA/PEI/γ-PGA by the cells, but L-glutamic acid had no effect. The pDNA/PEI/γ-PGA complex was taken up by a γ-PGA-specific receptor-mediated energy-dependent process.

Ma *et al*. (2009) reported the glucocorticoid-polyethyleneimine (GC-PEI)/pDNA complexes by conjugating glucocorticoids with low-molecular-weight PEI 1800. The result showed that receptor binding of five GC was different and transgene expression enhanced linearly with the increasing GC. In addition, confocal microscopy examination confirmed that GC-PEI/DNA complexes were more effectively translocated in the nucleus than PEI 25 K or PEI 1800 complexes and the cytotoxicities of the GC–PEI polymers were lower than that of PEI 25 K. These results demonstrated that transfection activity of GC–PEI polymer correlated with its GC, and this regularity might be useful for the development of a more efficient GC substituted polymer as a promising nuclear-targeting carrier.

Sun *et al*. (2008c) synthesized a novel cationic polymer, carboxymethyl dextran-graft-polyethylenimine (CMD-g-PEI), which was fabricated through grafting the 800 Da PEI to the biodegradable and biocompatible carboxymethyl dextran. The transfection efficiency of CMD-g-PEI at the N/P ratios over 30–70 was higher or comparable to that of 25 kDa PEI at its optimal N/P ratio of 10, while the cytotoxicity of CMD-g-PEI was much lower than that of 25 kDa PEI.

Zeng *et al*. (2009a) synthesized a novel gene vector biotinylated polyethyleneimine/avidin bioconjugates (ABP) by coupling biotin onto the backbone of high molecular weight branched PEI (25 kDa) and bioconjugating with avidin by the avidin–biotin strong affinity. The resulting gene vector ABP presented significant lower cytotoxicity and higher transfection efficacy in HepG2 cells, due to the biocompatibility of avidin and the interaction between avidin and HepG2 cells. They also prepared the

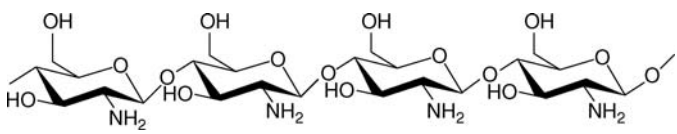
biotinylated disulfide containing PEI/avidin bioconjugate as gene vector, and found that transfection efficiency was specifically enhanced in HepG2 cells (Zeng *et al*., 2009b).

Zhang *et al*. (2009) have synthesized polyaspartamide-based oligoethylenimine brushes from polysuccinimide (PSI) via a ring-opening reaction with N-Boc protected ethylenediamine, tetraethylenepentamine, pentaethylenehexamine, and linear polyethyleneimine (Mn 423), respectively. Their *in vitro* cytotoxicity was significantly lower than that of branched PEI 25000. The four synthetic polycations showed excellent DNA binding ability and condensed DNA to form small-sized polyelectrolyte complexes with positive surface charge at higher N/P ratios. The transfection efficiencies increased with increasing the length of oligoethylenimine of the polymer side chains.

8.2.3 Chitosan-mediated gene delivery

Chitosan is produced by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.), to form a polymer composed of D-glucosamine and N-acetyl-D-glucosamine subunits linked by β(1,4) glycosidic bonds (Fig. 8.11). It can be used in the fields of medicine and healthcare, environmental protection, functional materials, and agriculture. The amino group in chitosan has a pKa value of ~6.5, thus, chitosan is positively charged and soluble in acidic to neutral solution, which makes chitosan bioadhesive, and easily bound to negatively charged surfaces, such as cell membranes. The biodegradability, biocompatibility, and cationic potential of chitosan have helped it become one of the most prominent, naturally derived non-viral vectors for gene transfer. Mumper *et al*. pioneered the early efforts for gene delivery using chitosan in the mid-1990s (Mumper *et al*., 1995).

Investigations have indicated that the molecular weight of chitosan polymers can strongly influence gene transfer efficiency. High molecular weight polymers can more effectively entrap DNA than low molecular weight analogues. In addition to molecular weight, several other factors have been shown to affect the transfection efficiency of chitosan polyplexes, including the N/P charge ratio, pH, the degree of deacetylation, and cell type. The optimum N/P ratio was shown to be 5 for chitosan polyplexes.



8.11 Chemical structure of chitosan.

Optimal transfection efficiency of chitosan polyplexes can be achieved between pH 6.8 and 7.0.

A great number of modifications to the polymer structure have been made to improve transfection efficiency of chitosan polyplexes. To improve the buffering capacity of chitosan-based polyplexes, two important modifications have been investigated. The chitosan polymer has been conjugated with varying ratios of urocanic acid. The same buffering capacity was achieved by conjugating chitosan with polyethyleneimine. Transfection efficiency of the chitosan-PEI derivative rivaled that of 25 kDa PEI, but cytotoxicity was significantly reduced for the chitosan-PEI derivative. Hydrophobic moieties such as deoxycholic acid, stearic acid, and alkyl chains were conjugated to chitosan in order to reduce the aggregation of chitosan polyplexes and improve interactions with cell surfaces. The thiolated derivative, which could form reducible disulfide linkages, was proposed to further improve transfection efficiency of chitosan. The delivery of chitosan complexes to specific cell types was achieved by conjugating chitosan to various cell-targeting ligands. For example, hepatic cell-targeting using galactose, lactose or a trisaccharide showed improved gene transfer efficiency in HepG2 cells.

Jiang *et al*. (2008) successfully prepared and evaluated a novel Gal-PEG-CHI-g-PEI copolymer as a new hepatocyte target gene carrier. The Gal-PEG-CHI-g-PEI has an enhanced ability to form complexes with DNA and has physicochemical properties suitable for a gene delivery system. This copolymer had low cytotoxicity and exhibited high hepatocyte specificity *in vitro* as well as *in vivo* when compared to PEI 25K and CHI-g-PEI.

Lu *et al*. (2008) synthesized three NMC-g-PEI copolymers by using chitosans with different molecular weights. NMC_{5K}-g-PEI and NMC10K-g-PEI showed comparable transfection activity and lower cytotoxicity as compared with PEI (25 KDa) in both 293T and HeLa cells, whereas NMC_{50K}-g-PEI showed higher cytotoxicity and lower transfection activity compared with NMC_{5K}-g-PEI and NMC_{10K}-g-PEI. The presence of fetal bovine serum did not affect the transfection activity of NMC_{5K}-g-PEI and NMC_{10K}-g-PEI remarkably but resulted in increased transfection ability of NMC_{50K}-g-PEI.

Lu *et al*. (2009) also synthesized a series of chitosan-based oligoamine polymers from N-maleated chitosan (NMC) via Michael addition with diethylenetriamine (DETA), triethylenetetramine (TETA), tetraethylenepentamine (TEPA), and linear polyethyleneimine (Mn 423) respectively. These polymers displayed good DNA binding ability. The structural difference of oligoamine side chains affected the buffer capacity, cytotoxicity, and transfection efficiency. The results showed that the gene transfection efficiency of these polymers was better than that of chitosan. Moreover, the transfection efficiency was dependent on the length of the

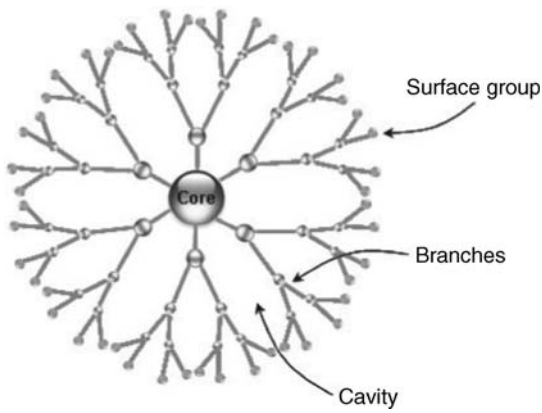
oligoamine side chains as well as the molecular weight of the chitosan derivatives.

Bowman *et al.* (2008) studied the oral gene delivery efficacy of DNA polyplexes composed of chitosan and Factor VIII DNA. Transgene DNA was detected in both local and systemic tissues following oral administration of the chitosan nanoparticles to hemophilia A mice. Functional factor VIII protein was detected in plasma by chromogenic and thrombin generation assays, reaching a peak level of 2–4% FVIII at day 22 after delivery. In addition, a bleeding challenge one month after DNA administration resulted in phenotypic correction in 13/20 mice given 250–600 μg of FVIII DNA in chitosan nanoparticles, compared to 1/13 mice given naked FVIII DNA and 0/6 untreated mice.

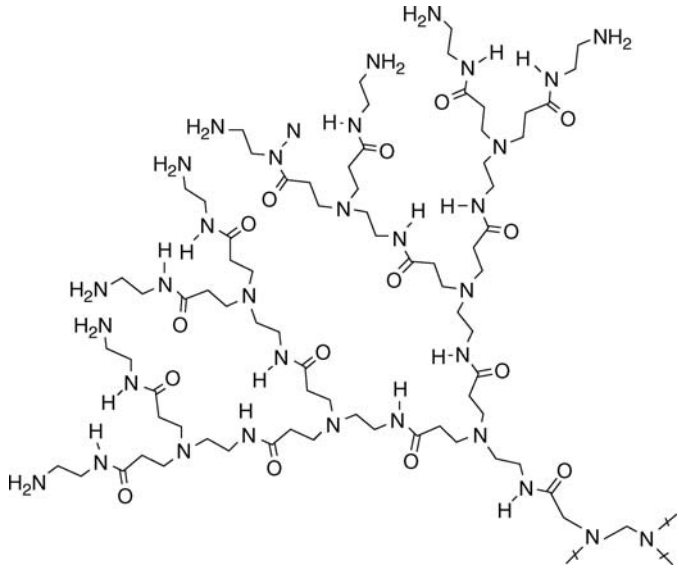
8.2.4 Dendrimer-mediated gene delivery

Dendrimers are highly branched and monodisperse macromolecules with symmetrical, nanometre-sized architecture, which are prepared by multistep synthetic procedures. They consist of a central core molecule, which acts as the root from which a number of highly branched, tree-like arms originate in an ordered and symmetric fashion. This type of architecture induces the formation of nanocavities, the environment of which determines their solubilizing or encapsulating properties, while the external groups primarily characterize their solubility and chemical behavior (Fig. 8.12).

The first exploration of dendrimers for gene delivery focused on the polyamidoamine (PAMAM) dendrimer, which is the most common class of dendrimers suitable for many materials science and biotechnology applications (Fig. 8.13). Unlike classical polymers, PAMAM dendrimers have a high degree of molecular uniformity, narrow molecular weight distribution,



8.12 Dendrimer structure.

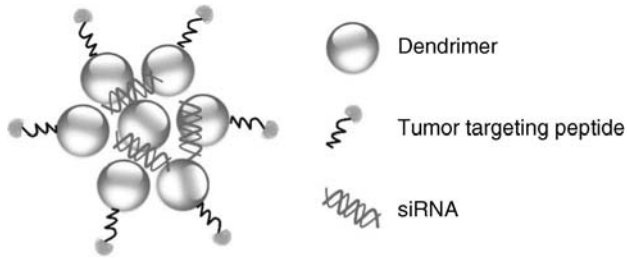


8.13 Chemical structure of PAMAM.

specific size and shape characteristics, and a highly functionalized terminal surface. They are normally based on an ethylenediamine or ammonia core, with four and three branching points respectively. Using a divergent approach, the molecule is built up iteratively from the core through addition of methylacrylate, followed by amidation of the resulting ester with ethylenediamine. Each complete iterative reaction sequence results in a new dendrimer generation (e.g. G3, G4) with terminal amine functionality, whereas the intermediate half generations (e.g. G2.5, G3.5) terminate in anionic carboxylate groups. Another commercially available dendrimer used for drug and gene delivery is based on polypropylenimine (PPI) units, using butylenediamine (DAB) as the core molecule.

Nanocavities of dendrimers can encapsulate a satisfactory amount of active drugs and DNA. The external groups of dendritic polymers can be modified, providing a diversity of functional materials that are employed for various applications, including drug delivery. Thus, commercially available or custom-made dendrimeric or hyperbranched polymers have been functionalized and are used as drug delivery systems, or gene delivery vectors.

Patil *et al.* (2009) designed and evaluated two internally cationic polyamidoamine dendrimers for siRNA delivery into cancer cells. The result suggested that a lesser degree of quaternization improved the cellular uptake of siRNA but did not considerably increase its gene silencing activity. In contrast, targeting of the dendrimer specifically to the plasma



8.14 Dendrimer-based tumor targeting siRNA delivery system.

membrane of cancer cells by luteinizing hormone releasing hormone (LHRH) peptide further improved internalization of siRNA by cancer cells and significantly enhanced its intracellular activity, leading to a substantial suppression of the expression of a targeted gene. Data obtained show the high potential of targeted internally cationic dendrimers as nanocarriers for efficient delivery of siRNA to cancer cells and their possible use in cancer chemotherapy (Fig. 8.14).

Mashayekhan *et al*. (2008) presented a serial passaging protocol that permitted the enrichment of undifferentiated embryonic stem (ES) cells by culturing them on a surface modified with a synthesized dendrimer having D-glucose as a functional ligand. The D-glucose-displaying dendrimer (GLU/D) surface caused mouse ES cells to form loosely attached spherical colonies, and the frequency of such colonies increased gradually with the number of passages. The spherical colony cells passaged four times on the GLU/D surface acquired more of the characteristics of undifferentiated cells than the cells cultured on a conventional gelatin-coated surface. The cells cultured on the GLU/D surface retained their germ-line transmission ability after four passages.

Nam *et al*. (2009) synthesized biodegradable polycationic PAMAM esters (e-PAM-R, e-PAM-K) that contain arginines or lysines at the peripheral ends of PAMAM-OH dendrimer through ester bond linkages. The PAMAM esters were readily degradable under physiological conditions (pH 7.4, 37°C), with more than 50% of the grafted amino acids hydrolyzed within 5 h. The arginine-conjugated PAMAM ester-mediated transfection of a luciferase gene showed better transfection efficiency than the branched 25 kDa PEI and PAM-R, and lower cytotoxicity, especially with primary cells such as human umbilical vein endothelial cells (HUVECs) and smooth muscle cells (SMCs).

Fu *et al*. (2008) encapsulated PAMAM/DNA complexes in water soluble polymer PHEA and then deposited on or sandwiched in fast degrading CA-(DLL)n films to mediate cell transfections. The expression of pGL3-Luc and pEGFP-C1 plasmids in HEK293 cells indicated that the PAMAM/DNA complexes deposited and sandwiched films could successfully mediate gene

transfections. Compared to the film with the conventional linear poly(DL-lactide) as the top layer, the film with CA-(DLL)n as the top layer exhibited a much higher gene expression level because CA-(DLL)n had an accelerated degradation, so that the PAMAM/DNA complexes could be exposed to the cells seeded on the film to mediate transfection via phagocytosis. During all transfections, the films did not exhibit any additional cytotoxicity to the cells.

8.2.5 Polypeptide-mediated gene delivery

Among various non-viral gene delivery methods, using natural or artificial polypeptides with certain biological functions is considered as one promising approach. Peptide-guided non-viral gene delivery has clear advantages over other gene therapy strategies. Unlike other vectors, peptides have reduced cytotoxicity and immunogenicity, along with a much greater biodegradability. Peptides can also serve many functions that other non-viral vectors cannot perform alone. There are peptides to condense DNA into compact particles, disrupt the endosomal membrane, escape proteasomal degradation, traffic DNA cargo to the nucleus, and target polyplexes to specific receptors. These properties may all be part of a single peptide sequence or a combination of peptides chemically conjugated to form a vector capable of packaging and targeting DNA for efficient delivery. Over the past 20 years, peptide-guided gene delivery has progressed from the use of heterogeneous mixtures of polylysine to the precise synthesis of defined-length homogeneous peptides. The use of peptides as non-viral delivery vectors is still in its infancy and has the great potential to grow exponentially, as new peptides are discovered that have the ability to achieve these goals alone or in combination with other systems. There are also four barriers that must be overcome by peptide vectors to achieve successful gene delivery. They must be able to condense tightly DNA into small, compact particles; target the condensate to specific cell surface receptors; induce endosomal escape; and target the DNA cargo to the nucleus for reporter gene expression.

Peptide-oligonucleotide conjugates offer a unique strategy of delivering genetic material into cells with high efficiencies and cell-specificity. These peptide vectors are able to deliver oligonucleotides into cells by utilizing short sequences of basic amino acid residues which readily cross the plasma membrane. In general, this conjugation method requires milder conditions than solid phase strategies, but also requires multiple purification steps that result in lower yields. Unlike the covalent attachment of peptide–oligonucleotide complexes, peptide–DNA complexes are generally formed by electrostatic interactions similar to those used to form lipoplexes or

polyplexes. Complexation of peptides and siRNA can also proceed via either electrostatic or covalent interactions.

Cationic peptides can efficiently interact with the negatively charged phosphate backbone of DNA through electrostatic interactions, and form nanoparticles with a positive charge that is able to interact with cell membranes and internalize into the cell to permit gene expression. Moreover, lysine and/or arginine-rich cationic peptides are able to condense DNA efficiently into small, compact particles that can be stabilized in serum. Attachment of a peptide ligand to the polyplex also allows targeting to specific receptors and/or specific cell types. Peptide sequences derived from protein transduction domains are able selectively to lyse the endosomal membrane in its acidic environment leading to cytoplasmic release of the polyplex. In addition, short peptide sequences taken from longer viral proteins can provide nuclear localization of condensates once they are in the cytoplasm. Several peptides that are able to perform these various functions to carry out peptide-guided gene delivery, such as polylysine-containing peptides, histidine-rich peptides, transactivator of transcription (TAT), Gly-Ala repeat, arginine–glycine–aspartate (RGD), nuclear localization signal peptide, and so on.

One of the first cationic peptides used to mediate gene delivery was poly-L-lysine (PLL). The polymerization degree of Lys, as a synthetic repeat amino acid chain, ranges between 90 and 450, and leads to the formation of a polypeptide chain with an acceptable degree of biodegradability, important in terms of cell physiology and controlled release of the DNA into cell nuclei. However, the high degree of polymerization is also directly related to cytotoxicity. In 1987, Wu *et al*. first presented that PLL coupled with asialoorosomucoid (asOR) formed soluble polyplexes, enabling gene expression targeted to those cells exposing the receptor for asOR on their surface.

Recent research studies show that many modifications of peptide length and sequence have been made to give variations to the original polycations in order to improve gene transfection efficiency. Suri *et al*. (2009) investigated the cell signalling events caused by lysine-functionalized rosette nanotubes (RNTs) (K-RNT) co-assembled with Arg-Gly-Asp-Ser-Lys functionalized RNTs (RGDSK-RNT) for induction of inflammation and apoptosis in human adenocarcinoma (Calu-3) cells. RGDSK/K-RNTs induced phosphorylation of P38 MAPK, which regulates secretion of TNF-α, activation of caspase-3, and apoptosis in Calu-3 cells. These results suggest that the RNTs could be used as a drug to induce apoptosis in cancer cells or as a versatile platform to deliver a variety of biologically active molecules for cancer therapy.

Sun and co-workers (2008b) studied the influence of RGD addition on the gene transfer of SS-PEI/DNA binary complexes in HeLa and 293T cells. It

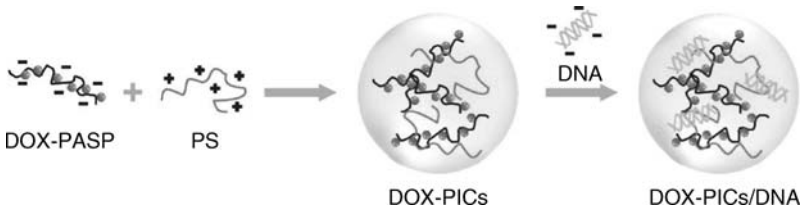
was found that the introduction of RGD would decrease the transfection efficiency in HeLa cells; however, the transfection efficiency alters slightly with the increasing amount of RGD peptide ranging from 3 to 20 mg in cultured 293T cells. The decreased transfection efficiency in HeLa cells was attributed to the binding interactions between the surplus RGD and the anb3 and anb5 integrins in HeLa cells, which would prevent the specific endocytosis of RGD in complexes and non-specific endocytosis of SS-PEI/DNA complexes in HeLa cells.

Xiang *et al*. (2003) have developed a self-assembled non-viral gene carrier, poly-L-lysine modified iron oxide nanoparticles (IONP-PLL), which is formed by modifying poly-L-lysine to the surface of iron oxide nanoparticles. IONP-PLL can deliver exogenous genes to cells *in vitro* and *in vivo*. After intravenous injection, IONP-PLL transferred reporter gene EGFP-C2 to lung, brain, spleen, and kidney. It transferred exogenous DNA across the blood–brain barrier to the glial cells and neuron of the brain.

Seow and Yang (2009) have designed a new class of triblock oligopeptides, NH_2-$I_5H_4R_8$-$CONH_2$ (I), NH_2-$F_5H_4R_8$-$CONH_2$ (F), NH_2-$W_5H_4R_8$-$CONH_2$ (W), and NH_2-H_4R_8-$CONH_2$ (H), and demonstrated their ability to deliver DNA efficiently into cells. These peptides yielded high *in vitro* gene transfection efficiencies, at levels that are comparable or even superior to those for PEI. In particular, the luciferase expression level induced by I within a mouse breast-cancer model was 13 times higher than that mediated by PEI. Each block offered essential functionalities, and both its nature and length could be further modulated to improve gene transfection and reduce cytotoxicity. The *ex vivo* immunomodulation of organs or tissues prior to transplantation was another application particularly amenable to this system.

Zeng and Wang (2005) used a recombinant DNA method to produce a polypeptide comprising a hairpin motif of nerve growth factor (NGF) as a targeting ligand and a 10-lysine sequence as a DNA-binding moiety. The recombinant polypeptide was composed of a targeting moiety derived from loop 4-containing hairpin motif of NGF and a DNA-binding moiety of 10-lysine sequence and expressed in *Escherichia coli*. The enhancement of gene transfer in PC12 was inhibited by pretreatment of free, unbound polypeptides, suggesting an NGF-receptor-specific effect of the polypeptide.

Mitsui *et al*. (2006) engineered recombinant fusion Ovalbumin (OVA) that contained three differrent protein transduction domains (PTDs), including the most efficacious known PTD (polyarginine (R9)-PTD). Results demonstrated that R9-PTD-containing OVA transduced dendritic cells (DCs) most efficiently, and that transduction efficacy was closely correlated with the extent of Agspecific $CD4^+$ and $CD8^+$ T-cell activation *in vitro* and *in vivo*. Repeated vaccination with R9-PTD-OVAtransduced DC in (OVA-expressing) tumor-bearing mice induced enhanced antitumor



8.15 Schematic illustration of the self-assembled DOX-PICs as drug and gene carriers.

immunity, and elicited complete rejection of tumors when DC was co-injected with adjuvants.

Simeoni *et al*. (2003) investigated the mechanism through which MPG (a bipartite amphipathic peptide) promotes gene delivery. They demonstrated that cell entry was independent of the endosomal pathway and that the NLS of MPG was involved in both electrostatic interactions with DNA and nuclear targeting. MPG/DNA complexes interacted with the nuclear import machinery; however, a mutation, which affected the NLS of MPG, disrupted these interactions and prevented nuclear delivery of DNA. It was found that this mutation yields a variant of MPG, which was a powerful tool for delivery of siRNA into mammalian cells, enabling rapid release of the siRNA into the cytoplasm and promoting robust down-regulation of target mRNA. These results supported the potential of MPG-like peptides for therapeutic applications and suggested that specific variations in the sequence may yield carriers with distinct targeting features.

Cheng *et al*. (2009) prepared a series of self-assembled polyionic complexes (PICs) via electrostatic attraction between protamine sulfate (PS) and poly(L-aspartic acid) (PASP) or doxorubicin (DOX)-conjugated PASP (DOX-PASP). *In vitro* gene transfection investigation revealed that the transfection efficiency of PICs/DNA complexes was comparable to that of 25 kDa PEI/DNA complex (N/P ratio 10). Importantly, the gene transfection efficiency of PICs/DNA complexes could be tuned by altering the weight ratio of PS/PASP. The suppression of the proliferation activity of HeLa cells could be achieved by replacing PASP with DOX-PASP, suggesting a great potential of PICs as effective carriers for combined delivery of drug and gene (Fig. 8.15).

8.2.6 Nanoparticle-mediated gene delivery

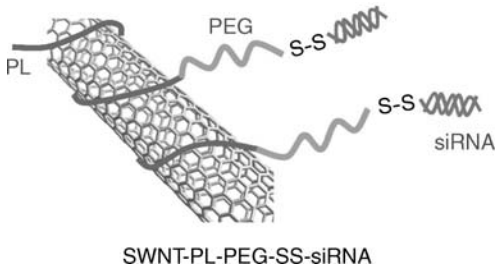
Recently, a series of multifunctional nanoparticles was developed that can be used in biomedical and pharmaceutical applications for diagnosis and therapy. Nanoparticles were investigated as non-viral gene vectors, including quantum dots (QDs), metallic nanoparticles, surface-modified

silica nanoparticles, carbon nanotubes, lipid-based nanoparticles, and polymeric hydrogels. Surface-modified nanoparticles are attractive candidates for gene delivery. Compared to cationic carriers, nanoparticles are inert and exhibit less cytotoxicity, and compared to liposomes, nanoparticles are more stable with respect to physical stresses.

Srinivasan *et al.* (2006) first showed that quantum dots could be covalently conjugated to plasmid DNA for transfection studies. However, certain requirements should be fulfilled by QDs: they should not exhibit any cytotoxicity, and they should be attached to a targeting moiety, allowing them not only to penetrate the cell membrane but also to reach their target site within the cell interior. Coating QDs with peptides has become more attractive. Walther *et al.* (2008) developed multifunctional fluorescent nanoparticles suitable for the non-viral delivery of negatively charged molecules like RNA. They incorporated the branched hCT-derived carrier peptide hCT (18-32)-k7 to the surface of luminescent QDs. Results indicated that more than one endocytotic uptake pathway in the internalization process. The QD–peptide bioconjugates exhibited no effect on cell viability and possess high stability inside living cells. The efficacy of this oligonucleotide drug delivery was highlighted by the successful intracellular transport of Cy-3 labeled RNA. These results proved that the multifunctional platforms were versatile tools for diagnostic and therapeutic imaging purposes applicable for biologically active siRNA or aptamer sequences.

Kikkeri *et al.* (2009) reported a simple and convenient method to prepare different sugar-capped PEGylated QDs that can be used for *in vitro* and *in vivo* applications. They showed that QDs capped with D-galactose are preferentially taken up via asialoglycoprotein receptor (ASGP-R)-mediated endocytosis *in vitro*. The uptake of Gal-capped QDs can be partially inhibited by knockdown of ASGP-R1. Moreover, they demonstrated in the mouse model that QDs capped with D-mannose and D-galactosamine sequester specifically in the liver. Upon intravenous injection of GalN-capped QDs, they found a significant increase in serum transaminases, indicating that liver injury was selectively mediated by GalN-QDs.

Carbon nanotubes (CNTs), owing to their unique mechanical, physical, and chemical properties, have been actively investigated for their applications in medical chemistry and biomedical engineering. Carbon nanotubes can be functionalized to achieve improved biological properties and functions. Recently, reports show that CNTs can take various cargoes such as small peptides, streptavidin, and nucleic acids and penetrate mammalian cell membranes into the cytoplasm via sidewall functionalization. However, the amount of genes or peptides conjugated by each CNT is still limited. More important, these genes or peptides taken by CNTs have to be released out and then take effect in the cancer cells. But the mechanism of



8.16 Carbon nanotubes for gene silencing.

the effects of CNTs on cells is still not well clarified. Therefore, the strategy of using less CNTs to take more genes or drugs into cells is an excellent choice to protect target cells.

Kam *et al.* (2005) coupled various biological molecules to phospholipids functionalized single-walled CNTs (SWNTs) via cleavable disulfide linkage. With this novel functionalization, they demonstrated transporting, releasing, and nuclear translocation of DNA oligonucleotides in mammalian cells with SWNT transporters. Results showed highly efficient delivery of siRNA by SWNTs and more potent RNAi functionality than a widely used transfection agent, lipofectamine (Fig. 8.16).

With the aim of improving the amount and delivery efficiency of genes taken by CNTs into human cancer cells, Pan *et al.* (2009) prepared different generations of polyamidoamine dendrimer modified multi-walled CNTs (dMNTs). The dMNTs fully conjugated with FITC-labeled antisense c-myc oligonucleotides (asODN); those resultant asODN-dMNTs composites were confirmed to enter tumor cells within 15 min by laser confocal microscopy. These composites inhibited the cell growth in time- and dose-dependent means, and down-regulated the expression of the c-myc gene and C-Myc protein. The asODN-G5.0 dMNTs exhibit the maximal delivery efficiency and inhibition effects on cancer cells among MNT-NH2-asODN composites, dendrimer-asODN composites, and dMNT-asODN composites, which may be helpful to solve the problem in antisense therapy.

However, several *in vitro* and *in vivo* studies have reported that CNTs were cytotoxic. For example, Jia *et al.* (2005) found that when SWNT and MWNTs were incubated with alveolar macrophages (AM), a significant (~35%) increase in cytotoxicity was observed after 6 h of exposure. Moreover, Warheit *et al.* (2004) reported that introducing 5 mg/kg SWNT into the intratracheal regions of rats produced a mortality of 15% within 24 h, compared to control rats with carbonyl iron or graphite particles (3~10 mm). While concern that CNTs may be cytotoxic dampens the enthusiasm of this material for biomedical applications, new approaches are being developed to mitigate their toxicity.

8.17 Structure of DODAB-AuNP/DNA complex.

Recently, gold nanoparticle (AuNP)-based gene delivery systems have attracted attention because of their functional versatility, biocompatibility, and low toxicity. Niidome *et al*. (2004) succeeded in preparing gold nanoparticles coated with 2-aminoethanethiol. The preparation of the cationic nanoparticles was easy, cost effective, and reproducible. Gold nanoparticle/DNA complexes at various weight/weight (w/w) ratios were added to HeLa cells. Significant gene expression was observed and its level was increased by increasing the charge ratio. At a w/w ratio of 17, the expression was 100-fold higher than in the case of DNA alone. Significant expression was also observed with other cell lines.

Li *et al*. (2009) found a transfection enhancer, nocodazole (NCZ) for dioctadecyldimethyl ammonium bromide (DODAB)–AuNPs mediated transfection. The results from flow cytometry (FCM) and luciferase assay showed that NCZ produced at least a three times increase in transfection efficiency. They also pursued the mechanism involved in the promotion of the transfection efficiency. The inhibition of the DODAB-AuNPs/DNA complexes being degraded in lysosomes might be the reason for the transfection enhancement. The results indicated that researchers can try to modify lipids on gold nanoparticles and pre-treat cells with NCZ before transfection, which might improve the subsequent efficiency (Fig. 8.17).

8.3 Limitations of bioactive materials in gene therapy

Despite significant progress having been made and a number of non-viral gene delivery systems having been rapidly explored over the past decade,

non-viral gene carriers are still less efficient than viral vectors, especially for *in vivo* gene delivery, which has directly limited the clinical applications of non-viral gene vectors. Current non-viral gene delivery systems have some defects, including low transfection efficiency, transient gene expressions, poor serum stability, higher cytotoxicity, and acute immune response *in vivo*. Hence, further intensive efforts are strongly required in order to improve the properties of presently available non-viral gene systems and find novel approaches for gene delivery, and the non-viral gene vectors should be much safer and more efficient in the future.

It is very important that researchers should identify the limiting factors which can lead to successful non-viral gene delivery. The major challenges for *in vivo* gene delivery are to overcome the limiting barriers and carry out strategies to enhance gene delivery efficiency with minimal tissue damage or side effects. A thorough understanding of the differences among various organs, tissues, and cell lines in response to physical impacts will provide clues on how to develop an effective vector or procedure applicable to humans.

It is essential to understand the relationship between the physicochemical properties of non-viral vectors and their transfection activities; accordingly, the limitations that exist in non-viral gene therapy could be investigated and avoided. The mechanism of *in vivo* gene transfer varies, depending on the route of administration. Most likely, the physical properties of the complex will change after *in vivo* administration. The extent of this change may be related to its interaction with biological fluids. When there is limited contact with biological fluids, such as with an intratumor injection, the polyplex may not change appreciably in its physical properties before it encounters the tumor cells. In contrast, when a polyplex is administered intravenously, its size, structure, and charge are expected to alter significantly before it reaches the target tissues or cells. Many studies have demonstrated the impact of physicochemical properties on processes such as distribution, interaction with biocomponents, absorption to target cells, and intracellular trafficking (Hattori and Hashida, 2005).

Since the formation of cationic carrier/plasmid DNA complexes depends on the electrostatic interaction between the cationic vector and the anionic DNA, the mixing ratio of these two components exerts effects on the physicochemical properties of the resultant complex, such as particle size and zeta potential of the polyplex, which has a strong association with transfection efficiency.

Small and compact complex particles play an important role in polycation gene delivery. Also, the particle size would apparently affect the cytotoxicity and transfection efficiency of gene vectors. It is known that the particle size depends on many parameters, including pDNA concentration, sequence of addition of polycation or pDNA, and ionic strength of solvent. Although

larger particle size could give DNA better protection from enzyme inhibition during gene delivery, it would limit organ access and modulate biodistribution of the complexes at the cellular level. For many gene therapy applications, the target cells will form a part of the parenchyma or interstitium of the organ and the access to these cells is restricted for particulate carriers after vascular administration. This is because macromolecules and particulate carriers can only extravasate from the vasculature at specialized sites, for example, the liver or spleen, where the endothelial lining has suitable gaps, so-called fenestrae, which allow particles of around 200 nm or smaller to pass.

The positively charged complex is also essential for binding the negatively charged cellular membrane, which facilitates the entering of a complex into the nucleus by cellular uptake. Because cellular uptake of a complex is considered to be a non-specific process that is based on the interaction of the excess positive charge of the complex and the negatively charged cell membrane, it is generally believed that higher surface charge enhances the interaction of the complex with the negatively charged cell membrane, and the positive ζ-potentials could stabilize the complexes against aggregation and guarantee a small complex size. Therefore, the cationic charge has to be optimized in order to yield optimum transfection activity in target cells under both *in vitro* and *in vivo* conditions. The stabilities of these complexes depend on the strength of the electrostatic interaction and thus on the total charge and the charge density of the carrier molecule. However, complex formation is not always easily controlled, as the process is influenced by stoichiometric as well as kinetic factors. Moreover, higher positive charges also lead to the unexpected higher cytotoxicity of the polycation, which may reduce the transfection efficacy and limit the clinical application.

It was found that pH of medium, serum, and molecular mass (Mw) of polymers would limit the transfection efficiency of complexes as well. Recently, more information has accumulated regarding how serum directly affects the biophysical properties and *in vivo* transfection efficiency of a complex. Initially, serum causes the complex to aggregate, with further interaction causing its disintegration, resulting in DNA release and degradation. The serum effect appears to be a general phenomenon; however, the rate of aggregation and subsequent disintegration is largely dependent on the structure of the polycation. Mw has been shown in numerous studies to be directly proportional to both cell toxicity and transfection efficiency. Most of the research has shown that the low cytotoxic effects typically correlate with lower Mw (less than 2 kDa) vectors and the enhanced transfection efficiencies typically associate with high Mw (larger than 25 kDa) vectors. The pH condition of the transfection medium is very important to achieve high transfection efficiency. Sato *et al.* (2001) reported that transfection efficiency of chitosan/DNA complexes at pH 6.9

was higher than that at pH 7.6. Dependence of transfection efficiency on pH of the culture medium is considered to be attributable to the protonation of amines in chitosan.

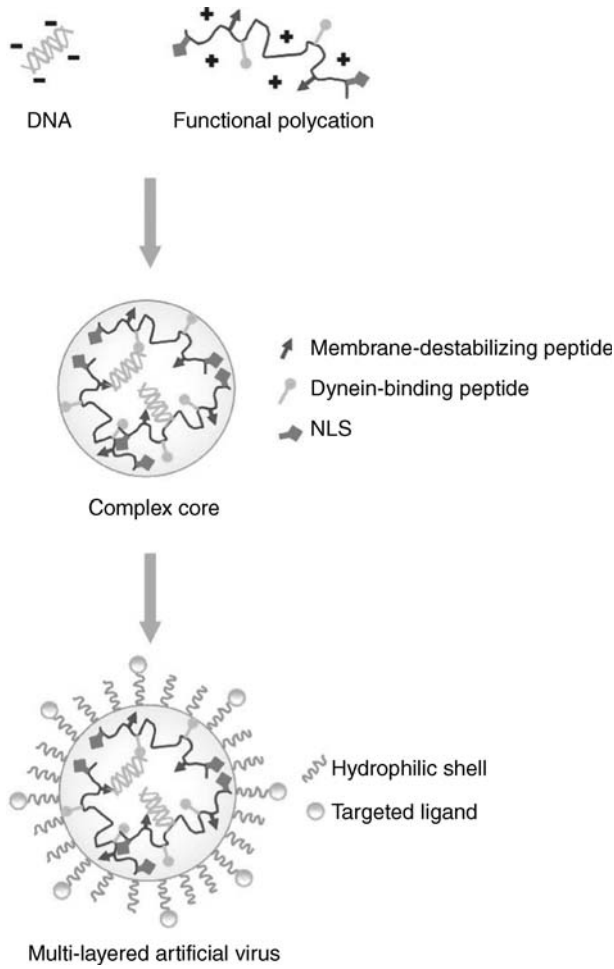
Accordingly, the clinical viability of a gene delivery system requires the unimpeded systemic delivery through the bloodstream and frequently to disseminated regions of the body. It has been shown that cationic polyplexes can (i) interact with blood components (e.g. serum proteins, blood cells) to form large, aggregated, embolytic entities; (ii) aggregate with other individual polyplexes due to exposure in varying physiological salt conditions; (iii) activate the complement system; or (iv) be recognized and cleared by the reticuloendothelial system. The induction of any one of these events can lead to premature elimination of the polyplex, preventing delivery of the genetic cargo to its final destination. By designing for sufficient serum stability to account for these clearance mechanisms, polyplexes may experience extended circulation times, which in turn improves the chance for extravasation into the targeted tissue and increases the opportunity for polyplex–cell interactions. Nowadays, many hydrophilic, non-ionic polymers have been developed to reduce polyplex aggregation, increase solubility, reduce surface charge, and prolong circulation in the bloodstream. Other barriers to systemic gene delivery include the rapid clearance of the complex by the reticuloendothelial system (RES) and the lack of target specificity (Wong *et al.*, 2007).

8.4 Future trends

In spite of some setbacks, the future prospect of gene therapy is bright. More efforts should be made in the field of non-viral gene delivery, as we have to confront challenges associated with cell targeting specificity, gene transfer efficiency, gene expression regulation, and vector safety. Hence, the future successful non-viral vectors for gene therapy will be biocompatible, have efficient and specific gene expression in target cells, and be manufactured in an economical way. They will be multifunctional systems; for instance, they will deshield on entry into the cell, release membrane-disrupting agents on entry to the endosome, and unpack DNA from the carrier on transport to the nucleus. The non-viral gene delivery systems of the future will be more efficient in targeting and transfection, and also they will have improved formulation and storage stability, enabling them to be used as a bedside medicine for various applications. Recently, many new approaches and ideas have been reported, which have indicated the development trend of non-viral gene delivery systems.

8.4.1 Artificial viruses

Nowadays, researchers try to mimic the properties of viruses with the aim of achieving their higher transfection efficiencies; these are called 'artificial viruses' because they have several features common to viruses, including targeting domains, protective coating, and endosomal release agents. Mastrobattista *et al*. (2006) proposed a core-shell concept of an artificial virus. The three main structural components of this artificial gene delivery system are: the plasmid vector, engineered for optimal expression; the artificial virus core, consisting of pDNA, condensing agents, and functional peptides; and finally the hydrophilic shell, exposing targeting ligands for cell-type-specific gene delivery (Fig. 8.18).



8.18 A conceptual model of the assembly of a multi-layered artificial virus.

Lim and co-workers (2008) have demonstrated that directed assembly of an artificial virus was possible by using rationally designed, preorganized β ribbons. A unique feature of the nanostructure was its capability to encapsulate siRNA within the non-charged carbohydrate surfaces, while preserving its discrete nanostructure. Considering the variety of supramolecular nanostructures that are currently, or soon would be, available, this type of approach provides a general means to construct various repertoires of controllable artificial viruses. It was anticipated that other functional properties of natural viruses could be further finely installed in this artificial virus, which would ultimately lead to the generation of safe and efficient artificial viruses for gene and drug delivery.

8.4.2 Nucleus-targeted gene delivery

Many extracellular and intracellular barriers need to be overcome during a successful delivery; the non-viral vector must be able to to protect DNA, target specific cells or tissues, and transport DNA into cytoplasma. After escaping the endosome, the condensed DNA must be efficiently transported to the nucleus, where the naked DNA must be released for efficient expression. Compared with achievements in overcoming other barriers to gene transfer, the methods for promoting nuclear localization remain vague. Some researchers indicated that reporter gene expression may be considerably enhanced if transfection occurs shortly before cell division due to the temporary breakdown of the nuclear envelope, which allows inclusion of DNA within the nuclear space. However, this behavior appears to be vector type-dependent and may be clinically limited, as most cells in the body are post-mitotic. Many studies also showed that synthetic vectors could minimize the complex size, which appears to improve delivery to the nuclear region. However, the only way to transport into the nucleus is to pass the nuclear pore complexes (NPCs) with only 9 nm inner diameter, so that free diffusion of non-rival gene vectors with larger sizes is unlikely (Brunner *et al.*, 2000; Martin and Rice, 2007;Wong *et al.*, 2007).

To ensure nuclear targeting, many nuclear localization signal (NLS) peptides have been developed that target the DNA to the nucleus and allow entry through the NPCs by active transport. The complexation of DNA with NLS peptide showed great improvement to transfection efficiency, compared with the vector that is unable to target the nucleus (Martin and Rice, 2007).

Moore *et al.* (2009) synthesized low-toxicity polyethylene glycol (PEG)-based vehicles with endosomal escape functionalities. Coupling one SV40 peptide, a classical NLS, or two TAT peptides, a non-classical NLS, to PEG–DNA-binding peptide (PEG–DBP) vehicles increased the transfection

efficiency of PEG-DBP/DNA particles 15-fold and resulted in similar efficiency to that of PEI.

Liposomes have been prepared loaded with DNA (green fluorescent protein (GFP)) and additionally modified with TATp and PEG, with PEG being attached to the liposome surface via both pH-sensitive hydrazone and non-pH-sensitive bonds. The pGFP-loaded liposomal preparations have been administered intratumorally in tumor-bearing mice and the efficacy of tumor cell transfection followed after 72 h. The administration of pGFP-TATp-liposomes with the low pH-detachable PEG resulted in at least a three times more efficient transfection, since the removal of PEG under the action of the decreased intratumoral pH leads to the exposure of the liposome-attached TATp residues, enhanced penetration of the liposomes inside tumor cells, and more effective intracellular delivery of the pGFP. This result can be considered as an important step in the development of tumor-specific stimuli-sensitive drug and gene delivery systems (Kale and Torchilin, 2007).

However, only moderate success has been achieved in overcoming this barrier, so future efforts may begin focusing more and more on surmounting this obstacle. In order to minimize residence time within the cytosol and promote transport toward and into the nucleus, more natural endogenous cytosolic factors and their associated cellular pathways should be found and recruited to facilitate the shuttling of either polyplexes or DNA.

8.4.3 Biocompatible polymers

Non-viral vectors should preferably be biocompatible and biodegradable to prevent carrier-induced toxicities and the accumulation of carrier components in the body. It is difficult to predict which polymer will be cytotoxic and which not on the basis of the structure of the cationic polymer. However, in general, low molecular mass cationic polymers are less toxic than high molecular mass polymers. Accordingly, more efforts should be made in order to facilitate DNA release where low molecular weight polymers are crosslinked or linearly linked together by degradable linkages (e.g. disulfide bonds, ester bonds) to form a high molecular weight polymer that can eventually degrade to its lower molecular weight components.

In addition to disulfide linkages, PEI derivatives with acid-labile ester linkages have been explored by many research groups to create biodegradable gene carriers. Kim *et al.* (2004) synthesized biodegradable PEI–PEG conjugates by reacting low molecular weight PEI (600, 1200, 1800 Da) with PEG succinimidyl succinate (2000 Da) to form polymeric structures. When condensed with pDNA, these PEI–PEG conjugates showed reduced cytotoxicity as compared to 25 kDa PEI and improved gene transfer as compared to 1.8 kDa PEI. Derivatives of the polysaccharide dextran have

been applied across the fields of chemistry and biology. Sun *et al.* (2008a) have synthesized (dextran-hexamethylenediisocyanate)-g-polyethyleneimines (Dex-HMDI)-g-PEIs) through grafting low molecular weight (800 Da) branched polyethyleneimine (PEI) to HMDI functionalized dextrans. The cytotoxicity of (Dex-HMDI)-g-PEIs was lower than that of 25 kDa PEI. The gene transfection efficiency of (Dex-HMDI)-g-PEI/DNA complexes at certain N/P ratios in 293T cells was higher than or comparable to 25 kDa PEI/DNA complex at its optimal N/P ratio of 10. In addition, comparing with (Dex-HMDI)-g-PEI with a high molecular weight dextran, (Dex-HMDI)-g-PEI with a low molecular weight dextran demonstrates lower cytotoxicity and higher transfection efficiency.

Wang *et al.* (2009) synthesized the polyethyleneimine (PEI)-grafted polycarbonates (PMAC-g-PEIx) as a kind of biodegradable polycations for gene delivery. Backbone polymer, poly(5-methyl-5-allyloxycarbonyl-trimethylene carbonate) (PMAC), was synthesized in bulk catalyzed by immobilized porcine pancreas lipase (IPPL). Then, PMAC-O, the allyl epoxidation product of PMAC, was further modified by PEIx with low molecular weight (x = 423, 800, and 1800). The molecular weights of PMAC-g-PEIx, measured by gel permeation chromatography with multi-angle laser light-scattering (GPC–MALLS), were 81 900, 179 900, and 200 600 g/mol, with polydispersities of 1.2, 1.4, and 1.7 respectively. *In vitro* experiments demonstrated that the PAMC-g-PEIx showed much reduced cytotoxicity and enhanced transfection efficiency could be found in comparison with PEI25K in 293T cells. Furthermore, pre-incubation of PMAC-g-PEI1800 showed a weakening binding capacity with DNA. The biodegradability of PMAC-g-PEIx can facilitate the efficient release of pDNA from polyplexes and reduce cell cytotoxicity. Their results suggested that PMAC-g-PEIx would be a promising non-viral biodegradable vector for gene delivery systems.

Besides the biodegradable PEI, PLL, PAA, and methacrylate-based macromolecules, various other novel degradable compounds have been synthesized and investigated for gene transfer. Ko *et al.* (2008) synthesized the new acid-degradable cationic nanoparticles using a monomer-to-polymer approach. The nanoparticles were designed to cause swelling and osmotic destabilization of the endosome, while cationic branches holding anionic DNA are cleaved from the polymeric backbone of the nanoparticles and make plasmid DNA accessible for efficient gene expression. Efficient release of plasmid DNA upon hydrolysis of the nanoparticles at the endosomal pH 5.0 and transportation of the released DNA to the nucleus of a cell were shown. *In vitro* studies showed significantly higher transfection efficiency by degradable nanoparticles than PEI polyplexes at very low concentrations. Preliminary pulmonary transfection of mice using degrad-

able nanoparticles demonstrated a remarkably higher expression of firefly luciferase at 70% lower concentration than using naked DNA alone.

In order to obtain permission for clinical application of non-viral gene vector mediated gene therapy, we need to make sustained efforts to combine those efficient synthetic vectors with therapeutic genes, to make clinical safety assessment, and to provide indications of how animal models correlate with clinical experience. It is very important to remember the mechanisms, applications, and limitations of current gene delivery systems, and to obtain more novel information from chemical, biological, and various other fields, so that vectors may be rationally designed to overcome systemic, cellular, and molecular barriers to genetic therapy. In conclusion, an ideal system for gene therapy should be biodegradable, non-toxic, non-immunogenic, able to arrive efficiently at target cells, suitable for high gene expression, and should remain stable during storage and *in vivo*. With extensive efforts being put into designing non-viral vectors with higher gene transfer efficiency, synthetic gene carriers may become superior to viral analogues in clinical trials in the near future.

8.5 References

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9

Bioactive materials in plastic surgery and body reconstruction

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Abstract: Bioactive materials have found wide application in plastic surgery and body reconstruction, including tissue augmentation, craniofacial reconstruction by utilisation of bone and skin substitutes and soft-tissue fillers, wound repair, and scar-reduction technologies. Limitations include the supply of blood to the matrix for tissue repair, the general issues associated with the tissue engineering when cell therapy is used, the optimisation of the mechanical properties of the biomaterials, and the understanding of the mechanism of implant failure during the product's life cycle. Future trends include the development of new technology to create a biomatrix for tissue repair, the introduction of new bioactive entities with specific functions for tissue repair, the development of new versatile bioactive materials to achieve balanced mechanical properties and functionality, or the delivery of cells or genes to enhance the tissue repair.

Key words: plastic surgery, body reconstruction, tissue repair, cosmetic surgery.

9.1 Introduction

The British Association of Plastic, Reconstructive and Aesthetic Surgeons defines plastic surgery as 'the combination of various surgical skills and techniques to attempt to restore normal, functional anatomy from the abnormal, whether the abnormality is congenital, traumatic or as a result of a disease process such as cancer or infection' [1]. The cosmetic component of plastic surgery often has higher public perception than the functional part of the plastic surgery [2] and it has been often referred to as cosmetic plastic surgery, which requires not only good surgical skill, but also a very well-developed understanding of patient personalities and their psychological

make-up, their ambitions, and their motivations. It also has an artistic aspect; an appreciation of form, the challenge to apply that judgement, and to seek perfection, according to Nicolle in the Sir Harold Gillies Memorial Lecture [3]. In the UK, aesthetic or cosmetic surgery is generally excluded from the NHS because of resource restrictions. Some requests, however, combine an element of reconstruction or the management of deformity with aesthetics and may be termed 'borderline' conditions, as shown in Table 9.1 [4].

Differing from plastic surgery, cosmetic surgery is defined as the use of surgical procedures, in the absence of disease or physical trauma, to alter the physical appearance of the body in pursuit of psychosocial benefit [5]. This is a customer-led business with great market needs. According to the latest statistics from the American Society for Aesthetic Plastic Surgery (ASAPS), overall, close to 10 million surgical and non-surgical cosmetic procedures were performed in the USA in 2009 alone, and the vast majority of the procedures are non-surgical, including the top five in the list: botox injection, laser hair removal, hyaluronic acid injections, microdermabrasion, and laser skin resurfacing. The top five surgical cosmetic procedures in 2009 were breast augmentation, liposuction, eyelid surgery (blepharoplasty), rhinoplasty (nose job), and abdominoplasty (tummy tuck) [6].

In this chapter, applications of bioactive materials are mainly covered in plastic surgery and body reconstruction. Bioactive materials involved in cosmetic surgery for enhancing appearance, such as soft tissue augumentation, are also discussed.

9.2 Applications of bioactive materials in plastic surgery and body reconstruction

Plastic surgery and body reconstruction include hand surgery, melanoma, treatment of burns, wound healing/scarring, nerve injury, cleft lip and palate for maxillofacial surgeons, breast reconstruction, head and neck surgery, laser surgery, lower-limb trauma, hypospadias, craniofacial surgery, haemangioma, and free-flap monitoring. Bioactive materials have found wide application as implants, structured supporting materials, scaffold for tissue engineering, and adhesives for tissue repair. In the field of cosmetic plastic surgery for the purpose of the rejuvenation and aesthetic enhancement of the patients, biomaterials include mainly dermal implants for non-invasive soft tissue augumentation and implants for rhinoplasty. The most commonly used biomaterials in plastic surgery and body reconstruction are summarised in Table 9.2.

Table 9.1 Borderline referral protocol [4]

	Aesthetic enhancement	
	Excluded	Allowable
Face	Surgery for wrinkles (rhytides, 'crows feet' and 'marionette' lines). Face lift. Neck lift. Brow lift and brow ptosis. Other purely aesthetic surgery.	Corrective surgery for structural or soft tissue anatomical anomaly resulting from congenital or acquired pathological condition. Correction of facial nerve palsy.
Nose	Rhinoplasty for aesthetic purposes. Minor nasal lumps, humps, and deviation, particularly when blamed upon childhood knocks.	Correction of congenital abnormalities including cleft lip and palate. Septorhinoplasty after nasal trauma where there is gross distortion of anatomy and sustained interference with airway.
Eyes and eyelids	Upper and lower blepharoplasty (correction of excess skin 'tired eyes', 'eyebags' or malar bags).	Post-traumatic or post-tumour reconstruction. As part of a procedure for ectropion or entropion. Where redundancy of the upper lids overhangs the pupil and genuinely interferes with the field of vision (this must be distinguished from congenital or acquired ptosis of the levator mechanism). During the correction of facial nerve dysfunction. Gross asymmetry.
Ears	Prominent ears (pinnaplasty) under the age of five years and over the age of 18 years. Below the lower age limit, surgery is being requested by the parents, the patients are not compliant with the post-operative regime and the surgical results are unpredictable. The upper age limit is debatable, since some patients with genuine concerns only present when parental opinion and, thus, control is no longer a factor.	Congenital abnormalities of the external ear (other than prominence). Prominent ears in patients over the age of five years and under the age of 18 years only. The degree of prominence is largely immeasurable, but it would be reasonable to refer all children who are being teased or perceive there to be a problem themselves (as opposed to their parents). Gross asymmetry.
Cranium and scalp	Male pattern baldness. Hair transplantation.	Congenital anomalies (craniofacial and cutaneous). Correction of post-traumatic bony and soft tissue deformity. Post-burn alopecia. Localised bony masses.

Table 9.1 (cont.)

Breast procedures		
	Excluded	Allowable
General	Patients desiring uplift and change in appearance rather than symptomatic relief. Body mass index (BMI) is greater than 30. BMI should be recorded in all referral letters.	Appropriate for relief of functional and psychological symptoms (neck, upper back, and interscapular pain, bra straps cutting in, sleeps in bra, inframammary intertrigo, unable to undress in front of partner or go swimming due to breast size, social isolation, and adverse comments from others). Symptoms with bra cup size at least E or EE. Obvious asymmetry (= greater than a cup size difference with difficulty finding bras to fit). Asymmetry following breast cancer surgery.
Augmentation	Post-partum related loss of volume and changes of appearance. Gender reassignment. Small, but natural breasts (see 'male chest' below).	Unilateral procedure to balance a contralateral breast cancer reconstruction. Congenital anomalies, such as Polands syndrome and constricted or 'tubular' breast, pectus deformity, or chest wall asymmetry associated with scoliosis. Asymmetry of more than one cup size. 'Male chest' appearance. Bilateral augmentation only in patients with very little or no breast development, who have never had children.
Inverted nipples	Treatment is aesthetic and surgical correction usually results in an inability to breast feed	
Change in breast shape	Mastopexy (tightening of skin and 'uplift' without volume change). Inverted nipples where there is no suspicion of underlying tumour.	Any surgery related to breast cancer or its sequelae. Surgery to contralateral breast during breast reconstruction or correction of asymmetry.
Gynaecomastia (Male breast reduction)	The overweight and obese. Body builders and sportsmen desiring reduction of perceived excess pre-pectoral tissue to enhance appearance at the gymnasium. The abuse of anabolic medication should be excluded.	Where there is true breast glandular development or obvious asymmetry. Breast development secondary to hormonal disturbance or drug complication. Suspicion of male breast cancer.

Table 9.2 Biomaterials used in plastic surgery and body reconstruction

Material type	Materials	Applications
Metals	Stainless steel Metallic wire Vitallium Titanium Gold	Head and neck
Polymers	Silicone	Soft tissue augumentation: nose, tracheal, chin, artificial skin
	PTFE PMMA Polyester Biodegradable polymer Natural biopolymer	
Bone cements	Hydroxyapatite Bioglass	Head and neck
Tissue adhesives	Cyanoacrylates Fibrin glue	General surgery

9.2.1 Metals

Metals have been utilised as biomaterials in orthopaedic structure applications for bone and joint repair. Metals used as implants need to meet the general requirements for safety and functionality governed by the stringent regulatory control discussed in previous chapters. Commonly used biomaterials include 316 stainless steel, gold, nitinol, vitallium, titanium, and alloys.

In plastic surgery and body reconstruction, craniofacial surgery often involves metal implants. Nitinol, which is strong, elastic, and possesses a thermomechanical memory property, has been used as nitinol craniofixators to fix craniocerebral injuries [7]. A new grip-like titanium device (Skull Grip) was found to be capable of providing optimal attachment of the flap to the skull and also enabling fast bony healing, to avoid possible pseudoarthrosis and/or osteolytic changes, after completion of a craniotomy for plastic surgery [8]. A thin titanium mesh combined with a prelaminated free radial forearm flap can be used to reconstruct a raised ear resulting from a traumatic defect [9]. Kirschner wires/mesh made of stainless steel or aluminium can be used with a hand table to stabilise the fingers and wrist in an optimal position. The material is inexpensive, easily available, autoclavable, light, sturdy, and yet mouldable [10]. Other plates and screws used in hand surgery have been found to be very biocompatible materials, have a low profile, can be contoured to suit individual anatomy, and provide considerably reduced rates of soft tissue irritation. These features afford surgeons the ability to fix complex injuries in a stable manner, thereby

instituting early rehabilitation in an effort to maximise individual outcomes [11]. Stainless steel springs have been found to be safe and efficacious for the treatment of sagittal synostosis, a type of craniofacial deformity [12, 13]. In addition to the focus on the metal types for plastic surgery and body reconstruction, considerable research has been devoted to studies on the interaction of the metal surface, cells, and tissue. Surface coating and morphology have a strong influence on the osteoblastic cell attachment and proliferation when bony tissue is involved [14–16]. Titanium osteofixation has been compared with poly(70L-lactide-co-30DL-lactide) plate osteofixation for the treatment of cleft lip and palate maxillary retrognathia and complications in orthognathic surgery, demonstrating a 5-year outcome stability [17].

For cosmetic effects, numerous biomaterials have been successfully utilised in facial skeletal implants, creating change impossible to obtain with soft tissue techniques alone [18].

9.2.2 Polymers

Polymers as bioactive materials for medical applications are discussed in various chapters in this book. In this chapter, the design of bioactive materials is considered, in accordance with the specific applications in plastic surgery, body reconstruction, and cosmetic plastic surgery.

Artificial skin for burns, skin substitutes or wound dressings

The basic concerns in burns reconstruction are for function, comfort, and appearance. Normal and hypertrophic scarring, scar contracture, loss of body parts, and change in colour and texture of injured skin are processes common to all seriously burned patients and yet unique to each [19]. Skin grafts are the standard bioactive materials for the reconstruction of damaged skin after the burn. Skin transplanted from one location to another on the same individual is termed an autogenous graft or autograft. It is classified as either split-thickness or full-thickness, depending on the amount of dermis included in the graft. Owing to the limitation of donor grafts, other skin substitutes, including allografts, xenografts, and tissue-engineered products, have been developed and applied in burn treatment in plastic surgery and body reconstruction, as shown in Table 9.3. The biomaterials used include synthetic and biodegradable polymers [20, 21].

Soft-tissue augmentation [26]

In plastic surgery, biocompatible materials are widely accepted as implants for soft tissue augmentation in the treatment of various body defects due to

Table 9.3 Lists of tissue-engineered skin products for plastic surgery of burn wound treatment

Product name	Design	Applications	Company
Biobrane	Silicone membrane coated on one side with porcine collagen and embedded with nylon mesh	Temporary coverage of partial-thickness wounds and toxic epidermal necrolysis (TEN), chronic wounds, or following skin resurfacing [21]	UDL Laboratories, Inc., Rockford, Ill
ICX-SKN	Collagen or polymer-based scaffold that is seeded with fibroblasts from a donor cadaver	Cellular dermal allografts use in coverage of partial- and full-thickness wounds	Intercytex Ltd, Manchester, UK
TransCyte	A nylon mesh incubated with human fibroblasts with an outer silicone layer as a temporary epidermis	Temporary coverage of deep partial or excised full-thickness wounds	Advanced Tissue Sciences/S&N/ Advanced BioHealing Inc.
Apligraf	Bovine collagen and neonatal fibroblasts combined with an epidermal layer formed by neonatal keratinocytes	Treatment of chronic wounds and donor sites, overlay dressing on split-thickness skin grafts to improve function and cosmesis	Organogenesis, Inc., Canton, Mass
Orcel	Bovine collagen sponge coated with neonatal allogeneic keratinocytes	Treatment of chronic wounds and donor sites, overlay dressing on split-thickness skin grafts to improve function and cosmesis	Ortec International, Inc., New York, NY
Epicel	A sheet of autogenous keratinocytes for grafting	Coverage of a large surface area defect	Genzyme Biosurgery, Cambridge, Mass
Laserskin	A hyaluronan ester sheet of autogenous keratinocytes with laser microperforated for grafting	Coverage of a large surface area epidermis defect [22]	Fidia Advanced Biopolymers, Abano Terme, Italy
Hyalograft 3D	Hyalruonic acid ester three-dimensionally seeded with fibroblast combined with Laserskin	Dermis, treating chronic ulcers, particularly diabetic foot and vascular ulcers, as well as burns	Fidia Advanced Biopolymers, Abano Terme, Italy
MySkin	Silicone sheet surface coating of $<$100 nm thickness, deposited by plasma polymerisation of acrylic acid suitable for autologous keratinocytes growth	Burn, chronic wound	CellTran

Table 9.3 (cont.)

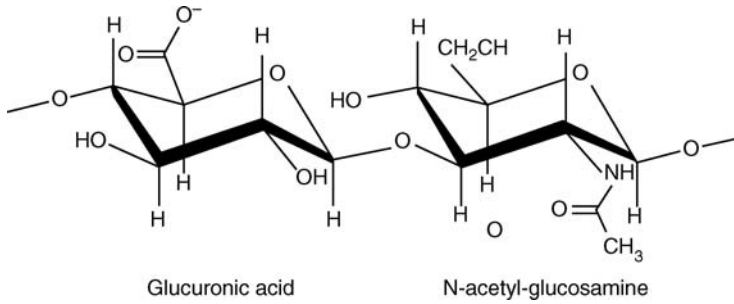
Product name	Design	Applications	Company
Cell Spray	Cultured epithelial autograft suspension	Partial-thickness injuries or in conjunction with dermal reconstruction technology to treat deep dermal or full-thickness injuries [23]	Avita Medical
Integra DRT	Synthetic bilayer acellular skin substitute composed of an outer silastic sheet (epidermal analogue) with a matrix composed of bovine collagen and glycosaminoglycan (dermal analogue)	Wound coverage of deep partial- or full-thickness burns	Integra Life Science
AlloDerm	Acellular dermal allograft	Deep partial- and full-thickness injuries [24]	LifeCell Corp
CSS (cultured skin substitute)	Culturing autologous fibroblasts and keratinocytes [25]	Full-thickess burn wounds	Shriners & U Cincinnati
Dermagraft	A three-dimensional, degradable, biocompatible, lactate/ glycolate scaffold-based, neonatal fibroblast culture	Treatment of chronic wounds such as diabetic foot ulcers (DFUs)	Advanced Tissue Sciences/ S&N and now owned by Advanced Biohealing Inc.

cancer, HIV, and other traumatic effects. In cosmetic surgery, non-surgical soft tissue augmentation is one of the most popular treatments for maintaining a young appearance. The most widely accepted biomaterials include synthetic and biodegradable polymers. In this section, some of the most commonly used polymers are reviewed

Hyaluronic acid

Hyaluronic acid (HA) is a natural, biocompatible, biodegradable glycosaminoglycan, which can be found in tissue as a component of the extracellular matrix and is well distributed throughout the mammalian body, especially in the synovial fluid (SF) of the joints, the umbilical cord, and the vitreous body of eyes [27]. It is a linear polysaccharide, having a repeating unit of disaccharide of glucouronic acid and N-acetyl-D-glucosamine, with a molecular weight ranging from 10^4–10^7, as shown in Fig. 9.1.

Hyaluronic acid can be produced from both animal sources and fermentation, using certain strains of *Streptococcus*. While it is found in



9.1 Chemical structure of hyaluronic acid.

large quantities in extracellular matrices, HA also contributes to tissue hydrodynamics [28], movement and proliferation of cells, and participates in a number of cell surface receptor interactions, such as CD44 [29]. In addition to its excellent biocompatibility, HA has a unique rheological property, acting as a hydrophilic viscoelastic hydrogel, due to the macromolecular physical entanglement. All these physical, physiological properties and functions provide the foundation for the acceptance of HA in many medical applications [30], where it has a long history of safety and efficacy [31] (see Fig. 9.2).

The first HA biomedical product, Healon, was developed in the 1970s and 1980s and is approved for use in ophthalmic surgery (i.e. corneal transplantation, cataract surgery, glaucoma surgery, and retinal attachment surgery) [32]. HA is used to treat osteoarthritis of the knee [33]. Such treatments are administered as a course of injections into the knee joint and are believed to supplement the viscosity of the joint fluid, thereby lubricating the joint, cushioning the joint, and producing an analgesic effect [34]. HA has also found application in drug delivery [35] and tissue engineering [36].

Dermal fillers have been popularly used for facial rejuvenation to remove wrinkles and treat the tissue volume loss. As a tissue filler, the dermal filler needs to be biocompatible, predictable, adjustable to the anatomy of the patient, long-lasting, reversible, and natural in appearance [37]. As tissue fillers, HA derivatives represent an alternative treatment option for the ageing face, particularly for facial lines, lip augmentation, and treatment of distensible atrophic facial scarring. Since 2003, the US Food and Drug Administration (FDA) has approved HA injections for filling soft tissue defects, such as facial wrinkles. These tissue fillers are analogous to collagen injections but have the advantages of longer lasting effects and decreased risk of allergic reaction [38]. Among the tissue fillers, Restylane, Hylaform, Juvederm, Belotero, and Puragen are commercially available HA derivatives marketed in Europe, while Restylane and Hylaform are also FDA approved. These fillers contain naturally occurring HA molecules that are

1934
Karl Meyer and John Palmer isolate a previous unknown chemical substance from the vitreous body of cows' eyes. The substance contained uronic acid, so they propose the name 'hyaluronic acid' from hyaloid (vitreous) + uronic acid.

1942
The first commercial use of hyaluronic acid (HA). Endre Balazs applies for a patent to use it as a substitute for egg white in bakery products. He goes on to become a leading expert on HA, making the majority of discoveries concerning HA during the next 50 years.

1964
TC Laurent created the first crosslinked HA, using Bis-epoxide as a crosslinking agent.

1980 and 1990s
The use of HA in medicine is extended to a number of areas, including treatment of joint pain due to arthritis, utilisation in fertility clinics, aiding eye surgeons with cataract operations, and tissue augmentation.

1996
Restylane is launched by Q-Med in Sweden, introducing the first generation of HA dermal fillers for cosmetic use.

2000
Dr Xiaobin Zhao files a patent for the preparation of multiple crosslinked HA, the technology behind DXL in Mentor Corporation, USA.

LEA Derm introduces the Juvederm range of products, the first homogeneous gels.

2004
After a decade of development and testing, Mentor receives CE marking for Puragen with DXL, the first double crosslinked form of HA, ushering in the era of fourth-generation HA technology.

2005
Mentor Corporation launches the sale of Puragen internationally.

9.2 A brief history of hyaluronic acid [31].

chemically crosslinked in order to increase the duration *in vivo*. The Restylane family, including Restylane, Perlane, and Restylane Fine Lines (Q-Med, Uppsala, Sweden; Medicis, Arizona, USA), consists of non-animal stabilised hyaluronic acid (NASHA), which is derived from a process using 1, 4-butanediol diglycidyl ether (BDDE) as a crosslinking agent to form ether crosslinks between the two hydroxyl groups of HA molecules [39]. The Hylaform family, including Hylaform Fine Lines, Hylaform Plus, and Hylaform (Inamed Corporation, California, USA), is made from Hylan B gel, whicn is derived from a crosslinking process, using divinyl sulphone (DVS), in which the crosslinking is also through the hydroxyl groups of HA to form sulphonyl-bis-ethyl-crosslinks between HA molecules [40]. The

Juvederm family, including Juvederm 18, Juvederm 24, Juvederm 24HV, and Juvederm 30 (Leaderm, France), is available in HA concentrations of 18 mg/ml (Juvederm 18) or 24 mg/ml (Juvederm24, 24HV and 30). These are crosslinked HA products, using BDDE as the crosslinking agent, similar to Restylane [41], but it is claimed that they are in a homogeneous gel form rather than in particle form [42]. A novel technology, termed double-crosslinking technology (DXL^{TM}), has been used to create two new products, Puragen and Puragen Plus (Mentor Corporation, California), in which HA is crosslinked via both ether-crosslinkage and ester-crosslinkage [43–45]. Another product, Belotero, is based on a technology named CPM (cohesive polydensified matrix), in which BDDE is used as a crosslinking agent. After the primary crosslinking with the ether-bond formation, low molecular weight (LMW) HA is introduced into the matrix for secondary crosslinking among HA molecules [46].

It is known that HA should be a good base for a soft tissue augmentation material but each HA-based dermal filler has its own distinct characteristics with variations in physico-chemical properties, such as rheological properties, gel particle size, and durability. Understanding the differences between tissue fillers will enable physicians to select the appropriate commercial products for each individual patient [47, 48].

Silicone

Medical grade silicone (polymethylsiloxane) has been utilised as the gel filling and also in the construction of the outer shell of the breast implant, which is used for the most popular cosmetic plastic surgery procedure – breast augmentation [49]. The history of development and clinical uses of silicone mammary gel implants reflects the history of the advancement of biomaterials used in the field of medical devices, especially in plastic surgery and body reconstruction. It is a very good example of the design and application of polymer chemistry in clinical applications. The basic entity silicone, polymethylsiloxane, is a linear polymer chain, which is oil absorbable and of migratory nature. Normally, the silicone implant is formed by lightly crosslinked silicone in gel form, mixed with a certain percentage of uncrosslinked silicone and encapsulated in a crosslinked silicone elastomer shell. The continuous diffusion of low-molecular-weight (LMW) polymethylsiloxane through the silicone shell leads to implant failure, including reduced mechanical strength of the elastomer shells, possible immunological effects due to the LMW silicone 'bleed and migration', and other adverse events, such as capsular contracture, pain, and disfigurement [50]. Therefore, the crosslinking chemistry plays a very important role in the control of the bleed and migration of the silicone gel to achieve two goals: (i) inhibition of silicone fluid swelling and weakening of

shells, and (ii) prevention of the release of silicone fluid into tissues by diffusion through the shell. These can be achieved by control of the gel or the shell. For example, the silicone gel can be developed into 'soft' but fully crosslinked silicone gels (with low crosslink density) and the silicone elastomer shell can be mechanically enhanced via (i) improvements in silica fillers (which at about 20% loading, induce a l0-fold increase in strength over unfilled silicone), and (ii) fibre reinforcement of silicone elastomers, perhaps based on stable low-modulus polymer fibres [50].

Other improvements have focused on the tissue and implant interaction via control of the design of the device, including surface properties, shape, and the texture of the silicone implant. Numerous designs, including double-lumen implants, textured-surface implants, alternative filler implants, enhanced cohesive silicone gel implants, and anatomically shaped implants, have evolved in the past decade to reduce the possible tissue response [51]. When silicone is implanted, as a foreign material, it triggers an inflammatory response, which leads to the formation of fibrosis (scar tissue) surrounding the implant. The scar capsule contracture is a well-known result of all foreign bodies and has been well recognised as the most common local adverse affect of the silicone implant. The tissue response is influenced by the molecular weight of the silicone. The intensity of the cellular and capsular response was lowest for silicone oil and increased as the material's molecular weight increased and its compliance decreased [52].

Although implants may cause local symptoms, rupture over time, or be associated with an immunological reaction, comprehensive epidemiological studies have concluded that there is no connection between breast implants and the known connective tissue diseases or between the implants and breast carcinoma. There is no increase in the risk of recurrence in mastectomy patients reconstructed with implants and no delay in the detection of recurrences. Recent laboratory studies in animals suggest that silicone may have anticarcinogenic effects [53].

A textured surface of a silicone implant, with the correct microdomain size and pattern, has been found to be capable of reducing formation of the capsule. In one study, the interaction of human fibroblasts with silicone surfaces was monitored by cell cycle analysis. Silicone was textured with 2, 5, and 10 mm wide grooves (2MU, 5MU, 10MU, respectively) or maintained smooth. Cells proliferated on the fibronectin-preadsorbed silicone, as demonstrated by increased coverage and occurrence of subpopulations in the S and G2/M phase of the cell cycle. Cells on SMT went faster into the S phase than cells on textured silicones. Cells on 10MU showed less proliferation than cells on 2MU and 5MU [54]. It has also been found that cells will react with the dimensions of textured surface configurations accordingly [55] and the topographic configuration size was recognised and preferentially responded to by macrophages. Specifically,

macrophage spreading was maximised on surfaces with 2–5 μm configurations, and a correlation was found between macrophage spreading and surface roughness with regular textures [56]. Smooth and microtextured silicone rubber implants were implanted subcutaneously in rabbits for 3, 7, 42, and 84 days. The textured implants possessed parallel surface microgrooves and ridges with a width of 2.0, 5.0, and 10.0 μm. All grooves had a depth of approximately 0.5 μm. It is remarkable that significantly more inflammatory cells were present in the smooth implant capsules than in the capsule surrounding the textured implants. However, considering the thickness of the capsule surrounding the implant, it remains doubtful whether mechanical interlocking did occur, as there is no significant differences between the thickness of the capsules surrounding the smooth implants and those surrounding the microgrooved implants. The fact that more vessels were observed around the textured implants during the study could indicate a higher rate of tissue repair [57]. To verify the effect of surface morphology on the capsule thickness, tissue reaction to one specific microgrooved surface with one specific roughened surface and with a smooth surface was studied. Results showed that there are no favourable effects of surface texturing on capsule formation around subcutaneous implants. Although randomly roughened micropatterning of surfaces led to thinner capsules, this texture did not lead to more mature capsules and even appeared to induce more inflammatory cells at the implant–tissue interface than did smooth surfaces [58].

Another way to reduce the capsule formation around the implant is to modify the surface by graft polymerisation. In brief, a silicone sheet was exposed to corona discharge at 15 kV for 2 min in a dry air atmosphere to introduce peroxide groups to the surface, and then immersed into 10 wt% aqueous solution of acrylic acid monomer in a test tube. The monomer solution, together with the silicone sheet, was thoroughly degassed and the test tube sealed. The test tube was placed in a water bath at 50°C for 1 h to effect graft polymerisation. The silicone sheet was removed from the test tube and immersed in distilled water at 70°C for 15 h to remove homopolymers formed around it. Grafted silicone sheets were stored in distilled water before implantation. Results indicate that the polyacrylic acid grafted silicone implant has the least capsule around the implants [59]. By surface ion beam assisted deposition (IBAD) technology for coating metallic or inorganic materials (silver (Ag), copper (Cu), and hydroxyapatite (HAp)/TiO_2) on the silicone surface, antimicrobial silicone rubbers of improved interfacial strength can be produced [60]. In addition, silicone and hydroxyapatite (HAp) composites have been developed for various medical applications, including body reconstruction [61, 62]. Table 9.4 lists the most common silicone gel breast implants utilised clinically worldwide and summarises the technology used.

Table 9.4 Commercial silicone gel implant

Silicone gel-filled breast implant brand name	Technology used	Manufacturer
Cristalline Paragel	Unique shell texturing technology with barrier technology and scientifically associated with the reduced risk of capsular contraction	Eurosilicone, France
Paragel	Barrier layer technology minimising gel diffusion	Eurosilicone, France
CoGel	Nagotex™ textured surface	Nagor UK
Silimed®	Textured round silicone gel. Implants consist of a high-strength silicone gel-filled implant whose envelope presents a textured surface with open pores	Sientra, California, USA
Natrelle®	Barrier shell technology resulting in a low-diffusion silicone elastomer shell, filled with a soft, cohesive silicone gel	Allergan, California, USA
Mentor MemoryGel™	Textured breast implants were designed to reduce the chance of capsular contracture	Mentor Corporation, California, USA

Polytetrafluoroethylene (PTFE)

Polytetrafluoroethylene is a synthetic polymer that is carbon and fluorine based (CF2-CF2) and non-biodegradable in the human body. It is biologically inert, adding to its appeal for use as an implant material. As it is so inert, there is no possibility of vascularisation to immobilise the implant in place firmly. In order to overcome this disadvantage, expanded PTFE (sPTFE) was developed. e-PTFE is a woven polymer consisting of fibrils of PTFE that are connected via nodes of PTFE, creating a microporous structure, with an average internodal space of 20 to 40 μm (in Gore-Tex). This should allow sufficient ingrowth of tissue to prevent migration, while preventing a more robust tissue incorporation that would occur with a porous implant or mesh material. It is permanent but removable if any complication occurs during its lifetime. ePTFE is FDA approved for facial augmentation [63].

Polytetrafluoroethylene and ePTFE can be used for tissue augmentation of the nose (rhinoplasty) [63, 64], cheek [65], chin [66], lips [67], nasolabial furrows (deep smile lines), and other deep creases, such as glabellar creases

Table 9.5 PTFE for plastic surgery tissue augmentation

Product	Characteristics	Applications	Manufacturers
Gore-Tex® soft tissue patch	e-PTFE microporous structure allowing for host tissue incorporation	Laparoscopic hernia repair and inguinal herniorrhaphy, as well as temporary bridging of fascial defects	Gore Medical
Purform 3D ePTFE	100% ePTFE	Chin, nasal dorsum	Surgiform Inc.
SurgiSoft® ePTFE implants	Dual porosity ePTFE implant [69]	Permanent facial filler, for lip and nasal labial folds augumentation	Surgiform Inc.
SFAM ePTFE sheets and blocks	Comparable to Gore-Tex SAM implants	Facial augmentation and reconstructive	Surgiform Inc.
Gore's SAM	e-PTFE microporous structure allowing for host tissue incorporation.	Facial augmentation	Gore Medical
ePTFE composite™ facial implants	e-PTFE microporous structure allowing for host tissue incorporation.	Facial augmentation	Implantech Inc.

(frown lines) [67, 68, 69]. The PTFE products used for cosmetic, plastic surgery and body reconstruction are listed in Table 9.5.

Polymethylmethacrylate (PMMA)

Polymethylmethacrylate, commonly called Plexiglas or Lucite, is used extensively in medicine as bone cement, dentures, and artificial eye lenses [68]. Microspheres of PMMA suspended in collagen have been developed as injectable tissue fillers for cosmetic surgery to treat deeper wrinkles and furrows, perioral lines, lip and philtrum augmentation, scar revision, and other subdermal defects [70]. Compared to HA dermal fillers, PMMA-based filler, Artecol, is injected with a 27-gauge needle into the dermal/subcutaneous junction. Injections tend to be painful, and erythema and swelling are common; in addition, a skin test is required before the injection, as it contains collagen [71]. Furthermore, there are some doubts about whether the size of PMMA beads (less than 60 μm) in the product will be phagocytised, which may lead to a chronic inflammatory response [72].

Apart from soft tissue repair as tissue fillers, PMMA has been used in plastic surgery for craniofacial trauma. A PMMA-based space maintainer can be used to create a soft tissue envelope with definitely preserved volume and well-healed surrounding tissues, ideal for the placement of a tissue-engineering construct designed for bone regeneration during the subsequent reconstruction stage [73].

Other polymers used for manufacturing soft tissue fillers include: poly-L-lactic acid (Newfill®, Sculptra®), polyalkylamide gel (Bio-Alcamid®), and calcium hydroxylapatite (Radiance®, Radiesse®). Poly-L-lactic acid (PLA) was approved by the FDA in August 2004 for the treatment of HIV-associated facial atrophy. It is a biocompatible, bioabsorbable synthetic polymer, belonging to the family of aliphatic polyesters. Polyalkylamide gel (Bio-Alcamid®) is a synthetic polymer-based injectable filler. It is an injectable endoprosthesis, considered to be intermediate between an injectable filler and a prosthesis. Calcium hydroxylapatite (Radiance®, Radiesse®) consists of microspheres of calcium hydroxyapatite in a carboxymethylcellulose gel carrier. When injected into dermis, collagen forms around the calcium hydroxylapatite, providing long-term, natural-looking fullness.

Craniofacial tissue engineering

Bioresorbable polymers and bioceramics play very important roles in craniofacial surgery. In fact, the first experiments in the use of these biodegradable polymer-based medical devices were carried out in cranio-maxillofacial surgery [74, 75]. Copolymers of L- and D-lactide, poly-L/D-lactide, SR-PLDLA (D:L 70:30) plates and screws are the first commercially available self-reinforced bioabsorbable devices intended for the craniomax-illofacial skeleton (Biosorb FX). They have been used in access to osteotomies in cancer surgery, in maxillofacial trauma surgery, in the fixation of bone grafts, and in orthognathic procedures in both mandible and maxilla [76, 77]. Another copolymer with very encouraging clinical application results, particularly for paediatric craniomaxillofacial surgery, is poly-lactic-glycolic acid (PLGA) (Biosorb PDX, or Lactosorb), the copolymer of 80% PLA and 20% PGA [78, 79].

Tissue engineering in the maxillofacial region is an attractive alternative to autogenous and alloplastic materials. Injectable bioactive materials have been used in craniofacial tissue engineering, as the injectable scaffolds have the ability to fill irregularly shaped tissue defects in a manner more non-invasive in comparison with most current surgical techniques utilised in the treatment of traumatic injury, tumour resection, or congenital deformity [80]. For the injectable tissue-engineering system, the bioactive materials need to provide early mechanical support commensurate with that of the

tissue being replaced, allow cells to survive, proliferate, and differentiate, and provide for the controlled release of any drugs or growth factors delivered simultaneously. Above all, this matrix must be biocompatible and ideally will be biodegradable, such that, with time, regenerated tissue will replace the biomaterial component of the system, resulting in functional, healthy tissues, approximating those of the premorbid individual and avoiding long-term implant failure, which would require subsequent retrieval [78]. For example, apatite-coated PLGA microspheres were fabricated by incubating PLGA microspheres in simulated body fluid (SBF), which may be useful for bone regeneration through minimally invasive surgical procedures in orthopaedic applications [81]. In-situ polymerised injectable hydrogel has been evaluated for the treatment of orbital floor injuries. The hydrogel is based on the polymerisation of 5-ethyl-5-(hydroxymethyl)-b,b-dimethyl-1,3-dioxane-2-ethanol diacrylate (EHD) and poly(ethylene glycol) diacrylate (PEGDA). Results demonstrated that the unloaded hydrogel was initially bordered by a fibrin clot and then by fibrous encapsulation. Bone morphogenic protein-2 (BMP-2) loaded hydrogels, independent of concentration, were surrounded by fibroblasts at both time points. Histological analysis also demonstrated that significant bone growth was present at the 2.5 μg BMP-2/implant group at 28 days [82]. Injectable HA-based hydrogel was prepared by using acrylated HA and poly (ethylene glycol) tetra-thiols via a Michael-type addition reaction. Human mesenchymal stem cells were cultured in cell-adhesive RGD peptide immobilised hydrogels, together with BMP-2. Cells cultured in the RGD/BMP-2-incorporated hydrogels showed proliferation rates higher than that of a control or RGD-immobilised hydrogels. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) showed that the expression of osteoblast marker genes, such as CBFa1 and alkaline phosphatise, was increased in HA-based hydrogel, with the expression level dependent on the molecular weight of HA, RGD peptide, and BMP-2. This study indicates that low molecular weight HA-based hydrogels can be applied to tissue regeneration as differentiation guidance materials of stem cells [83].

Bioceramics and their polymer composites have received great interest, beyond that related to their utilisation as injectable scaffolds in craniofacial tissue engineering. Mechanically, bioceramics are much stronger than polymers, and this property plays a critical role in providing mechanical stability to constructs, before the *in vivo* formation of new bone matrix. However, ceramics and glasses are very fragile because of their intrinsic low fracture toughness and high flaw sensitivity. Bioactive ceramics and glasses can be combined with polymers to form composite biomaterials for osseous regeneration [84, 85]. For example, a composite in the form of a fibre has been produced for tissue-engineering applications, especially for craniofacial tissue repair. The composite comprised: (a) a sol–gel derived bioactive glass

comprising less than about 30 ppm of nitrate species; and (b) a biocompatible, biodegradable material [86]. Polycaprolactone (PCL), a semicrystalline linear resorbable aliphatic polyester, is a good candidate as a scaffold for bone tissue engineering, owing to its biocompatibility and biodegradability. The poor mechanical properties have been enhanced and osteoconductivity promoted by the addition of hydroxyapatite (Hap) particles [87, 88]. In this respect, Hap cement is suitable for use when aesthetic or reconstructive augmentation of the non-stress-bearing craniofacial skeleton is desired. BoneSource™ has been used for craniofacial tissue repair [89–92].

Regenerative medicine for craniofacial plastic surgery can be achieved using stem cell technology. PLGA scaffold seeded with marrow mesenchymal stem cells (MSC) is osteogenic and can be used for bone augmentation [93]. Calcium phosphate cement (CPC) and CPC-chitosan scaffolds have been shown to be non-cytotoxic and supported cell growth and proliferation. A reported study on the response of hMSCs cultured on CPC for the delivery of stem cells for bone regeneration indicates the scaffold has promise as a delivery vehicle for stem cells and osteoinductive growth factors to promote dental and craniofacial bone regeneration [94]. Tissue transplantation has been found in plastic surgery since the first successful hand transplantation in France in 1998. However, ethical, social, and psychological issues are raised when considering face transplantation. The long-term results of the recently performed partial face transplantations will be critical in assessing the future application of partial or total face transplantation [95].

9.3 Limitations of bioactive materials in plastic surgery and body reconstruction

Plastic surgery has advanced and expanded rapidly over the last 30 years, and is concerned with the restoration of form and function. However, the search for an ideal biomaterial acting as an implant, and complying with the complex requirements for plastic surgery and body reconstruction, remains a challenge, as many limitations exist in clinical application.

One limitation is angiogenesis, the formation of new blood vessels from an existing vascular bed into the new implanted constructs, in order to form new tissue. It is an integral factor in determining the success or failure of many procedures in plastic and reconstructive surgery. It is clear that in order to produce an effective functioning vascular system, a complex controlled cascade of events must take place. To ensure initial vessel formation is followed by vessel maturation and stabilisation, any therapeutic system must use a coordinated sequence of cytokine release,

including the factors vascular endothelial growth factor (VEGF) and Ang-1. The optimum mode of delivery of these factors is yet to be determined, to ensure a safe, effective, and sustained cytokine effect. Gene therapy has emerged as a promising vehicle for the delivery of such a sustained cytokine effect. However, further studies are required in order to assess the safety and efficacy of such therapy [96]. Another advanced approach is based on stem cell technology. Adult stem cells and stem cell-derived tissues can be useful therapeutically in replacing cells and even tissues in humans. However, one of the most striking and yet unsolved problems remaining is the lack of a sufficient number of stem cells available for the treatment of patients in various surgical areas, covering cardiac, transplant, gastrointestinal, plastic, foetal, and neurosurgery. Therefore, there is an urgent need for a supply of phenotypically and functionally well-defined stem cells of high and standardised quality for safe clinical application [97].

Cosmetic surgery is portrayed as risk free, with no reference to potential problems or complications. The misguided perceptions and unrealistic expectations of cosmetic surgery can lead to problems due to the nature of the implant and the procedures [2]. The major factors that contribute to complications of facial implant surgery, for example, include the composition of the materials, surgical technique, and facial region [98]. For other soft tissue augmentation, although breast implants have not been associated with an increased risk of breast cancer, they may interfere with routine mammography. Therefore, women with breast augmentation may be more likely to be diagnosed with advanced disease [99, 100]. Another implication is infection-related implant failure. According to a large epidemiological retrospective cohort study with long-term follow-up in Denmark, infection rates of 2.0–2.5% were found for cosmetic breast implants [101]. Formation of an acellular collagenous sheath around a foreign material usually follows the placement of a prosthesis. This will form a capsule around the implant. Capsular contracture is the leading long-term complication that occurs after breast implantation [102]. Studies suggested that immune mechanisms towards the foreign materials rather than bacterial flora may play a key part in capsular contracture [103].

An ideal graft or implant material needs to be biocompatible and possess physical properties and long-term stability, devoid of complications, to meet the following criteria:

- biocompatible and non-toxic;
- functionally effective with appropriate physical properties (e.g. mechanical properties, elasticity, viscosity, hardness);
- resistance to infection;
- no inflammatory response;
- cost effective;

- easy removal;
- bioresorbable/permanent according to use;
- no migration;
- no transmission of disease;
- easy to apply clinically;
- non-invasive, if possible.

To meet all these criteria for an implant remains a challenge for the future.

9.4 Future trends

Regenerative medicine has recently witnessed considerable activity in basic and translational research, with advances now making their way into plastic surgery practice. Ideal reconstructive goals, such as a complete return to original form and function, are frequently not achieved completely. Regenerative techniques now in clinical use and at the translational research stage hold promise for custom-tailored constructs, with the potential to regenerate tissue in the host, without significant donor site morbidity. These techniques may provide better structure, aesthetics, and function than the best options currently available [104].

Tissue engineering of complex tissues, such as those found in the craniofacial region, is a demanding task. Research will focus on the incorporation of multiple cellular phenotypes and the enhancement of the cellular interactions towards tissue repair, possibly stimulating their behaviour by supplying bioactive factors. Furthermore, the problem of insufficient vascularisation must be overcome, since most tissues are strongly dependent on blood supply for growth. Creating stratified tissue architectures and recreating the physiological structure–function properties of the native tissues is the ultimate goal. The choice and design of a tissue-engineering scaffold can significantly aid in this process, not only by serving as a delivery vehicle for cells and bioactive signalling factors, but also by providing the ability to interact with and guide tissue growth. Cell–material interactions and mass transport by control of the porous structures of the scaffold are only some of the important parameters that need to be incorporated into the design. Additionally, it is necessary to take into account that the location and form of defective tissues require special treatment. For those head–neck and facial delicate regions, aesthetic considerations are important and there should ideally be minimal scar formation.

As the popularity of non-surgical and minimally invasive procedures continues to grow, in the future, manufacturers and surgeons will develop new techniques and products that advance the science, produce even better results, and lessen recovery time. For example, HA is non-immunogenic,

biodegradable, natural, and long-lasting via control of the crosslinking process, resulting in near-ideal filling agents for cosmetic tissue augmentation. Variations in manufacturing processes, mechanical properties, such as viscosity, elasticity, and hardness, ease of injection, and body residence time provide the users with more options. Innovation is also predicted in the development of techniques utilising gene therapies to mould a face or other surface feature, without performing repeated surgery [105]. In this respect, it is important to develop drug delivery systems (DDSs) enabling a therapeutic gene to be delivered specifically to the target cell at an appropriate timing, for a certain time period, and bioactive materials will play very important roles in this [106].

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10

Bioactive materials in drug delivery systems

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Abstract: In this chapter, the focus of drug delivery systems is on bioactive materials. By manipulating the processes for producing bioactive materials, which contain bioactive elements, the release profile of bioactive molecules can be adjusted. Bioactive materials include natural biopolymer-based biomatrices, such as collagen, chitosan, and hyaluronic acid; bioceramics, such as hydroxyapatite and bioactive glass; and synthetic biodegradable polymers, such as PLA/PGA copolymers. Bioactive materials as carriers for the delivery of bioactive molecules have achieved clinical application in many fields, including oncology, orthopaedics, dental materials, cardiovascular, urology, wound care, and dermatology. The limitations for bioactive materials used in drug delivery include the difficulties in achieving a controlled release profile, owing to the structures of the materials, the complexity of the regulatory issues, the selection of the drugs, the difference between *in vitro* and *in vivo* conditions, and the assessment of device stability. Future trends in this field will focus on the improvement in design of delivery vehicles, using structurally well-defined bioactive materials in order to achieve sustained drug release, together with high efficacy.

Key words: drug delivery system, controlled release, nanomedicine, drug delivery implant.

10.1 Introduction

Theoretically, an ideal drug delivery system (DDS) should deliver a drug to a specific site in a specific time and release pattern. At present, 95% of all new potential therapeutics have poor pharmacokinetics and biopharmaceutical properties [1]. Therefore, there is a need to develop suitable DDSs that distribute the therapeutically active drug molecule only to the site of action, without affecting healthy organs and tissues.

The most commonly and commercially available DDS is based on

Table 10.1 Biomaterials used as carriers for drug delivery

Biomaterials	Examples
Natural – biodegradable	Hyaluronic acid Collagen Elastin Polysaccharides
Synthetic – biodegradable	Polyesters Polyanhydrides Polyortho esters PLGA
Synthetic – non-biodegradable	Silicone Poly(ethylene–co-vinyl acetate) (PEVA) Polyacrylate

nanoscaled bioactive materials, under the modern term of nanomedicine, including liposomes [2, 3], micelles [4, 5], nanoemulsions [6, 7], nanoparticles [8–10], nanogels [11], and dendrimers [12]. Other than the nanomedicine DDS, bioactive materials used in drug delivery systems are mainly focused on natural biopolymer-based biomatrices, such as collagen, chitosan, and hyaluronic acid; bioceramics, such as hydroxyapatite and bioactive glass; and synthetic biodegradable polymers, such as poly-lactic acid/poly-glycolic acid (PLA/PGA) copolymers, with the medical applications focused on these biomaterials used as implants for drug delivery. Biomaterials used as carriers for drug delivery are listed in Table 10.1.

10.2 Applications of bioactive materials in drug delivery systems

The utilisation of bioactive materials as carriers for the release of drugs has achieved wide clinical applications.

10.2.1 Oncology

Cancer chemotherapy is not always effective. Difficulties in drug delivery to the tumour, drug toxicity to normal tissues, and drug stability in the body contribute to this problem. Polymer DDSs can release drugs in a sustained, continuous, and predictable fashion. Commercial examples include Decapeptyl® [13], Lupron Depot® [14], Zoladex® [15] for treating prostate cancer, and Gliadel® for the treatment of recurrent malignant glioma [16].

Malignant gliomas represent 13–22% of brain cancers. Regardless of the treatment method, the median survival time is less than 1 year. Systemic chemotherapy has a limitation due to the blood–brain barrier (BBB) causing

low efficacy [17]. Approaches to overcome the limitation are the administration of drug-containing microparticles or the placement of an implant-drug into the brain. As the release of the incorporated drug from these systems can be controlled over periods of weeks to months, one or two administrations are generally sufficient. Thus, the risk of infections can be significantly reduced [18]. For example, different types of paclitaxel-loaded, poly(lactic acid-co-glycolic acid) (PLGA)-based microparticles and lipidic implants, which can be directly injected into the brain tissue, were produced for brain cancer treatment [19]. Study indicates that paclitaxel release from glycerol tripalmitate-based implants (initial loading = 29% w/w) was very slow in phosphate buffer pH 7.4: except from an initial burst of approximately 12%, almost no drug was released within the first 3 weeks. For the PLGA-based microparticles with 20 and 40% theoretical drug loading, similar to the glycerol tripalmitate-based implants, the drug release was very slow: less than 35% paclitaxel was released within the first 4 weeks [19]. The release profile is influenced by the polyvinyl alcohol (PVA) concentration used to make the microparticles, due to the change of particle size. This means there are effects of different formulation and processing parameters on the resulting drug release kinetics.

Unrelieved cancer pain remains a significant problem worldwide. The vast majority of patients with cancer pain can be successfully managed with oral analgesics. However, parenteral administration of opioids is required in some patients with disorders of the gastrointestinal tract. The high opioid requirements can lead to toxicities associated with intermittent dosing schedules. There is a clinical need to design a slow release system for cancer pain relief. Ethylene vinyl acetate (EVA) copolymer discs, containing 50% hydrornorphone by weight, were demonstrated to be a highly biocompatible, non-biodegradable, polymeric device, which releases hydromorphone subcutaneously at a constant rate for 4 weeks [20].

Viadur (leuprolide acetate implant, ALZA Corporation, Mountain View, California), a 1-year leuprolide delivery system for palliative treatment of advanced prostate cancer, was developed to maintain steady serum leuprolide concentrations and improve long-term medication compliance, offering the potential for more effective disease management. The implant uses osmotic pressure to deliver leuprolide acetate continuously at a controlled rate for 1 year [21].

The implants used in cancer treatment can be based on biodegradable and non-biodegradable polymers. The main biomaterials used in this field have been reviewed by Fung and Saltzman [22].

In addition to the above-mentioned implant DDS, the more advanced type of DDS consists normally of multicomponents, which include a targeting moiety and supplementary active ingredients, in addition to the carrier and drug. The carrier combines all the components of the DDS and

provides the required characteristics of the whole DDS, i.e. solubility, molecular mass. A targeting moiety enforces the specific delivery of a drug to the targeted organ, tissue, or cell. Acting as a penetration enhancer, the targeting moiety also improves the cellular uptake of the entire DDS. The most effective and widely used approach is based on the coupling of a drug carrier with a targeting moiety specific to certain cancer cells. Many different types of targeting moieties are being used experimentally and in clinical trials [23–25]. A system containing poly(ethylene glycol) (PEG) polymer as a carrier, camptothecin (CPT) as an anticancer drug/cell death inducer, a synthetic analogue of luteinising hormone-releasing hormone (LHRH) peptide as a targeting moiety/penetration enhancer, and a synthetic analogue of BCL2 homology 3 domain (BH3) peptide as a suppressor of cellular anti-apoptotic defence has been investigated [26].

A thermo-responsive gelling system has been used for a DDS as the polymer system can form gel in situ. Normally, the aqueous polymer solution is in the sol state below room/body temperature, but it will turn into gel at body temperature. The thermo-responsive polymers include Poloxamer or Pluronic hydrogels, composed of poly(ethylene glycol-*b*-propylene glycol-*b*-ethylene glycol) [27] and a symmetric triblock copolymer, with an architecture of BAB or ABA type, in which A is the hydrophilic poly(ethylene glycol) (PEG) and B is the biodegradable hydrophobic polyester, for instance, PLGA [28]. Anticancer drugs can be included in the system as an injectable for drug delivery [29].

A pH-responsive polymeric carrier has also been studied for the delivery of anticancer drugs to the tumour tissue site, as it is slightly more acidic (pH~6.8) than the normal tissue [30, 31]. The encapsulated drugs will target the tumour sites and be released upon the change of pH medium at the tumour sites. In addition to the pH difference at tumour sites, the pH difference at endosomes (pH 5.0–6.0) and lysosomes (pH 4.0–5.0) can help to maximise the anticancer drug, DOX, delivery to the tumour tissues, using pH-responsive micelles [32].

The pH-responsive polymers are normally designed to incorporate pH-responsive moieties into the polymer chain or onto the surface. For example, the block copolymer of hydrophilic methyl ether poly(ethylene glycol) (MPEG) and pH-responsive/biodegradable poly(β-amino ester) (PAE) was produced, using a Michael-type step polymerisation, resulting in an MPEG-PAE block copolymer, by which anticancer drug camptothecin (CPT) can be efficiently encapsulated into the pH-responsive polymeric micelles (pH-PMs) by a simple solvent casting method [33]. The administered drug can be localised in the tumour site. Other pH-responsive polymer drug delivery systems include poly(ethylenimine)/sulfonamine micelles [34], poly-(L-histidine) or sulfonamide-containing pullulan [35, 36], and various polymeric micelles/polyplexes, etc. [37, 38]. A review of

stimuli-responsive nanocarriers for drug and gene delivery has been published, in which the pH-responsive materials covered include pH-responsive polymeric nanocarriers, pH-responsive polymer–drug conjugates, pH-responsive liposomes, pH-responsive micellar delivery systems, and pH-responsive dendrimers [39].

10.2.2 Orthopaedics

There is a wide interest in the use of bioceramics for bone tissue engineering purposes. Scaffolds made of osteoconductive bioceramics alone or of composites can be feasible for tissue engineering. The ceramic, which enables implantation for load-bearing applications, can be used as a carrier for drugs, in which release patterns and efficiencies strongly depend on the type of ceramic/cement used. In general, ceramics show a higher initial release than cements, which have a more sustained release pattern [40].

Bioceramics, such as CaP ceramics and CaP cements, have been used to deliver growth hormone [41], bone morphogenetic protein [42], transforming growth factor-beta [43], and insulin-like growth factor [44]. Mesoporous bioactive glasses have been investigated as DDSs, owing to their adjustable pore diameter and high specific surface area [45, 46]. In addition to bioceramics as carriers for drug delivery, other biomaterials, such as polymethylmethacrylate (PMMA) bone cement [47] and collagen [48], have been used to deliver antibiotics to bone tissues. The use of antibiotic-impregnated bone cement in the treatment of osteomyelitis was very successful. However, the use of cement blocks inhibited drainage of secretions from the debridement area and the same cement was also very difficult to remove if redebridement was necessary. To overcome these drawbacks, a system with antibiotic-impregnated cement beads strung on steel surgical wire has been developed. These bead chains were flexible and were impregnated with gentamicin. The antibiotic beads were designed to treat localised infections residing in bone and soft tissues [49–51].

Aliphatic polyesters, including PLA, PGA, and poly-dioxanone (PDS), are the few US Food and Drug Administration (FDA) approved polymers routinely used for producing drug delivery devices. These polyesters, with poly(caprolactone) (PCL), polyanhydrides, and polyphosphazenes (POPs), substituted with amino acid esters, are the most extensively investigated polymers, essentially because of their good hydrolysability and biocompatibility [52].

Porous chitosan matrices, chitosan–poly(L-lactide) (PLLA) composite matrices, and chitosan coated on PLLA matrices have been fabricated by freeze-drying and crosslinking aqueous chitosan solution. Porous chitosan matrices combined with ceramics and constituents of extracellular matrices have been prepared and examined for their bone regenerative potential.

With these materials, drugs or growth factors can be incorporated for tissue local delivery [53, 54].

In order to have a good affinity to bone tissue when the drug is targeted, PLGA nanoparticles were modified with both alendronate and PEG, as alendronate has a specific adsorption to hydroxyapatite (Hap). Research indicates that the DDS using PLGA nanoparticles modified with alendronate can target bone tissue specifically [55].

To repair large bone defects based on bone tissue engineering, biological factors, such as cells, growth factors, or genes, are typically required to regenerate bone defects effectively [56, 57]. The DDS for the delivery of these biological factors has been a hot topic and interesting area for many years [58–61]. The future relies on a good combination of DDS components for delivery of the factors and bioactive materials certainly play a very important role for bone tissue regeneration.

10.2.3 Wound care and dermal drug delivery

DDS-based bioactive materials to tackle the biofilm formation and infections are briefly described in Chapter 5. The main challenge in designing a wound dressing device for the release of low molecular weight (MW) hydrophilic antibiotics is to overcome the rapid discharge of the drug from the device. The technical requirement for local antibiotic release should exhibit a considerable initial release rate in order to respond to the elevated risk of infection from bacteria introduced during the initial trauma, followed by a release of antibiotics at an effective level, for sufficiently long to inhibit latent infection [62]. Approaches to overcome the drawbacks include using hydrophobic materials or a biomatrix to trap or bond the hydrophilic antibiotics in order to slow the release of the bioactive substances [63, 64].

For skin regeneration by tissue engineering, incorporating growth factors into appropriate vehicles can achieve controlled release of factors at the target area over an extended period of time until the new tissue forms, as it is necessary to enhance and guide both tissue organisation and neovascularisation, which normally occurs with the long-term tissue growth process [65]. The biomaterials used for the DDS for wound care or skin tissue repair are generally based on natural biopolymers such as collagen [66], gelatin [67], fibronectin and fibrin [68, 69], alginate [70], and chitosan [71, 72].

Development of successful topical/transdermal DDSs has been limited in scope, due to the significant penetration barrier provided by the stratum corneum (SC), the topmost skin layer. The most applied polymers on skin belong to various classes, for example, cellulose derivatives, chitosan, carageenan, polyacrylates, polyvinyl alcohol, polyvinylpyrrolidone, and silicones [73, 74]. Nanoparticles based on biocompatible PLGA [75], poly(ε-

caprolactone) [76], chitosan [77], a combination of chitosan, poly(gamma-glutamic acid) and (gamma-PGA) [78], and hyaluronan [79] have also shown promise in dermal drug delivery. The emerging fields of gene and stem cell therapy in wound healing have also received great attention for the treatment of chronic and acute wounds [80].

10.2.4 Cardiovascular system

Restenosis after an initial successful angioplasty of an atherosclerotic plaque remains the major limitation of coronary angioplasty in humans. Some 30–50% of patients undergoing coronary angioplastic procedures develop reocclusion within 3–6 months [81]. A form of nanoparticulate drug delivery dosage offers the possibility of a catheter injectable therapeutic approach, with sustained release capabilities, to tackle the problem. The typical formulation of nanoparticles is described as follows: PLGA and therapeutic agent are dissolved in an organic solvent (chloroform, methylene chloride, ethyl acetate, or co-solvent). This organic phase is emulsified in an aqueous phase containing PVA, acting as an emulsifier, to form an oil-in-water emulsion. Organic solvent evaporation is achieved by stirring at room temperature. In the case of water-soluble drugs and protein molecules, a multiple water-in-oil-in-water emulsification solvent evaporation technique is used. A water-soluble drug or protein is first dissolved in water and emulsified in an organic polymer solution to form a water-in-oil emulsion. This primary emulsion is further emulsified in an aqueous PVA solution to form a multiple emulsion. Following evaporation of the organic solvent(s), nanoparticles are formed, which are then recovered by ultracentrifugation, washed repetitively with water or buffer, and lyophilised [82, 83].

It is now virtually proven that drug eluting stents delivering anti-proliferative agents to the vessel wall can reduce restenosis [84–86]. For example, a novel family of biodegradable nanostructured hybrid polymers to be used as paclitaxel-loaded stent coatings, termed polyhedral oligosil-sesquioxane thermoplastic polyurethanes (POSS TPUs), with enhanced mechanical properties, has been designed [87, 88]. By altering the copolymer segments, the drug release profile can be adjusted. The Cypher™ stent is composed of a polymer matrix loaded with sirolimus and a polymer topcoat serving as a diffusion barrier. Polymer blends containing poly(ethylene-covinyl acetate) and poly(butyl methacrylate) were reported to release 100% of sirolimus during approximately 1 month [89, 90].

In addition to the DDS for the treatment of restonosis, the controlled release drug delivery implant has also been used for prevention of bioprosthetic heart valve calcification [91], prevention, and treatment of cardiac arrhythmias [92].

10.2.5 Urology

The kidney, bladder, and prostate are easily accessible for minimally invasive interventions. Intravesical drug delivery (IDD), or direct delivery of the drug into the bladder through a catheter, has proved to be an effective method to ensure maximal delivery of therapeutic agents at the site of the disease and to minimise systemic side effects. Based on these methods, nanoparticles, microparticles, or small-scale implants as drug carriers can simply be deployed and retrieved using percutaneous or endoscopic systems in outpatient settings, with minimal patient discomfort and maximal therapeutic benefit. Chang *et al.* [93] studied poly(ethyl-2-cyanoacrylate) (PECA) epirubicin-loaded nanoparticles (EPI-NP) against bladder cancer cell lines (T24, RT4). PECA is a biocompatible, biodegradable, mucoadhesive polymer that can have strong interactions with cells and tissues. The nanoparticles in conjunction with a surfactant (Tween80 or Pluronic) had sizes less than 200 nm and greatly improved the penetration of epirubicin into the bladder walls, since commercial aqueous formulations of EPI have very low efficacy. Tissue analysis showed that the EPI-NP formulation had higher penetration and accumulation in the tissues than the free drug. Intravesical DDSs can be made more effective using materials which are mucoadhesive; i.e. they can attach to the mucous membrane of the urothelium. A number of biomolecules, such as chitosan, carbomers, and cellulose derivatives, have been identified as having mucoadhesive properties. Study of the use of chitosan carriers for mitomycin C (MMC) to serve as a drug reservoir on the bladder surface indicates that the higher MW of chitosan has better adhesion to the bladder tissue [94]. The sulfated form (N-sulfonato-N,O-carboxymethylchitosan, sNOCC) was used to encapsulate and transport the anti-inflammatory agent (5-aminosalicylic acid, ASA) into the bladder wall, in rat models, using protamine sulfate-induced cystitis. An optimised combination of 3% sNOCC with 5-ASA was found to reduce inflammation and urinary frequency to the greatest extent [95].

In-situ-forming hydrogels (ISFH), such as the thermoresponsive, biodegradable, PLGA–PEG–PLGA-based ones (OncoGel), have been used for incorporation and local delivery of paclitaxel to solid tumours in animal models [95]. Results showed that there was targeted delivery of the drug to tumour tissues, with sustained release. Other ISFHs, such as the commercial Pluronic F127 in-situ gelling system, have been further improved by incorporation of biodegradable drug-loaded polymeric nanoparticles to enhance the availability of poorly soluble drugs and, thereby, allow sustained, localised delivery of therapeutic agents [96]. By adjusting the composition of Pluronic F68 and PVA, an ISFH can be produced for drug delivery. With 30wt% F-68 aqueous solution and 15wt% PVA aqueous solution, F-68/PVA complex gel was prepared and its swelling transition

was observed at approximately 37°C [97]. The commercial products Eligard® and Atridox®, which contain leuprorelin and doxycycline respectively in an ISFH named Atrigel®, have received approval by the FDA [98]. The therapeutic benefit of sustained delivery afforded by thermosensitive hydrogels was demonstrated by delivering misoprostol, an anti-inflammatory drug. It was able to protect the bladder against cyclophosphamide-induced cystitis [99].

Transdermal devices are classified as rate-controlled reservoir/membrane patches and matrix diffusion-controlled patches, which can be used for urological application. For example, hypogonadism can result from failure of the testes or ovary (primary) or failure of the pituitary/hypothalamic axis (secondary) to secrete appropriate amounts of gonadotropic hormones. Hypogonadism can significantly impair a patient's quality of life. Clinical manifestations are treated with hormonal replacement therapy (HRT), in which testosterone is the most commonly used hormone. It is usually administered transdermally using reservoir patches or topically applied gels [100].

With more extensive use of medical devices in ageing populations, accompanied by serious infection problems associated with these medical devices, design and study of improved methods for direct, controlled, and local release of drugs to prevent device-related infections remains a compelling priority. Innovative, effective drug/device combination products are required for improved performance of medical devices, decreasing health care costs, avoiding systemic administration of high levels of antimicrobial drugs, and reducing further risks of antibiotic resistance. Two main strategies have been utilised to reduce the incidence of device-related infections: anti-adhesive biomaterials, using physicochemical surface modification methods, including non-drug-containing coatings, films, and ion treatments, and direct incorporation of drugs into or onto the medical device, either immobilised or released [101]. A detailed description is given in Chapter 5.

10.3 Limitations of bioactive materials in drug delivery systems

In this chapter, bioactive materials-based DDSs have been reviewed. The bioactive materials used include nanomaterials, biodegradable or non-biodegradable materials, and hydrogels. For a targeted DDS, the nanomaterials offer great promise but there are still many limitations. One limitation is the selection of the ligand, which has specific affinity to the targeted site for drug delivery. Another limitation is the binding process for linking the ligand to the drug carrier, which normally involves chemical

attachment. The process is difficult to control and is not practical on the industrial scale. A third limitation is the complexity of the actual *in vivo* application of the DDS with designed targeted system. The biological system has a great influence on the site target action using a DDS, preventing an ideal clinical result in many cases. Finally, the limitations on understanding the nanotechnology itself require various stages of research and development, in particular the toxicity of nanoparticles, as they tend to accumulate in vital organs [102, 103].

While the concepts of the environmentally-sensitive hydrogels, such as pH-responsive and temperature-responsive hydrogels, are sound, the practical applications require significant improvements in the hydrogel properties. The most significant weakness of all these external stimuli-sensitive hydrogels is that their response time is too slow. The development of fast-acting hydrogels is necessary but is normally difficult to achieve. It will compromise the mechanical properties of the hydrogel, as the hydrogel needs to be thin and small. Environmentally-sensitive hydrogels for drug delivery applications also require biocompatibility. The preparation of novel polymers and crosslinking agents, with improved biocompatibility and biodegradability, would be essential for successful application [104].

The DDS in regenerative medicine and tissue engineering plays a very important role, especially for the delivery of growth factors and cells for tissue repair. Both growth factor and stem cell delivery strategies provide a greater understanding of the neovascularisation process and the early results are encouraging. However, many questions still need to be addressed for the ultimate realisation of clinical therapies. A major obstacle to growth factor delivery is the insufficient or indeterminate dosing and unresponsiveness to specific growth factors. Many angiogenic factors are pleiotropic, with different activities depending on concentration. Multiple growth factors involved in vessel formation are highly regulated and specifically timed. Furthermore, most proteins have relatively short half-lives. It is unlikely that the uncontrolled release of a single growth factor will achieve successful neovascularisation. The majority of work using local injection of angiogenic growth factors, as recombinant polypeptides or encoding genes, results in transient elevation of growth factor concentration and formation of capillaries that rapidly regress. For stem cell therapy, extensive cell death after transplantation and lack of well-described mechanisms underlying improved angiogenesis are primary challenges. A possible solution addressing many challenges is a combination strategy: the combination of two or more growth factors or the combination of both stem cells and growth factors. For full realisation of the latter strategy, a flexible, tailored delivery platform will be required [105].

Other general limitations with DDS are manufacturing issues, such as stability, batch to batch reproducibility, sterilisation method, low drug

entrapment, particle size control, production of large batch sizes, and short circulation half-life of vesicles.

In the cardiovascular system, drugs introduced into the vessel wall by local catheter delivery systems may be rapidly washed out over minutes to hours, particularly if they have no specific intramural binding properties. The successful translation of this concept to a viable clinical strategy has been technically challenging. Major obstacles that have been encountered include: (i) the design of devices that enable delivery of adequate quantities of drug to the vessel wall, without either injuring the wall or compromising flow, (ii) the development of delivery vehicles that allow retention of the administered drug within the local environment for periods of time adequate to ensure a therapeutic effect, (iii) the optimisation of strategies enabling the transfer of genetic material into cells within the vessel wall, and (iv) the development of sustained delivery polymeric coatings for stents that do not produce thrombosis or an inflammatory tissue response [106].

In summary, to achieve an ideal practical DDS, the properties of the drug, the characteristics of the material/device, selection of *in-vivo* model, and the patient status are all important factors for safe and effective drug delivery. A major challenge is to define the optimal dose, time, rate, and site of delivery.

10.4 Future trends

According to BCC Research, the worldwide market for advanced DDSs totalled $134.3 billion during 2008. The total was estimated to rise to $139 billion in 2009. BCC Research projects an increase to $196.4 billion for 2014, which would represent a compound annual growth rate of 7.2% in the five-year term [107]. The implantable/injectable drug delivery market generated record revenues of $9.8 billion worldwide in 2006 [108]. However, challenges in this area still remain, including:

- overcoming biological barriers, such as getting drugs across cell membranes and the blood–brain barrier;
- issues with solubility for many small-molecule drugs;
- drug delivery of biopharmaceuticals and vaccines, due to stability, and additional difficulties getting through biological barriers.

In order to develop an ideal drug delivery system or device, the following areas are being targeted for improvements [109]:

- improved efficacy;
- reduced side effects;
- continuous dosing (sustained release);
- reduced pain from administration;
- increased ease of use;

- increased use compliance;
- improved mobility;
- decreased involvement of healthcare workers;
- improved safety for healthcare workers;
- reduced environmental impact (elimination of CFCs).

Currently, many technologies have been developed for DDSs and some of them have reached the market [110]. A biomimetic-based approach is best for DDSs. This is an ideal DDS, having a non-protein adsorbing surface, a predictable drug release profile, and an interaction with only the disease site. Artificial cell approaches may be a solution for this type of DDS. Reconstruction of biomembranes and the transport of proteins over a polymeric capsule may find a place in the design of artificial cells. Modification of micro/nano particles with hydrophilic non-immunogenic polymers has been successfully applied to obtain long circulating drug carriers [111].

In the aspect of selection of biomaterials for drug delivery in tissue engineering, naturally-derived materials can mimic properties of the natural extracellular matrix and thus show great potential for numerous tissue-engineering applications. In particular, owing to their excellent cytocompatibility and protein-incorporation properties, naturally-derived materials have attracted interest in combining multiform applications in a single biomaterial, as both scaffold material and as a vehicle for active biomolecules.

For cell and drug delivery systems, efforts should focus on developing or improving new or combined processing strategies to obtain novel vehicles, with biomimetic morphology at the nano- and micro-scale levels, preferably those providing biological functions and having the capability to deliver relevant molecules, such as various growth factors, in a controlled, manner. It is hoped that collaborative research among the biomaterial, pharmaceutical, biological, and regenerative medicine fields will lead to more advanced, naturally-derived, materials-based cell and DDSs [112].

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