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Jan W. Gooch

Biocompatible Polymeric Materials and Tourniquets for Wounds

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Biocompatible Polymeric Materials and Tourniquets for Wounds

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Dr. Jan W. Gooch
Visiting Senior Research Engineer
Georgia Institute of Technology
Atlanta, GA
USA
Jan.Gooch@gtri.gatech.edu

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Preface

The topics contained in the book fall squarely within the realm of Combat Casualty Care, one of the pillars or mission areas for Army medical research. Specifically, the book deals with Far Forward Resuscitation, prevention and management of blood loss, wound dressing, prevention of infection, and prevention of adverse tissue responses and impaired healing. These aspects of treatment are critical to prevent death, treatment complications, and disability. They are the important aspects of early interventions in the battle area or during medical evacuation. Information covering so many aspects of this type of care are not usually found in a single source. This book will compile a large amount of important information that is current and state of the art. It will help to define the Standard of Care.

The use of polymeric materials is emphasized here because advances in the uses of biocompatible polymers for injuries and wounds have outpaced the dissemination of information. Innovative polymer technology was applied to the common combat and other trauma wounds associated with damaged soft tissue and bleeding.

The *golden hour* is the first 60 minutes following trauma or the onset of acute illness. The chances of survival are great if surgery or advanced trauma life support can be provided within that hour. Combat wounds, as well as accidental or inflicted civilian wounds, often occur where immediate medical assistance is not available, and emergency care becomes the responsibility of the victim of wounds. Therefore, self-care or care-giving can be of critical importance and too often the tools and methods have not been available.

On the battlefield, a properly applied tourniquet can be an effective means of controlling severe extremity wound hemorrhage. Much confusion exists among soldiers, medics, and military medical officers on a number of tourniquet related issues including:

- What is an appropriate combat tourniquet?
- When must an appropriate be applied?
- When and by whom should a tourniquet be removed?
- Under what conditions should a tourniquet be removed?

There are five levels of care for wounded warfighters.

Level I

Includes self-aid, buddy aid, and combat lifesaver skills. Also includes emergency medical treatment provided by combat medics and corpsmen and advanced trauma management provided by physicians and physician assistants. Highest level treatment capability: Army medical platoons (battalion aid stations) and USMC shock trauma platoons.

Level II

Includes physician-directed resuscitation, advanced trauma management, emergency medical procedures, and forward resuscitative surgery. Supporting capabilities may include basic laboratory, radiology, pharmacy, dental, limited blood products, and temporary patient holding facilities. Highest level treatment capability: Army division-level medical support, USMC Level II asset is the surgical company, and USAF EMEDS Basic and EMEDS +10.

Level III

Includes resuscitation, initial wound surgery, postoperative care, and more advanced ancillary services. This may also include restoration of functional health (definitive care). Highest level treatment capability: Army combat support hospitals, Navy and USMC fleet hospitals, and USAF EMEDS +25.

Level IV

Includes rehabilitative and recovery therapy for those who may return to duty if convalescence from injury does not exceed the established theater evacuation policy. This level of care is becoming less prevalent in contemporary warfare and battlefield patient management. Highest level treatment capability: Army field hospitals, general hospitals, and combat support hospital echelon above corps.

Level V

Includes the full range of acute convalescent, restorative, and rehabilitative care. Highest level treatment capability: permanent military or Veterans Affairs hospitals, or civilian hospitals that have committed beds for the National Defense Medical System.

This series of research efforts were designed to effectively support the individual warfighter wounded forward of hospitals and possible field aid stations as described in Level I. The scope of this investigation spans:

- Barrier dressings (liquid and particulate) for soft tissue wounds
- Sutureless tissue adhesives
- Antibacterial nanoemulsions
- One-hand operated and automatic tourniquets for the battlefield

Barrier dressings are envisioned to be self-applied to warfighters in order to protect exposed wounds from infection without clinical treatment. Sutureless tissue adhesives have been produced that can be quickly applied to the damaged soft tissue to close the open wounds; and ruptured vessels to restore blood transport that would otherwise require days of healing using sutures. Antimicrobial nanoemulsions have been reported to be capable of disinfecting surfaces in the work, residential and clinical environment contaminated with microbial agents dangerous to humans. Cognizant of anthrax contamination in public facilities, this capability could be useful for rapid decontamination of buildings. A new one-handed and self-applied tourniquet is needed by the warfighter to tourniquet each limb if injured in battle. New designs with automatic features are capable of providing low weight and cube, but quick and effective tourniqueting of any limb can be conveniently carried by the individual warfighter.

Each effort is addressed independently comprising a separate reference section with appended figures, tables, and literature.

Atlanta, GA Jan W. Gooch

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Georgia Institute of Technology, Atlanta, GA
Dr. F. Joseph Schork, Professor and Chair Department of Chemical & Biomolecular Engineering, University of Maryland

Contents

Acronyms and Abbreviations

MRMC	Medical Research and Materials Command
USAISR	United States Army Institute of Surgical Research

Chapter 1
Scope, Objectives, and Summary of Investigations

1.1 Scope and Objectives

This series of research efforts were designed to effectively support the individual warfighter wounded forward of hospitals and possible field aid stations. The scope of this investigation spans:

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1.2 Summary of Results

The results of the investigations were encouraging and continue to warrant further development including testing on humans and eventual commercialization. Each investigation produced a different set of results, which follow:

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1.2.1 Barrier Dressing for Wounds

Efficient barrier dressings are imperative for protecting injured tissue as can be appreciated by observing the robust bacterial growth shown in Fig. 1.1 that was initiated by placing the author's bare-hand onto a porcine blood agar plate and incubated at 37°C for 7 days. The environment is robust with organisms that reside on human skin and soil as well as ubiquitous airborne organisms.

Through experimentation with different dressings, it was discovered that any dressing designed to serve as a barrier to bacteria must possess the following numerous and special properties esoteric to soft tissue:

- Impervious to diffusion of nutrients and bacteria through the barrier which excludes any material with excess of about 20% water content (e.g., hydrogels)
- Aerobic bacteria will penetrate a noncontiguous (cracks, pinholes, etc.) barrier material such as a polymeric film
- A successful barrier cannot allow diffusion of nutrient to diffuse from the wound through the barrier to nourish the bacteria to promote growth and eventual penetration of the barrier dressing
- Surface energy of the "dried or cured" barrier would preferably be greater than water (e.g., lipohilic oil) to repel wetting, and therefore, repulsion of bacteria which is hydrophilic, but the wet dressing must be adherent to the soft hydrophilic tissue
- Liquid barrier dressing would preferably be a single component material that does not require catalysis or evaporation of a solvent

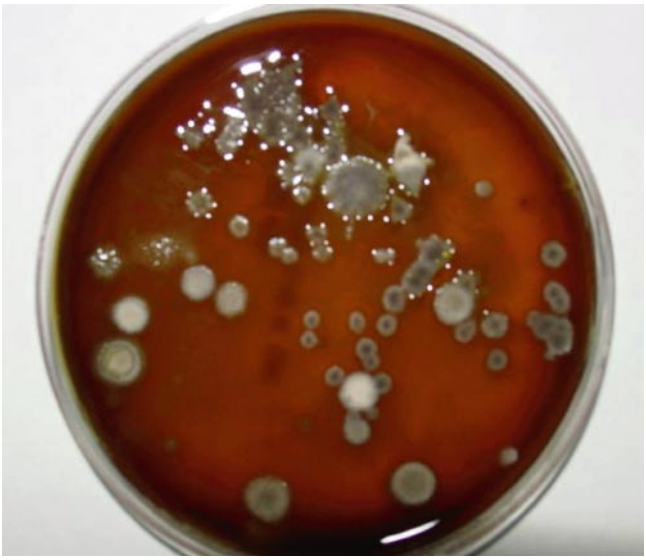

Fig. 1.1 Example of the ubiquitous presence of bacterial growth on porcine agar plate originally transferred from the palm of the author's hand and incubated for 7 days at 37°C

- The barrier dressing must be adherent to natural soft tissue that is always moist with bodily fluids and water and without an inflammatory response. Ideally, the barrier dressing will allow semipermeation of water vapor and gases from the tissue through the barrier dressing which will prevent the tissue from becoming too moist (maceration) to allow proper healing, and prevent irritation (itching, etc.)
- An aerosolized spray application is preferable because of the ease of transferring the barrier material to the tissue without abrasion
- Barrier materials must be biocompatible with soft tissue (in vitro) applications
- Barrier must be flexible (viscoelastic) as tissue to prevent fracture leading to disbanding from tissue
- Barrier materials must be easily removed at anytime without irritation to the wound or discomfort to the patient
- Cured or dried dressing barrier would be transparent so that the caregiver could observe the progress of wound healing
- Liquid dressing would be low in weight and cube, which would dictate that a small volume would dress a large area of the body

The hydrophilic copolymers synthesized from acrylic monomers and 2-octylcyanoacrylate barrier dressings were successful in providing protection for excised rats that were treated and inoculated with bacteria. The particulate dressing was not successful in providing protection for excised rats because the dressing was porous due to the lack of a continuous film and high water content in excess of 70%.

Miniemulsions (acrylate polymers particles in aqueous media) were also unsuccessful in providing a continuous film due to an inability for individual emulsified particles to form a continuous film on a moisture surface. Water separating the particles could not evaporate at a rate to allow a film to form before forming isolated aggregates (clumps) of polymer particles, and therefore, a continuous film necessary to provide a protective barrier dressing could not be formed from an emulsion even after adjusting the formulation. However, changing the aqueous media to an organic solvent produced improved results with delivery of the dressing in ethanol, acetone, and dichloromethane.

1.2.2 Bacteria Permeability of Dressing Materials

A study of different dressing materials for bacteria barriers was performed during the use of different materials and three species of aerobic bacteria. Anaerobic bacteria were used in the study due to laboratory policy. The dressing material was applied over nutrient agar, allowed to cure or dry followed by inoculation with bacteria (separate agar plate for each species of bacteria). The dressing materials that prevented bacteria penetration were solvent-borne polyacrylates, polybutyrals, and 2-octylcyanoacrylate. Films formed from emulsions and hydrogel type particulate materials allowed bacteria to penetrate to the agar nutrients and colonize as demonstrated in Fig. 1.2, which shows permeation of water-soluble dye through an aqueous gel of collagen.

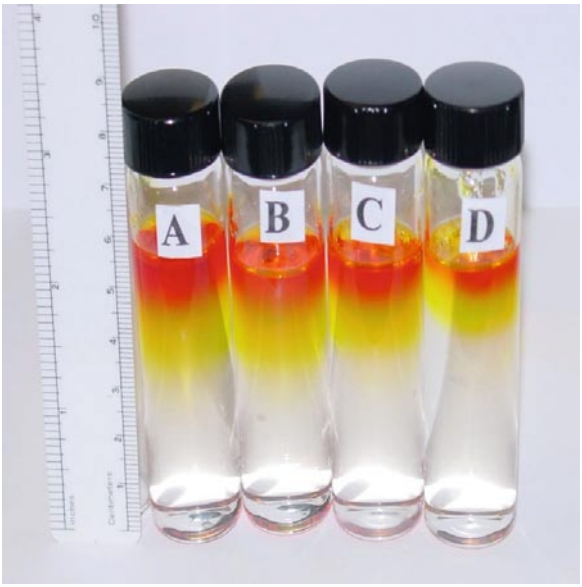

Fig. 1.2 Demonstration of permeation of FD&C Red Dye through collagen gel over 48 h: A–D vials represent 95%, 85% and 65% water by weight; the diffusion of the dye (consists of *red* and *yellow* dyes) is nearly constant regardless of the water concentration

1.2.3 Antibacterial Aqueous Emulsions

Recent developments in antibacterial "nanoemulsions" gained the attention of those wishing to decontaminate surfaces that could have been threatened with spore forming bacteria such as anthrax. Patented nanomulsions were reproduced from the inventors' published literature and patent disclosures. Marginal activity against bacteria was observed when challenged in the Baker Dilution Method or Kirby-Bauer Plate method. The additives used in the nanoemulsions were commonly known to have antibacterial activity when employed in proper concentration. The nanoemulsions do not oxidize as sodium hypochlorite or have a deleterious effect on permeants, and therefore, the attractiveness of a liquid material that does not discolor or otherwise damage surfaces as walls and furniture.

During the course of evaluating the patented sporicidal nanomulsions, new emulsions were discovered accidentally that did have antibacterial activity when challenged using the Baker Dilution Method and marginally using the Kirby-Bauer Plate Method. The technology is totally different from the reported nanoemulsions, but nonetheless, effective against some common bacteria when wetted. The discovery consisted of dissolving low molecular weight fatty acids in higher molecular weight fatty acids of specific viscosities and shearing them in water/surfactant to stable aqueous emulsions. The resulting emulsions were stable, safe, and effective against some bacteria and fungi.

1.2.4 One-Handed and Automatic Tourniquets

A tourniquet that could be applied effectively with one hand after suffering a wound and occlude blood vessels to slow or cease bleeding with automatic systolic pressure control was designed. As important as the design of an effective tourniquet was the necessary study of tourniquets used in past under combat conditions. Also, a study of clinical tourniquets was useful because they do not usually damage tissue and nerves as do combat tourniquets. It was preferable to design a tourniquet that would prevent excessive bleeding and minimally damage tissue and nerves. In addition, the battlefield often consists of soil, mud, rain, sand, and widely ranging temperatures.

A safe and effective combat tourniquet should possess the following properties as learned from these studies and experiences shared by the US Army medical personnel

- One-hand operation, application and tightening
- Force applying to tourniquet must be convenient for the injured soldier under any physical conditions including laying in fox hole or other position
- Occlusion pressure must be finely adjustable by the operator to stop bleeding while not over straining the limb and causing damage to tissue and nerves
- Functional under any climate, terrain and weather condition including rain, mud, sand and widely varying temperatures
- Option for automatic tourniquet adjustment using battery-powered pneumatic bladder
- Low in weight and cube

The above conditions were satisfied with a ratchet-buckle and single flexible 1.5 inch strap. The force capable of being applied through the lever-ratchet mechanism provided more than enough force from thumb or fingers to tourniquet any size limb. The strap was stressed so that it formed a "bow" shape in the center and formed the favorable round shape that is preferable for decreasing strain on tissue and nerves. The ratchet-buckle consisted of spikes to penetrate a fiber-woven belt that was very successful in defeating mud, sand, and blood, example shown in Fig. 1.3.

Fig. 1.3 Cinch-buckle for adjustable one-hand tourniquet

Chapter 2
Barrier Dressings for Wounds

2.1 Introduction

The skin is the largest human organ and is the essential interface between the host and its environment. Among the major functions of this organ are mechanisms that provide heat loss or heat retention; water loss or water retention; elimination of waste via exfoliation; protection against penetration of ultraviolet light; touch location of physical objects; and, perhaps most obviously, protection of underlying tissues from microbial pathogens contacted in the environment. Methods to establish an artificial barrier function over damaged skin by use of bandages, compresses, poultices, and other devices have been recorded during all phases of medical history. Today, there are adequate methods and devices for skin closure and/or bandaging at medical institutions capable of providing definitive surgical care, for example, hospital emergency and operating suites. The general availability of such facilities and emergency medical transportation systems are basic infrastructure components of modern societies. The wide variety of sizes, shapes, materials, and mechanical devices necessary to accomplish this level of wound care, however, is dependent upon an extensive logistic and storage base.

In the civilian setting, natural disasters such as tornadoes, hurricanes, fires, and explosions produce large numbers of casualties that cannot be treated immediately in emergency rooms and hospitals. A delay in medical treatment in the aftermath of disasters is often not available to remote geographic locations due to long distances from facilities, loss of power and essential services, or just the huge numbers involved and the enormity of the situation.

In the military setting, especially in the far forward edges of combat and other military casualty care, such medical logistical assets are impractical and only become available in proportion to the increasing level of care provided at the various staging facilities of the evacuation process. Under current planning instructions, major conflicts involving large numbers of combatants for extended periods of hostility are not expected. Rather, smaller operations with limited external logistical requirements and defined humanitarian/peace-keeping objectives are anticipated. With this scenario, rapid evacuation, as has been the U.S. Army's practice in most recent wars, may be delayed or not be available and therefore injuries will be managed

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locally for longer postinjury periods. This new time factor requires changes in front line medical capabilities.

The following study was performed under the following protocols at the US Army Institute of Surgical Research, Brooke Army Medical Center, Texas:

- Antimicrobial and barrier effects of dressings
- Rat excision model of wound infection – a type study

The "Tissue Sealant and Adhesive 6th, 7th and 8th Annual Conferences" sponsored by the Cambridge Healthtech Institute were excellent sources of information for materials on polymeric materials for barrier dressings.

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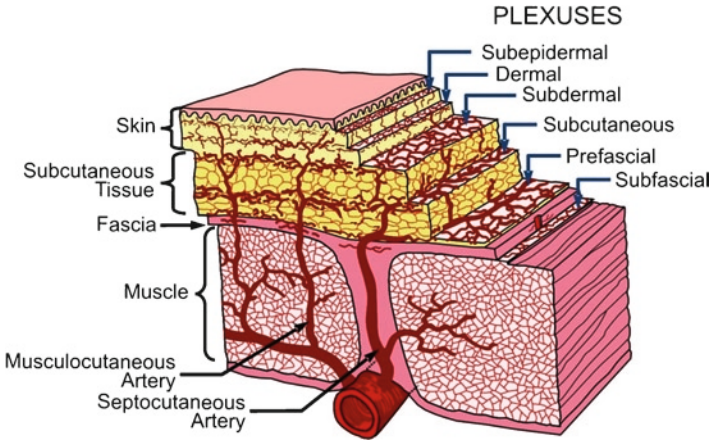


Fig. 2.1 The cutaneous macrocirculation of humans (McCarthy 1990)

Adhesives are usually designed to bond dry surfaces as wood and metal, but very specially designed adhesives are formulated for bonding moist tissue surfaces. The human skin is moist and contains sweat glands that allow moisture to permeate the full thickness of the skin. If injured, the subcutaneous tissue hemorrhages because blood vessels are severed. Vessel diameter increases with tissue depth as can be observed in the Fig. 2.1, and any tissue adhesive or protective barrier must be capable of bonding to a moist surface and possibly applied over a bloody field.

From a review of the literature, the following major properties are important to the caregiver using protective barrier dressing materials and adhesives as barriers.

- Barrier to bacteria to prevent infection
- Water vapor permeable to prevent maceration and promote wound healing
- Adhesion of the barrier dressing to injured tissue
- Flexibility of the barrier dressing on strained (stretching/contracting)
- Convenient to apply
- Acceptable environmental and shelf stability (combat conditions include −40°C to 60°C)

Results of the investigations of self-adhesive dressings for wound healing using occlusion and nonocclusion water vapor properties have been extensively reported in the scientific literature. The self-adhesive dressings have developed since Winter (1962) published “Review of classic research: Moist wound healing” using Large White pigs. Winter reported that the scab formed over a wound to the denuded skin retarded epithelization, and prevention of scab formation increased the rate of healing. The conclusion reached was that when a wound was kept moist with a barrier over the wound, the epithelization of the denude surface was twice as fast compared to just a scab over the wound.

Pollack (1979) reviewed Winter’s conclusions and also reviewed the influence of oxygen tension, humidity, temperature and infection on wound healing. Pollock

reviewed several papers on the environmental factors for wound healing and to select the optimal properties of a dressing. Realizing that some of the influences were subject to the advantageous manipulation of the practitioner, general conclusions were:

- Humidity: A semipermeable membrane type dressing is the best choice for wound healing and preferably a self-adhering dressing
- Temperature: Lower environmental temperatures decrease wound healing
- Infection: Bacteria inflammation has an inhibitory effect on wound healing
- Oxygen: Atmospheric oxygen and increased levels of oxygen (hyperoxia) exposed to the wound generally improves wound healing

Lionelli and Lawrence (2002) reviewed wound dressings and provided the criteria for desirable dressings:

- Protect wound from bacteria and foreign material
- Absorb exudate from wound
- Prevent heat and fluid loss from wound
- Provide compression to minimize edema and obliterate dead space
- Be nonadherent to limit wound disruption
- Create a warm, moist occluded environment to maximize epithelialization and minimize pain
- Be aesthetically attractive

In the same publication, a dressing that limits pain is most desirable which usually means an occlusive or water vapor barrier. However, a semipermeable dressing would be preferable that would control the amount of water vapor loss. A dressing that conforms to any contour, and a dressing that does not firmly attach to the tissue or interfere with the natural healing process are most desirable for superficial and full thickness skin wounds as well as deep tissue wounds. Further, a dressing that limits body water loss to less than 35 g of water vapor transmitted per m^2 per hour is considered low enough to maintain a moist environment for most wounds.

Visscher et al. (2001) reported the effects of semipermeable films and water vapor transport (WVT) on human skin following a standardized wound (81.8 $g\,m^{-2}\,h^{-1}$ WVT), induced by tape stripping, by measuring transepidermal water loss (TEWL), skin hydration, rate of moisture accumulations, and erythema. Wounds treated with semipermeable films (25.3–64.3 $g\,m^{-2}\,h^{-1}$ WTV) underwent more rapid recovery than wounds or wounds under complete occlusion (0.0–0.5 $g\,m^{-2}\,h^{-1}$). Barrier films that produced intermediate levels of skin hydration during recovery produced the highest barrier recovery and general healing. Sites exposed to highest levels of hydration showed the poorest recovery indicating that occlusion retards healing. The results supported the hypothesis that semipermeable wound dressings augment barrier repair and skin quality by providing an optimized water vapor gradient during the wound healing process. Mechanistically, superficial wounds can be considered in two groups: Those in which the epidermis is penetrated or denuded, and those in which the epidermal barrier is damaged but not breached. In the former case, wound healing proceeds by epithelization with migration of cells from hair follicles,

sweat-glands, and from around the edges of the wound. In the latter case, repair is subtle and involves augmentation of epidermal DNA and lipid synthesis by mechanisms linked to the transepidermal water gradient. The ultimate result of both types of wound healing is the restoration of barrier integrity with the formation of an intact stratum corneum epidermis.

The medical and life science journals historically contain voluminous publications regarding wound healing and dressing information; not all of which can be presented here, but the acceptable views of the scientific community are represented.

Research in the following sections focuses on self-adherent dressings that follow the general guidelines learned from the literature and experiences. Barrier dressings selected from a review of commercial and invented dressings were challenged by application to excised rats, followed by inoculation with infectious bacteria to determine the efficacy of the dressings against infection from the environment while evaluating the rate of wound healing.

The role of the liquid applied barrier dressing is to provide a biocompatible protective "coating" over the tissue for the purpose of protecting it from bacteria and environmental contamination. From a physical and dynamic point of view, the barrier coating must include proper stress–strain physical properties for reasons shown in Fig. 2.2. The skin (epidermis and dermis) and subcutaneous soft tissue are not smooth and stretch and retract (stress–strain) as the body moves to lift an arm or leg, for example. The barrier must experience the same stress–strain and flexing phenomena and remain adhered to the tissue, otherwise the barrier would disbond

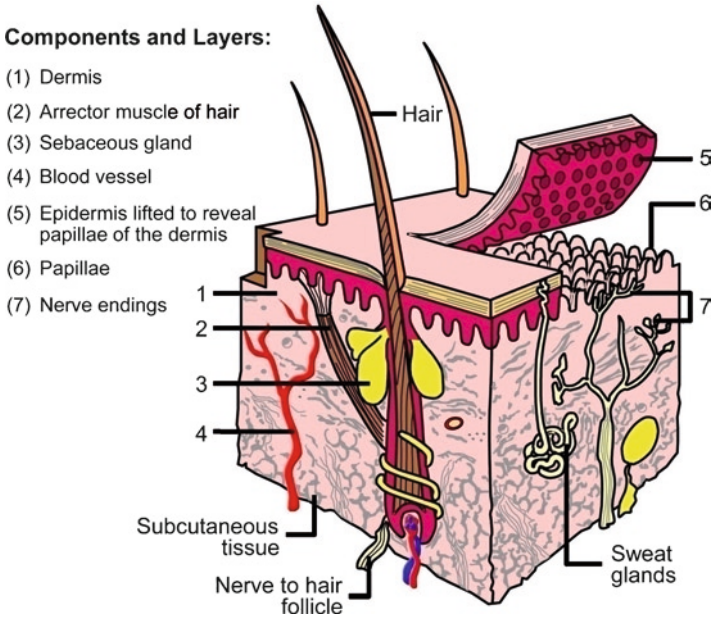


Fig. 2.2 The cross-sectional diagram of human skin tissue

and make the healing tissue vulnerable to attack from microbes and susceptible to dehydration. According to Lionelli (2003), the barrier should not adhere to the tissue as to impair healing. A detail figure of the epidermis in shown in Fig. 2.3, and it can be seen that no blood vessels exist in the epidermis and only secretion from vessels provide moisture and nutrition to the epidermis.

The wrinkled or corrugated texture of the skin is illustrated in Fig. 2.4. The skin elongates under lateral stress and relaxes when stress is removed. A liquid bandage applied to the skin must be able to adhere to the skin and possess a stress/strain property similar to skin in order to remain attached to the skin.

An illustration of the application of an emulsified liquid bandage is shown in Fig. 2.5. The microscopic view of this film formation shows microscopic spherical particles coalescing to form a continuous film. The advantage of the emulsion is that it is a waterborne and contains no solvents (i.e., organic solvents) which is preferred over organic solvents for the biocompatible property.

Table 2.1 lists and defines the terminology of mechanical stress–strain testing. Table 2.2 shows the values for procine skin. The typical stress–strain relationship for human skin is shown in Fig. 2.6 and the E for modulus value is shown as the slope on the linear segment of the curve.

Multiple stress–strain tests on the same sample of human skin is shown in Fig. 2.7. The stress necessary to stretch (stain) the same skin is less with each successive pulling or stressing of the skin sample. This phenomenon is typical of the skin to decrease in strength or modulus with continued stretching – it is the physical nature of skin to fatigue with repeated stretching or pulling.

Stress relaxation phenomenon is illustrated in Fig. 2.8 where it can be observed that a greater stress (pull or stretch) requires a greater period of time to relax or release the stress when both ends of a sample are restrained.

A working knowledge of the physical properties of skin is essential because a liquid dressing (bandage) applied directly to skin will be attached to the skin

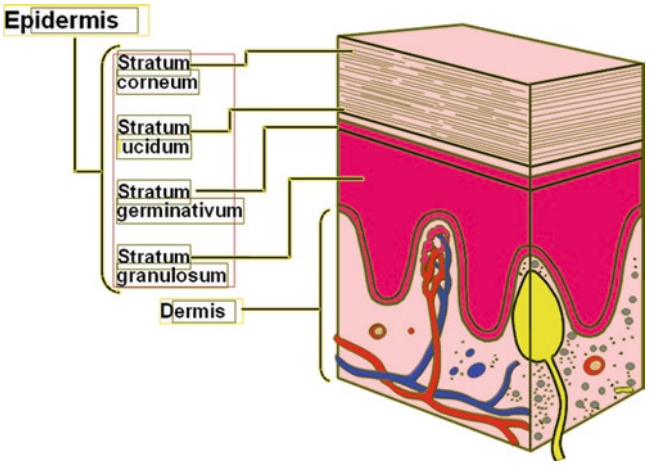


Fig. 2.3 The cross-sectional view of human skin epidermis

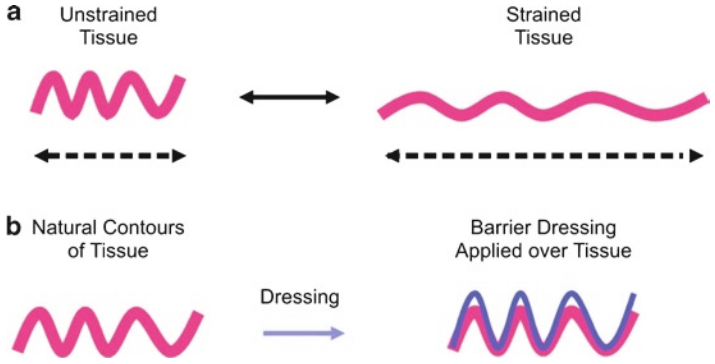


Fig. 2.4 The cross-sectional view of the elongation of the tissue (**a**) by applied lateral stress to demonstrate the change of shape in natural tissue, and the cross-sectional view of skin and soft tissue is shown in (**b**) with a barrier dressing that is liquid applied over the tissue. The barrier dressing must conform to the surface contours of the tissue

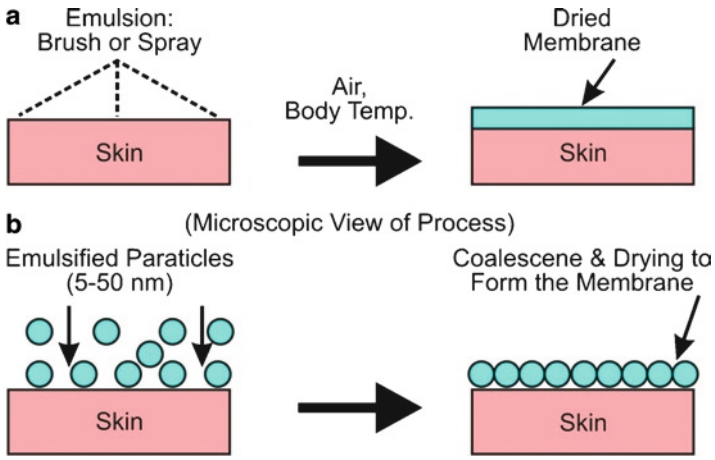


Fig. 2.5 Application of emulsion to skin and formation of protective membrane

Table 2.1 Stored/loss modulus and tan θ

Term	Symbol	Definition
Stored modulus	E′	The elastic component of a material, e.g., collagen fibers
Elastic modulus	E′	The viscous component of a material, e.g., tissue fluids
tan θ	E″/E′	The index of viscoelasticity

Table 2.2 Moduli and tan θ of porcine skin

Modulus	37°C	25°C
E′	6.583×10^7	9.409×10^7
E″	1.296×10^7	$1.801E^7$
tan θ	0.197	0.191

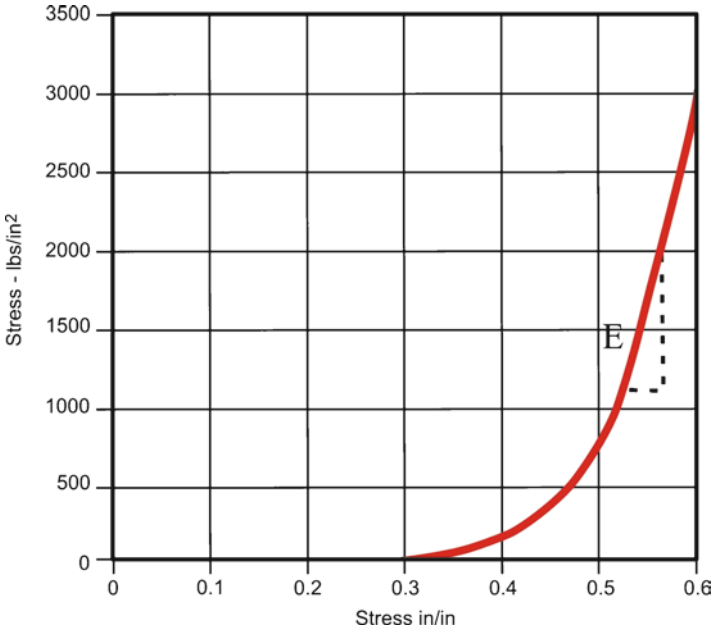


Fig. 2.6 Stress versus strain for human skin (McCarthy 1990). Reprinted with permission of Elsevier Health Science

surface and must move physically with the skin or the dressing will separate from the surface and cease to provide protection from infection.

2.1.1 Disinfection Fundamentals

Infection means that microorganisms have entered a wound, in this case, and caused damage by destruction of healthy cells in tissue. Disinfection means reducing the number of pathogenic organisms on objects or in materials so that they pose no threat of disease or harm to humans or animals. The growth and death rates of microorganisms are both logarithmic relationships. The effectiveness of antimicrobial agents on the death rate of microorganisms is important. Microorganisms treated with antimicrobial agents obey the same laws regarding death rates as those dying from natural causes (Black 2005). The principles of antimicrobial materials and their mechanisms are explained below for the purpose of understanding infection sources and control

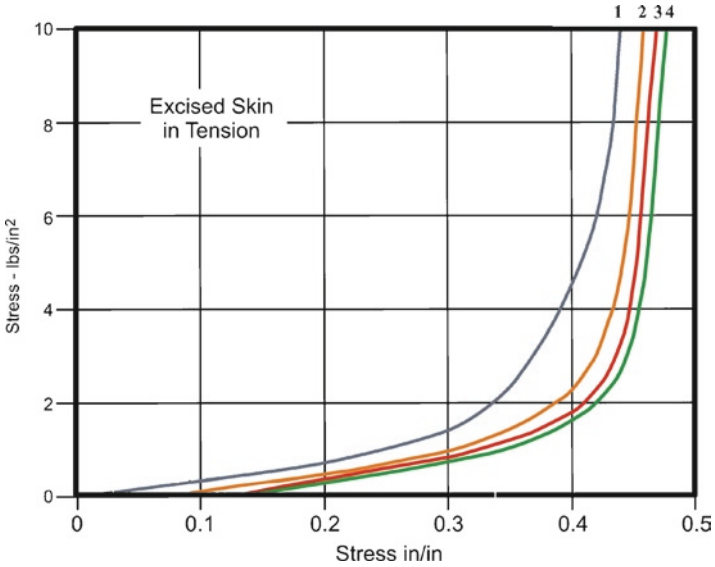


Fig. 2.7 Stress–strain successive plots of human excised skin (1, 2, 3, and 4, *l left to right*). (McCarthy 1990). Reprinted with permission of Elsevier Health Science

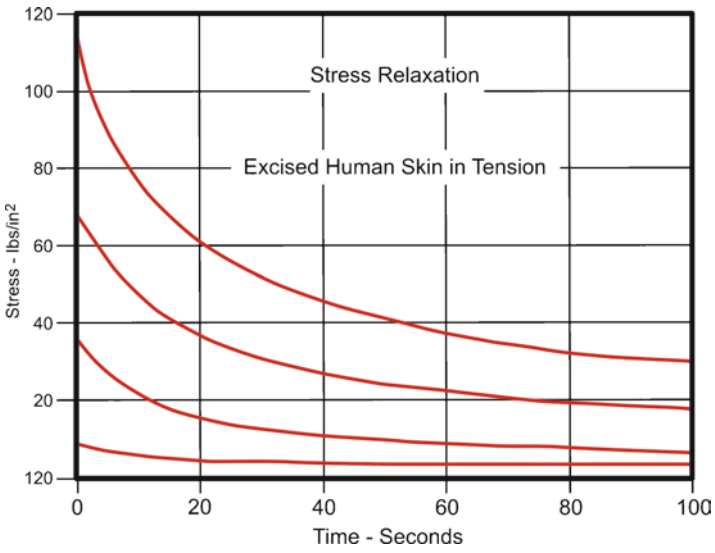


Fig. 2.8 Stress relaxation plot of human excised skin in tension, greater tension requires greater time to stress-relax, (McCarthy 1990). Reprinted with permission of Elsevier Health Science

(see Appendix – Antimicrobial Activity and Resistance), but the successful wound dressing will provide a physical barrier to microorganisms and will prevent infection from occurring. Antimicrobial terminology is explained in Table 2.3.

Table 2.3 Terminology of disinfection

Term	Definition
Antiseptic	A chemical agent that can be used topologically on tissue to destroy or control the growth of microorganisms
Disinfectant	A chemical agent used on objects as clothing to destroy or control the growth of microorganisms
Sterilization	The destruction of all microorganisms in or on a material (as bandages) or liquid (such as irritant fluids)
Germs:	All microorganisms: Bacteria, fungi, protozoa, spores, and viruses
Germi – *static*	An agent that inhibits the growth of a microorganism (e.g., bacteristatic)
Germi – *cide*	An agent that kills microorganisms (e.g., bactericide)

2.1.2 Antimicrobial Agents and Disinfection

The effectiveness of an antimicrobial agent is affected by concentration, pH, temperature, and time. Phenol (carboxylic acid) has been the standard disinfectant to which others are compared and evaluated under the same conditions. The result of this comparison is the Phenol Coefficient (Black 2005). Two organisms, *Salmonella typhi*, a pathogen of the digestive system, and *Staphylococcus aureus*, a common wound pathogen, are typically utilized to provide *phenol coefficients*. A disinfectant with a phenol coefficient of 1.0 has the same effectiveness as phenol, <1 is less effectiveness than phenol and >1 is more effective than phenol. Phenol coefficients are reported separately for each organism because the effectiveness of an agent is usually different for each microorganism.

2.1.3 Mechanisms and Kinetics of Disinfection

Chemical agents destroy or inhibit the growth (approaching zero) of microorganisms by participating in a single or multiple reactions that damage cell components. The bactericide is more effective than the bacteristatic agent because the kill rate of bacteria is greater than the growth rate. Microorganisms are composed of membranes (prokaryotic and eucaryotic) and protein (virus capids). The types of reactions fall into major groups: proteins and membranes.

2.1.3.1 Proteins

Much of the cell is composed of protein, and its enzymes are protein. Alteration of the protein is referred to as *denaturation*. In denaturation, hydrogen and disulfide

bonds are disrupted, and the functional shape of the protein molecule is destroyed. An agent that denatures proteins also prevents them from performing their natural functions. Proteins are temporarily denatured when treated with heat, mild acids, or bases, and sometimes their original structures returns to normal. Most proteins are denatured permanently when agents are used in strong enough concentration or degree of temperature to denatured them permanently. Naturation is "-static" is the normal protein structure returns and "-cidal" if the denaturation is permanent. Reactions that denature proteins include hydrolysis, oxidation, and the attachment of atoms or chemical groups.

2.1.3.2 Membranes

Membranes contain proteins that are susceptible to all of the reactions involving the denaturation of proteins. Membranes also contain lipids and their function can be disrupted by agents which dissolve lipids such as organic solvents such as ethanol. Surfactants reduce surface energy and may emulsify lipids. Membranes are negatively charged and are affected by cationic agents such as quaternary ammonium salts and biguanide compounds including chlorhexidine. Penetration of the cell wall to the protoplasm for prokaryotic and nucleus for eukaryotic cell is usual and results in the leakage of the components from within the cell.

2.1.3.3 Other Reactions

UV and ionizing radiation affect DNA and RNA. Strong oxidizing agents that penetrate the cell wall also alter DNA and RNA. Ultrasonic energy vibrates the cells until they disintegrate.

2.2 Solvent-Based Polymer Barrier Dressings

2.2.1 Preparation

Polymers were purchased from stock (Aldrich Chemicals Co. and DuPont Corporation). These materials were dissolved in ethanol and other solvents, but ethanol was a preferable medium because it is not toxic and often used in clinical environments. The identification of the polymers follows.

- Polyvinyl acetate, 500 kg/m (Aldrich no. 38,793-2)
- Polybutryal [poly(vinyl butyral-*co*-vinyl alcohol-vinyl acetate)] (Aldrich no. 19,097-7)
- Poly(vinyl alcohol-*co*-vinyl acetate-*co*-itaconic acid), no. 48,022-3.
- Polyvinyl alcohol (DuPont Evanol^{R} products, no. 90-50)

Table 2.4 Formulation of solvent-based barrier dressings

Component	% Weight
Mix of polymer and plasticizer	
Polymer: polyvinyl acetate or polybutyral	13.07
Diethylene glycol benzoate (10.1% based 12 on polymer weight)	1.32
Ethanol	85.45
Mix of chlorhexidine	
Ethanol (1.2% based on polymer weight)	0.16

The following Table 2.4 lists the components of the basic formulation that were successful in dissolving polyvinyl acetate and polybutyral in ethanol with an adjusted viscosity for proper application to tissue.

The polyvinyl acetate and polybutyral polymers were useful for forming films, but the other materials were not. Polyvinyl alcohol is only soluble in water and not capable of being blended with other polymers. Also, any polymer with a large percentage of polyvinyl alcohol will absorb too much water and become permeable to bacteria.

2.2.2 Formulation of Solvent-Based Dressings

Material properties and solubilities are contained in Appendix D. The formulations from Table 2.4 were bottled in a 2-ounce Boston Round Bottle with 18 mm Flip Cap purchased from the United States Plastic Corporation. These containers were convenient for flow-on type application directly on the excised rat tissue.

2.2.3 Results of Testing Barrier Dressings on Rats

The barrier dressing in Sect. 2.2.1 was tested by excising the dorsal skin of Sprague–Dawley rats and applying the liquid dressing over the excised tissue. A measured aliquot part of prepared *Staphylococcus aureus* bacteria suspension was applied over the barrier dressings, the exposed excised tissue, and chlorhexidine (5%)-treated excised tissue. Multiple rats were used for the dressing and control cases, and the results were reported statistically in terms of Fisher's Exact Test of the Chi-Square Test, two-tail *P* value with Bonferni's correction (see Appendix – Chi-Square Test). The cases were as follows:

- Polyvinyl acetate-vinyl alcohol copolymer dressing
- Polyvinyl acetate-vinyl alcohol copolymer dressing with 1.2 chlorhexidine preservative
- Polyvinyl butyral terpolymer dressing with 1.2% chlorhexidine. Preservative (based on polymer weight)

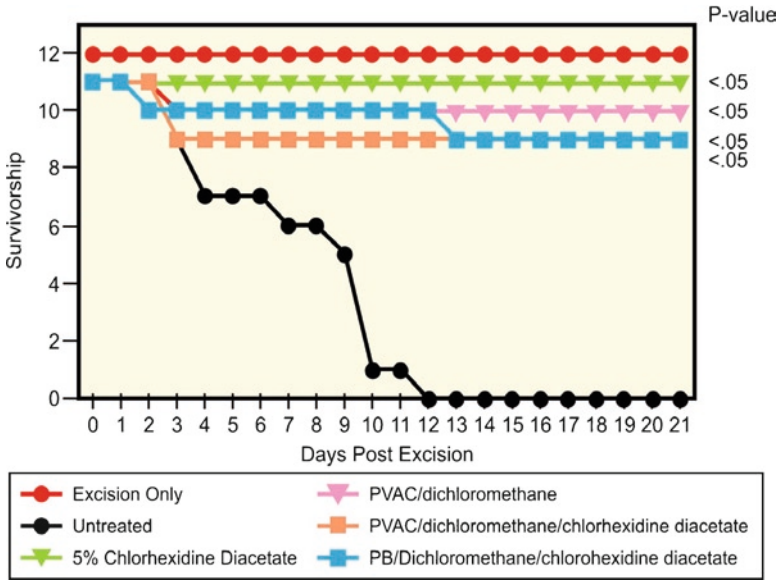


Fig. 2.9 Results of testing barrier dressings on rats

- 5% chlorhexidine treatment
- Neither treatment and nor dressing, only exposed excised tissue

The *P*-value of <0.05 (out of 1.0) is the boundary probability that rats will experience mortality (see Appendix for clarification of *P* value). From the inspection of Fig. 2.9 all of the dressings, nontreated-unexposed, and chlorhexidine-treated rats possessed a *p*-value of <0.05 which is acceptable by this model. All rats that were not exposed to the bacteria and were neither treated nor barrier dressed experienced unacceptable *P*-values.

2.3 Polyacrylate Miniemulsions and Nonaqueous Suspensions

2.3.1 Miniemulsions

Water-based barrier dressings are attractive for application to injured tissue because of the biocompatibility between water and tissue. The concept of a water-based dressing initially consisted of latex-type particles of polymer suspended in an aqueous emulsion. The emulsion would be liquid applied to the tissue, water would evaporate and the particles would coalesce to form a continuous film. The rate of evaporation of water is slow compared to solvents as ethanol that was recognized to be a limitation to application time (time to place on the tissue and harden). The following description of miniemulsions (miniEP) involves a “batch” type

emulsification of acrylic monomers with additives compared to a continuous process of "emulsion polymerization" (EP) that is different as a process and reaction kinetics. The reason for the miniemulsion approach is that all materials including additives (including water insoluble-antibacterial agents) can be formed in a single formulation and reaction compared to emulsion polymerization that requires all materials be water-soluble or dispersible. For example, a miniemulsion can include water-insoluble fatty acids and oils whereas an emulsion-polymerized polymer cannot.

The mechanisms of conventional emulsion and miniemulsion polymerization appear to be similar, but in some ways they are significantly different. A conventional unseeded (i.e., no small particles added at the beginning) batch emulsion polymerization reaction can be divided into three intervals. Particle nucleation occurs during Interval I and is usually completed at low monomer conversion (2–10%) when most of the monomer is located in relatively large (1–10 μm) droplets. Particle nucleation is believed to take place when radicals formed in the aqueous phase grow via propagation and then enter into micelles or become large enough in the continuous phase to precipitate and form primary particles which may undergo limited flocculation until a stable particle population is obtained. Significant nucleation of particles from monomer droplets is discounted because of the small total surface area of the large droplets. Interval II involves polymerization within the monomer-swollen polymer particles with monomer supplied by diffusion from the droplets. Interval III begins when the droplets disappear–or at least reach a polymer fraction similar to that of the particles–and continues to the end of the reaction. Because nucleation of particles can be irreproducible, commercial emulsion polymerizations are often "seeded" with polymer particles of known size and concentration, manufactured specifically for use as seed particles. In this proposal, for the purpose of clearly distinguishing between convention emulsions and miniemulsions, the term *macroemulsion* will be used for the former. In addition, a "latex" will be defined as a polymerized monomeric emulsion, while the term *emulsion* will refer to an unpolymerized monomeric emulsion.

Miniemulsion polymerization involves the use of an effective surfactant/costabilizer system to produce very small (0.01–0.5 micron) monomer droplets. The droplet surface area in these systems is very large, and most of the surfactant is adsorbed at the droplet surfaces. Particle nucleation is primarily via radical (primary or oligomeric) entry into monomer droplets, since little surfactant is present in the form of micelles, or as free surfactant available to stabilize particles formed in the continuous phase. The reaction then proceeds by polymerization of the monomer in these small droplets; hence there may be no true Interval II.

The size of the monomer droplets plays the key role in determining the locus of particle nucleation in emulsion and miniemulsion polymerizations. The competitive position of monomer droplets for capture of free radicals during miniemulsion polymerization is enhanced by both the increase in total droplet surface area and the decrease in the available surfactant for micelle formation or stabilization of precursors in homogeneous nucleation.

When an oil-in-water emulsion is created by the application of shear force to a sizes. In order to create an emulsion of very small droplets, the droplets must be

stabilized against coalescence and diffusional instability (Ostwald ripening). Stabilization against coalescence is effected by adding an appropriate surfactant. If the small droplets are not stabilized against diffusional degradation, they will disappear (Ostwald 1901), increasing the average droplet size, and reducing the total interfacial area. Jansson (1983) has shown that this disappearance can be very fast for small droplets. In creating a miniemulsion, diffusional stabilization is achieved by adding a small quantity (1–2% wt/wt based on monomer) of a highly monomer-soluble, water-insoluble stabilizing agent. Both long chain alkane such as hexadecane (HD) and long chain alcohols such as cetyl alcohol have been used as stability agents in miniemulsions (Barnette and Schork 1987; Chamberlain et al. 1982; Choi et al. 1985). Polymer, chain transfer agent and comonomers have been used successfully as well (Reimers and Schork 1996a, b). These stabilizing agents have been referred to as cosurfactants, although they may not actually play a surfactant role, and as hydrophobes. The preferred term, *costabilizer*, will be used here.

Research (Fontenot and Schork 1993a, b) indicates that miniemulsion polymerization can provide benefits over the current process technology of conventional emulsion polymerization. Among these are a process which is much more robust to contamination and operating errors, a more uniform copolymer composition when used for copolymerization, and a final product which is far more shear-stable than the product of conventional emulsion polymerization.

Gooch (1997, 2002) emulsified fatty acids and oils and autoxidized them under greater than atmospheric pressure of oxygen or air to produce a network polymer or "gel" that did not flow after applying the emulsion to a surface and allowing the aqueous phase to evaporate. By this method, the viscosity and crosslink density was adjusted to control rheological properties, including viscosity and flow-out.

2.3.1.1 Preparation

The preparation of miniemulsions for barrier dressings consisted of the follow considerations as disclosed in Georgia Tech Research Corporation International Application No. PCT/US03/06409, and listed below.

- Composition: Acrylic hydrophilic network polymer, biocompatible
- Form: Miniemulsion (miniEP), aqueous dispersion
- Application: Brush, roller or Spray
- Dried barrier dressing: 10–25 μm Semipermeable hydrophilic
- Antimicrobial: Powder or liquid added to miniEP

Theoretically, the films are applied to skin, they remain in excess of 5 days (naturally slough off with dead skin cells), and they are washable due to the interaction between the hydrophilic polymer and moist skin. A scheme is shown in Fig. 2.10 to demonstrate the application process of the emulsion to the injured skin or tissue (a), and the microscopic view of film formation on the skin is shown in (b). The object of the coalescence of particles is to form a continuous film to protect the tissue from environmental contamination and abrasion, but allow the

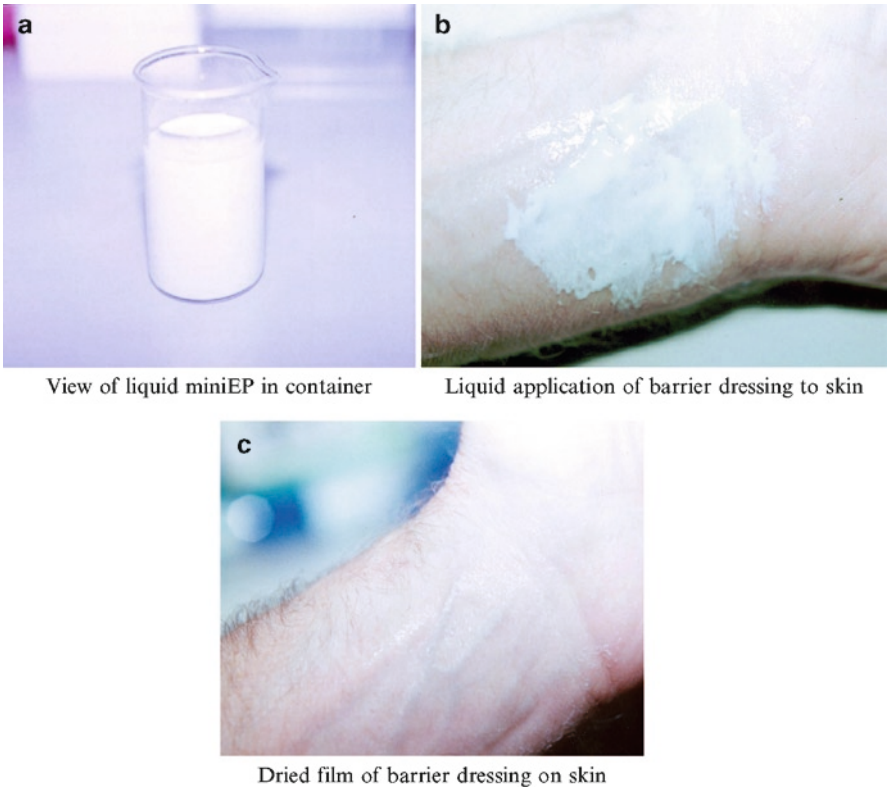


Fig. 2.10 View of (**a**) actual liquid miniEP, (**b**) liquid applied miniEP as a barrier dressing to skin, and (**c**) clear dried film on skin

skin to be permeable to water vapor and air. The composition and procedure is listed in Tables 2.4.

Optional II: Same basic composition and mass as above, but adjusted for the following postaddition of antimicrobial agents (e.g., chlorhexidine, etc.).

Antimicrobial agents 0.5–10.0 of total solids
Emulsification and polymerization procedure:
Prepare monomer component at STP conditions
Prepare mixture of monomers, initiators, hydrophobe/plasticizer mix thoroughly with mechanical mixer
Continue to keep dispersed (25°C) until used
Prepare aqueous component at STP conditions
Mix polyvinyl alcohol polymers in water and completely dissolve while mixing with mechanical stirrer and adjust temperature to dissolve all polyvinyl alcohol (PVA) until the solution is clear, then reduce to temperature to 20–25°C
Mix surfactants, in (a) while agitating with mechanical mixer at 20–25°C, mix until solution is clear and avoid entrained air

(continued)

(continued)

Feed 1 into 2 while sonicating or using dispersion equipment capable of producing 25–100 nm particles
Prepare emulsion for synthesis reaction: After 1 and 2 are completely emulsified, place mixture in kettle
Purge kettle with nitrogen for 30 min and reduce N_2 flow
Stir contents of kettle and initiate heat to reach 85–87°C for 30–45 min
Maintain temperature 75–77°C and notice exotherm, maintain cooling/heating control while continuing to agitate; too high temperature will cause excessive viscosity >2,000 cps
Maintain temperature (about 4 h) until 99% conversion is obtained and determined by oven percent nonvolatile tests; aliquot samples from the kettle are placed in convection oven at 105°C to determine volatile components.
After monomer conversion is complete, raise temperature to 85°C for 2 h.
Adjust pH sodium bicarbonate and ammonium hydroxide

Table 2.5 Composition of hydrophilic miniEP emulsion for skin barrier

Component	Function	Weight percent
Option I: (Patent comment: following materials may be used or equivalent materials may be substituted)		
2-Ethyl hexylacrylate	Monomer	9.40
Dioctyl maleate	“	7.52
Vinyl acetate	“	30.97
Itaconic acid (methyene succinic acid; 2-methylene-butanedioic acid)	Monomer	0.09
Polyvinyl alcohol (low M_w, η)	Viscosity/wetting adjustment	1.54
Water	Aqueous phase	45.42
Nonionic surfactants: alkylaryl polyether alcohol	Surfactant/emulsifier	0.54
Nonlylphenoxypoly (ethyleneoxy) alcohol	“	1.28
t-Butyl hydroperoxide	Initiator	0.10
Postaddition initiator	Monomer scavenger	0.14
Potassium persulfate		0.23
Sodium bicarbonate	pH Adjustment/buffer	0.13
Ammonium hydroxide (28%)	pH Adjustment	0.25
Hydrooxymethane (sulfinic acid, sodium salt, sodium formaldehyde sulfoxylate)	Reducing agent	0.13
Low M_w hydropbobic fatty amides, aminoethylethanolamines	Defoamer (Optional)	0.80
Benzoate esters Oxydiethyl dibenzoate, Ethanol, 2,2-oxybis, -dibenzoate, Propanol, oxybis,-dibenzoate	Hydrophobe and plasticizer	1.46
Total		100.00

The emulsion formed from the formulation in Table 2.5 is shown in Fig. 2.10, emulsion in glass beaker “a”, liquid application to skin in Fig. 2.10b and dried transparent on skin in Fig. 2.10c.

The dried film was very "wearable" and comfortable even after showing and exercising. Due to the nature of film formation on moist tissue, the film contained a significant amount of water (>20%) because the copolymer is hydrophilic and slightly crosslinked to absorb water. The formation of a film on a moist surface by the water-based emulsion is not conducive to a perfectly continuous dense film as is demonstrated in Fig. 2.10. The particles can be seen coalescing with each other, but they do not form a perfectly coherent and, therefore, not a continuous film as viewed in (c).

A transparent and visibly uniform film formed within 5 min over injured rat tissue shown in Fig. 2.10.

2.3.1.2 Testing on Rats

The testing procedure involving colonized bacteria *Pseudomonas aureus* in the protocol was too challenging for the water-based dressing and resulted in unacceptable mortality of rats. In follow-up tests, FD&C dyes were observed to penetrate the thickness of the dressing to the tissue, which means that the barrier was permeable to bacteria. Chlorhexidine added to the aqueous and polymer phase of the emulsion produced acceptable results in Kirby-Bauer tests, but not inoculation tests with bacteria on excised rats.

The conclusions drawn from these experiments are that the barrier dressing must possess the following properties:

- Very continuous and consistent film
- Nonporous film
- Nonwater absorbing film or barrier when applied over tissue to thwart the penetration of bacteria

2.3.2 Nonaqueous Suspensions

Due to the lack of success with excised rats in Sect. 2.3.1, the water-based barrier dressings were abandoned for this protocol, and polymers prepared in ethanol for application to tissue were prepared.

Special properties of polyvinyl acetate are the very high hydrogen bonding and adherence to moist surfaces, and the self-plastization by absorption of less than 10% by weight of water. The absorption of water is sufficient to make the polyvinyl acetate flexible with natural body movement, pliable on moist tissue, permeable to water vapor and air, but not permeable to bacteria. Therefore, copolymers comprising polyvinyl acetate were synthesized for nonaqueous barrier dressings. Monomers were included to lightly crosslink the polyvinyl acetate.

Table 2.6 Composition of nonaqueous suspension barrier dressing

Component	Weight
Monomers	
Vinyl acetate	48.39%
Dioctyl maleate	0.22%
2-ethylhexyl acrylate	0.27%
Initiators	
AIBN	1.10%
Ethanol	8.00%
Solvent	
Ethanol (ethyl alcohol)	42.02%
Total	100.00

Note 1: Polymerize at 70°C to 100% conversion
Note 2: Average particle size was 300 nm
Note 3: Total solids after polymerization – 49.98% @ 120 cP viscosity

2.3.2.1 Preparation

The nonaqueous suspensions were prepared using the basic formulation in Table 2.6.

2.3.2.2 Barrier Dressing Formulation

The nonaqueous dressings successfully formed continuous and nonpermeable films on agar plates and porcine skin (removed from the animal). The laboratory DD&C dye test showed no penetration into the dressing, agar, or tissue.

This dressing material can be sprayed, brushed, or rolled onto a surface while the ethanol evaporates. The most successful for wet tissue is brush-on, and spray-on for just moist tissue.

The disadvantage of ethanol is that it is absorbed in the tissue and does not allow perfect "spreading" over the surface of the tissue before hardening, and repeated applications must be made. However, all dressings in this report experienced a similar spreading effect and produced "pin-holes" after the first application.

2.3.2.3 Testing on Rats

The barrier dressings formed over agar plates and porcine tissue were successful. No Kirby-Bauer tests have been performed on excised rats to date. The dressing forms a water-impermeable barrier, is impermeable of FD&C dyes, and materials with these properties are impermeable to dyes (see Figs. 2.11 and 2.12).

An important property of nonaqueous suspension is the increased percent solids (49.28% wt./wt.) compared to polymers solved in ethanol (e.g., polyvinyl acetate in ethanol, <27%) that means more active ingredient can be delivered the wound and less dressing must be carried.

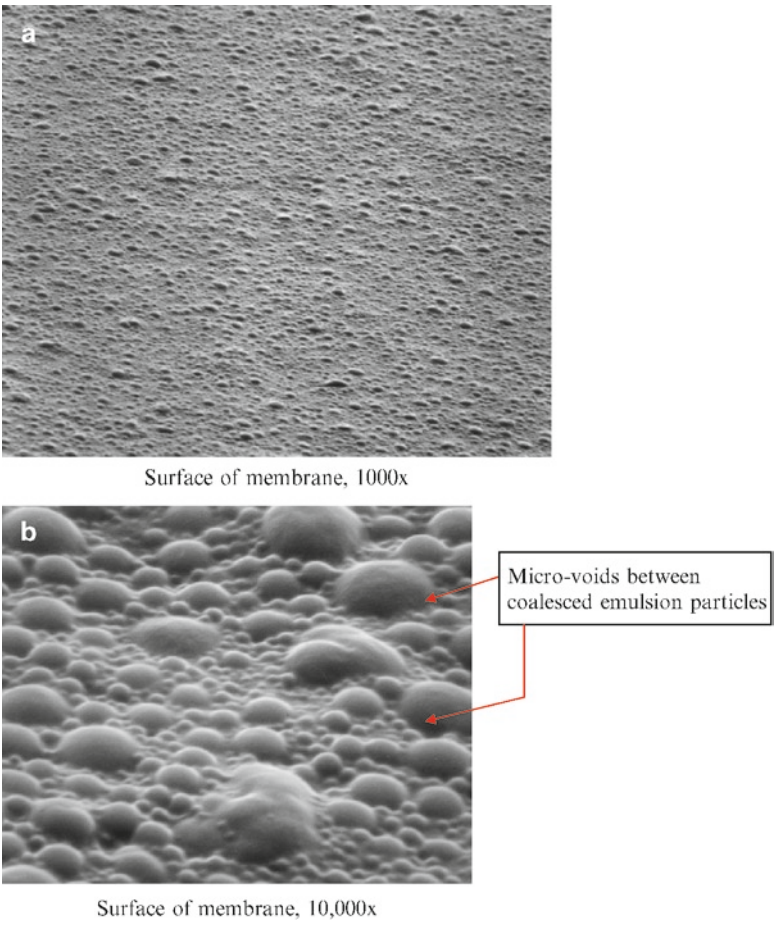


Fig. 2.11 Topological scanning electron micrographs of dried barrier dressing showing the spherical particle texture of the miniEP film surface

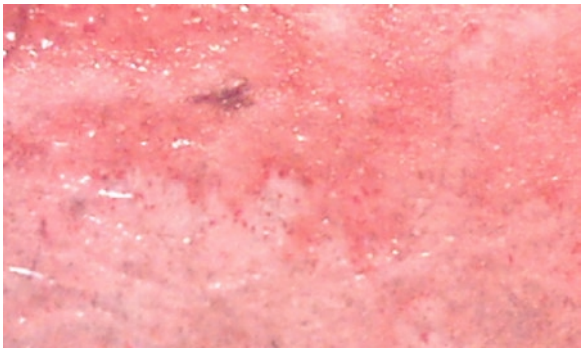
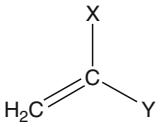


Fig. 2.12 Transparent skin barrier over excised rat skin

2.4 Cyanoacrylate

2.4.1 Technical Discussion

Alkyl cyanoacrylate adhesives (Coover et al. 1990) are unique among the many classes of adhesives, in that they are the only single component, instant bonding adhesives that cure at ambient conditions without an external energy source. These compounds were first synthesized in 1947 by Alan Ardis of B. F. Goodrich Company who reported "hard clear glasslike resins" when heat cured. Their adhesive properties remained undiscovered until the early 1950s, when scientists at Eastman Kodak Company inadvertently bonded the prisms of an Abbé refractometer together while characterizing a cyanoacrylate monomer. The first commercial result of cyanoacrylates was Eastman 910^{R}, a methyl ester-based adhesive which was introduced in 1958 and later "super glues" usually composed of 2-ethyl cyanoacrylate. These fast-setting materials are commonly known as cyanoacrylate alkyl esters, and they are hard and brittle by nature. The cure speed and adhesive strength tend to decrease with increasing alkyl chain length whereas the flexibility increases. The reactivity of cyanoacrylates is directly traceable to the presence of two strong electron-withdrawing groups (designated X and Y) shown below,



where *X* = –CN and *Y* = –COOR.

In the case of 2-octyl cyanoacrylate monomer, R = –C_8H_{17}, it is polymerized rapidly via an anionic mechanism. Anionic initiators include weak bases (e.g., H_2O) at ambient temperatures and water being the most important for barrier dressings. The reaction is highly exothermic and the actual mechanism of reaction is depicted in Fig. 2.13. This reaction will continue until all the available monomer is consumed or until growth is interrupted by the presence of an acidic species. It is the highly electronegative characteristics of the nitrile (–CN) and alkoxycarbonyl (–COOR) groups that account for the high reactivity of the double bond in the monomer such as weak bases (e.g., water) initiate rapid polymerization. In general, relatively low molecular weight chains are formed via this mechanism. Since virtually all materials have a thin layer of moisture adsorbed (tissue is very hygroscopic) onto their surfaces, it is easy to explain the reactivity of the alkyl-2-cyanoacrylate. When a film of cyanoacrylate adhesive is spread onto a surface for bonding purposes, polymerization occurs rapidly as carbanions are generated at a very rapid rate as a result of the contact between the liquid adhesive and adsorbed water molecules. The water molecules' hydroxyl groups effectively act to initiate polymerization. When excessive films of water reside on a surface, the liquid cyanoacrylate (adhesive) polymerizes over a film of water, and may float off without adhering to the surface, and for this reason, a surface (adherent) must be relatively dry.

Narang et al. (2003) reported that 2-octylcyanoacyrlate-based films are excellent microbial barriers. Dermabond Topical Skin Adhesive and Liquid Bandage^{R} are

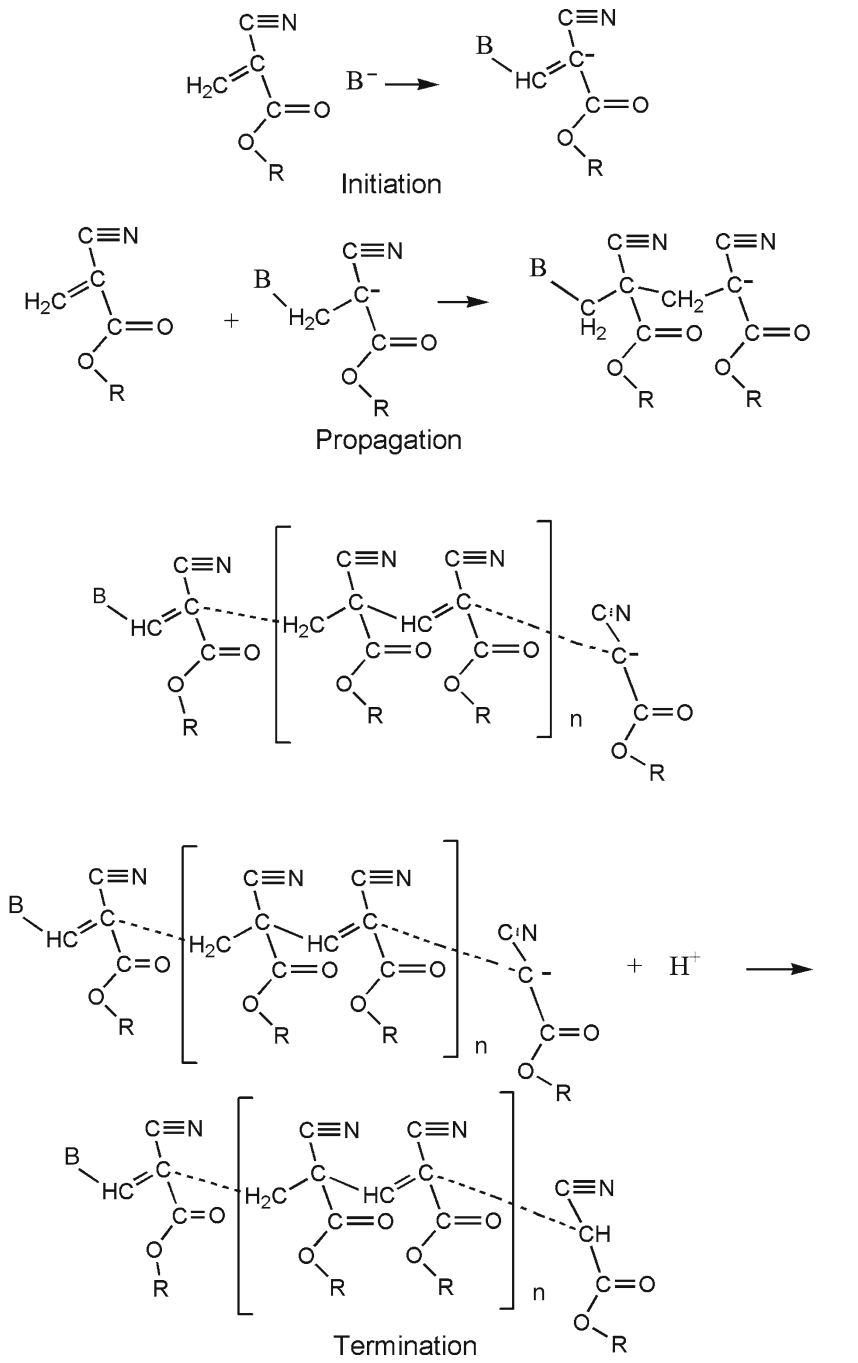


Fig. 2.13 The three basic steps in producing a polymer from the cyanoacrylate monomer via anionic polymerization (moisture curing)

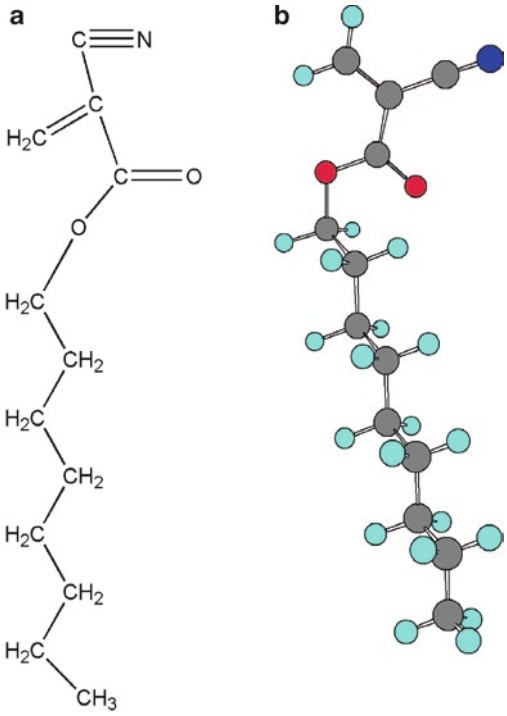


Fig. 2.14 2-Cyano-acrylic acid nonyl ester (also 2-octyl cyanoacrylate), *gray* is carbon, *blue* is hydrogen, *red* is oxygen, and *dark blue* is nitrogen

formulated products manufactured by Closure Medical Corporation for ETHICON, INC. consisting of 2-octyl cyanoacrylate shown in Fig. 2.14, preservatives and plasticizers. Liquid Bandage^{R} is formulated with a greater percentage plasticizer to provide greater flexibility in the cured film. Generally, the curing time for a cyanoacrylate adhesive decreased as the –R group explained above increases in the number of carbon atoms or chain length, and the flexibility increases proportionally. The cyanocrylate material used in the formulation of Dermabond contains an –R = 8 which is four times longer than the chain length for the original 2-ethyl cyanoacrylate (R = 2). The advantage of the R = 8 group (ASTM D-790) is increased flexibility with the sacrifice of cure rate. The longer –R group plasticizes the polymer and prevents the cyanoacrylate from becoming denser and more rigid. The three-dimensional model of 2-octyl cyanoacrylate shows clearly the long –octyl group extending from the vinyl group (site of polymerization) in correct atom-bond conformation.

The polymerized 2-cyano acrylate ($n = 8$) shown in Fig. 2.15a is a simple model to demonstrate a polymer of $n = 8$, and the correct atom-bond configuration of substituents and conformational molecular structure is shown in Fig. 2.15b in a planar or two-dimensional form, and the three-dimensional stick and ball models in Fig. 2.16a and b rotated about the *Y*-axis 90°.

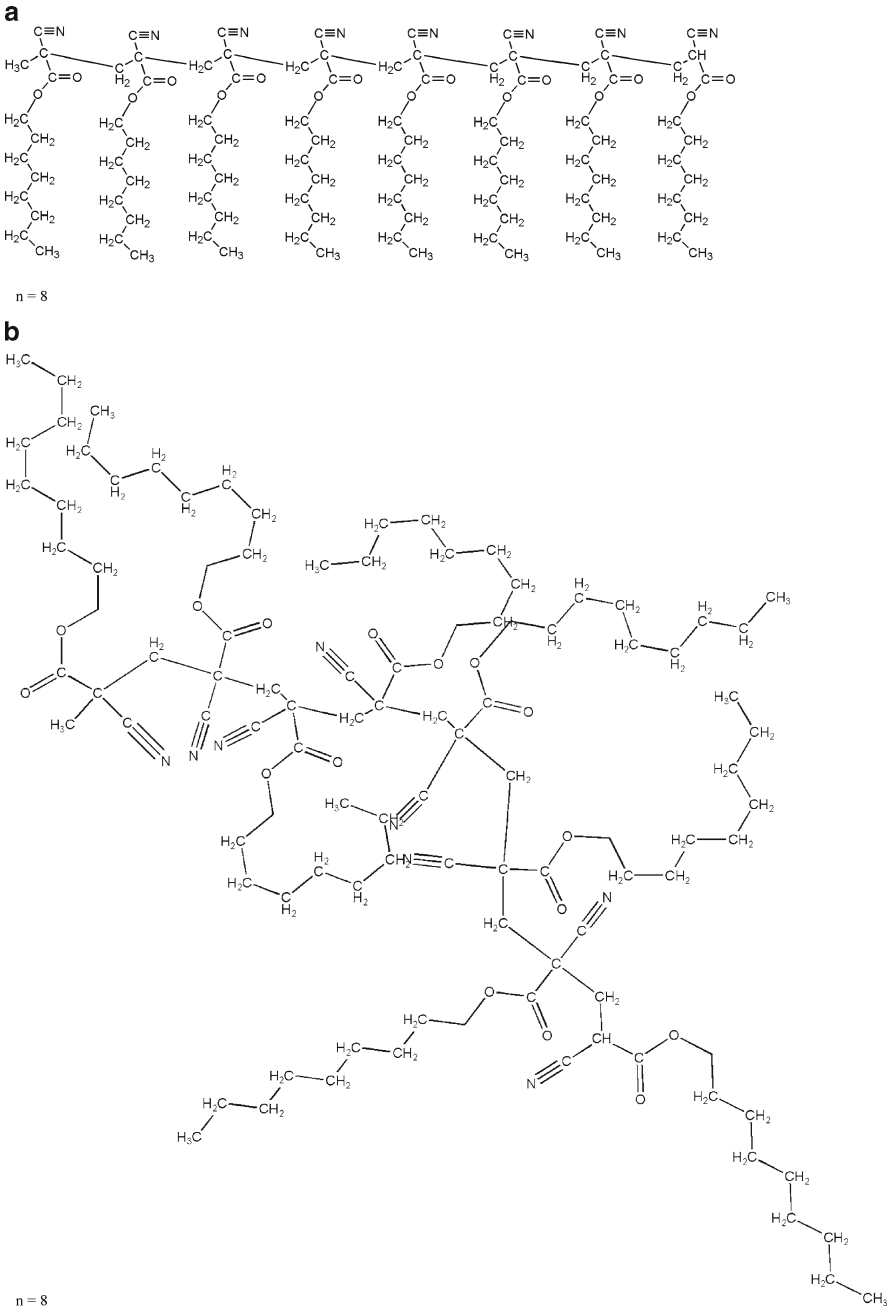


Fig. 2.15 Polymerized 2-octyl cyanoacrylate of eight-repeat units in (**a**) simple written chemical structure and (**b**) three-dimensional representation of bonds and atoms

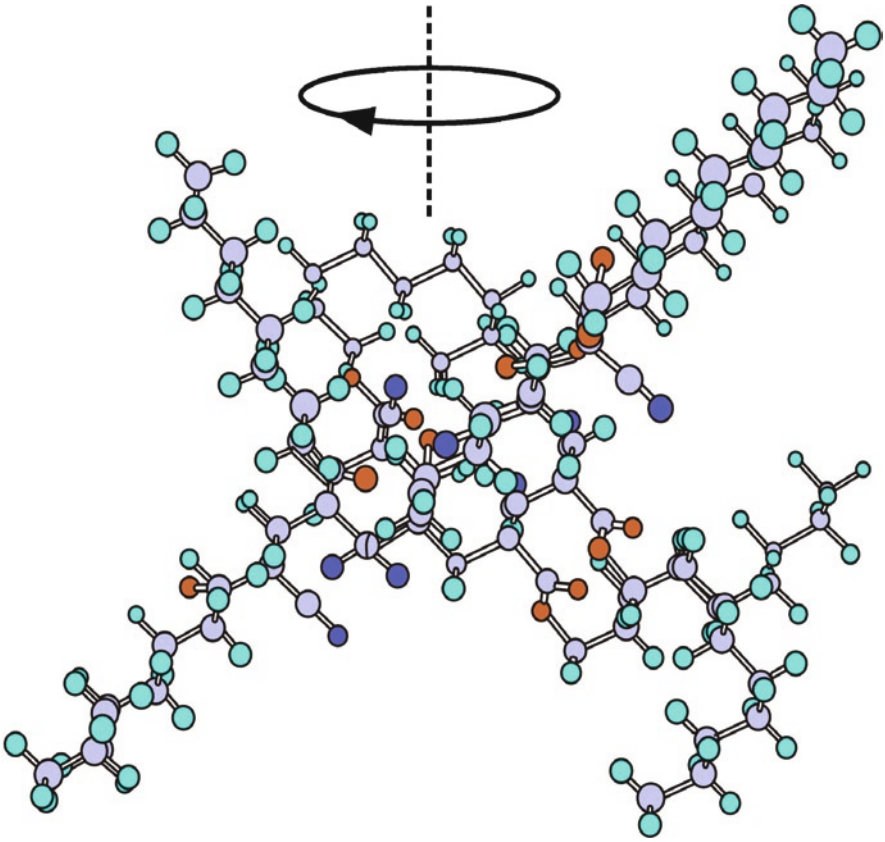


Fig. 2.16 Stick and ball three-dimensional molecular structure of 2-octyl cyanoacrylate ($n=8$) in 0° rotation about Z-axis

The long extensions of the –R groups are three-dimensionally arranged around the polymer back-bone chain, and the "floppy" chains prevent the formation of a lattice and therefore crystallization. Plasticization with materials ester-benzoates would further separate the molecules and provide more flexible hardened cement.

The monomer of the cyanoacrylate is slightly water-soluble and therefore water dispersible, but quickly polymerizes to a hard dispersed particle in water. Cyanoacrylates disperse easily in ethanol, but harden slowly. The water solubility property is important for allowing the cyanoacrylate monomer to penetrate tissue, which then polymerizes to a hard cement providing a strong bond, but an impenetrable film of cement. The natural flow of water through tissue has a proclivity to build pressure under a water vapor impenetrable material covering the tissue surface and push-off the material. Liquid Bandage and Dermabond are formulated from 2-octyl cyanoacrylate monomer and a preservative, but Liquid Bandage is formulated with significantly more plasticizer to provide correspondingly more flexibility for application to skin. Also, dilution with preservative chemicals retards the rate of polymerization. A water vapor penetrable material that is also a barrier

to bacteria would be preferable for a tissue covering or wound barrier. The author recommends the following literature ion this subject: Addad (1996), Maw et al (1997), Mertz et al (2001), Mouron et al (1996), Ottenbrite (1994), Sepe (1998), Sichina (1988), Segal (1985), Sorokin (1998), Surgical Applications of Tissue Sealants and Adhesives 6th Annual Conferences (2001–2004).

2.4.1.1 Results of Testing on Rats

The protocol was structured to test the barrier properties of the Liquid Bandage[R] product on rats when challenged with inoculations of bacteria, and the results successful.

2.5 Particulate Applied Barrier Dressing

2.5.1 Introduction

A low weight and cube and long shelf-life dry-particulate treatment for wounds received in the field would be an advantage for the soldier or caregiver if the treatment could be self-applied for this purpose. A sprinkle-particulate-type dressing was envisioned that would adhere to the tissue and absorb up to 130–180 times its volume in blood and fluid while forming a protective film barrier over the wound. Excessive fluid over the wound that typically hinders other bandages would be immediately absorbed into bandage to form a continuous hydrogel-type barrier dressing. Also, antimicrobial agents could be formulated as particulate into the dry dressing.

The class of hydrophilic and water-absorbent polymers are superabsorbent polymers (SAP). Super absorbent polymers are polymers that can absorb and retain extremely large amounts of a liquid relative to its own mass.

Water absorbing polymers, sometimes referred to as hydrogels, absorb aqueous solutions through hydrogen bonding with the water. An SAP's ability to absorb water is a factor of the ionic concentration of an aqueous solution. In deionized and distilled water, SAP may absorb 500 times its weight (from 30 to 60 times its own volume), but when put into a 0.9% saline solution, the absorbency drops to maybe 50 times its weight. The presence of valent cations in the solution will impede the polymer's ability to bond with the water molecule.

Superabsorbent polymers are now commonly made from the polymerization of acrylic acid blended with sodium hydroxide in the presence of an initiator to form a polyacrylic acid, sodium salt (sometimes referred to as cross-linked sodium polyacrylate). Some of the polymers include polyacrylamide copolymer, ethylene maleic anhydride copolymer, cross-linked carboxy-methyl-cellulose, polyvinyl alcohol copolymers, cross-linked polyethylene oxide, and starch grafted copolymer of polyacrylonitrile to name a few. The latter is one of the oldest SAP forms created.

Today, superabsorbent polymers are made using one of two primary methods; suspension polymerization or solution polymerizations. Both processes have their advantages over the other and both yield a consistent quality of product.

Solution-based polymerization is the most common process used today for SAP manufacture. This process is efficient and generally has a lower capital cost base. The solution process uses a water-based monomer solution to produce a mass of reactant polymerized gel. The polymerization's own reaction energy (exothermic) is used to drive much of the process, helping reduce manufacturing cost. The reactant polymer gel is then chopped, dried, and ground to its final granule size. Any treatment to enhance performance characteristics of the SAP is usually accomplished after the final granule size is created.

The suspension process is practiced by only a few companies because it offers a higher degree of production control and product engineering during polymerization step. This process suspends the water-based reactant in a hydrocarbon-based solvent. The net result is that the suspension polymerization creates the primary polymer particle in the reactor rather than mechanically in postreactions stages. Performance enhancements can also be during or just after the reaction stage.

The total absorbency and swelling capacity are controlled by the type and degree of cross-linking to the polymer. Low density cross-linked SAP generally has a higher absorbent capacity and swells to a larger degree. These types of SAPs also have a softer and more cohesive gel formation. High cross-link density polymers exhibit lower absorbent capacity and swell. The gel strength is firmer and can maintain particle shape even under modest pressure.

The largest use of SAP is found in personal disposable hygiene products, such as baby diapers, adult protective underwear and feminine napkins. SAP was discontinued from use in tampons due to the 1980s' concern over a link to toxic shock syndrome. They are also used for blocking water penetration in underground poser or communications cable, horticultural water retention agents, control of spill and waste aqueous fluid, artificial snow for motion picture and stage production. The first commercial use was in 1978 for use in feminine napkins in Japan and disposable bed liners for nursing home patients in the USA.

In the early 1960s, the United States Department of Agriculture (USDA) was conducting work on materials to improve water conservation in soils. They developed a resin based on the grafting of acrylonitrile polymer onto the backbone of starch molecules (i.e., starch-grafting). The hydrolyzed product of the hydrolysis of this starch-acrylonitrile copolymer gave water absorption greater than 400 times its weight. Also, the gel did not release liquid water the way that fiber-based absorbents do.

The SDA gave the technical expertise to several USA companies for further development of the basic technology. A wide range of grating combinations was attempted including work with acrylic acid, acrylamide, and polyvinyl alcohol (PVA).

2.5.2 Copolymer Chemistry

Polyacrylate/polyacrylamide copolymers are originally designed for use in conditions with high electrolyte/mineral content, and there is a need for long-term stability including numerous wet/dry cycles. Uses include agricultural and horticultural.

With the added strength of the acrylamide monomer, used as medical spill control, wire & cable waterblocking.

2.5.3 Solution Polymers

Solution polymers offer the absorbency of a granular polymer supplied in solution form. Solutions can be diluted with water prior to application, and can coat most substrates or can be used to saturate. After drying at a specific temperature of a specific time, the result is a coated substrate with superabsorbent functionality. For example, this chemistry can be applied directly onto wires & cables, though it is especially optimized for use on components such as rolled goods or sheeted substrates.

2.5.4 Super Absorbent Fibers

Besides granular super absorbent polymers, ARCO Chemical developed a super absorbent fiber technology in the early 1990s. This technology was eventually sold to Camelot Absorbents (Now bankrupt). Another fiber-spinning SAP technology was developed by Courtalds & Allied Colloids in the UK in the early 1990s. These acrylic-based products, while significantly more expensive than the granular polymers, offer technical advantages in certain niche markets, including cable wrap, medical devices, and food packaging.

2.5.5 Uses

- Wire & Cable Waterblocking
- Filtration Applications
- Spill Control
- Hot & Cold Therapy Packs
- Composites & Laminate
- Medical Waste Solidification
- Mortuary Pads
- Motionless Waterbeds
- Candles
- Diapers and Incontinence Garments
- Waste Stabilization & environmental Remediation
- Fragrance Carrier
- Wound Dressings
- Fire Protection

- Surgical pads
- Water Retention for Supplying Water to Plants
- Controlled Release of Insecticides & Herbicides
- Grow-In-Water Toys
- Magic Tricks Such as the 3 Cup Water Monte

Sodium polyacrylate also named acrylic sodium salt polymer or simply ASAP (repeating unit: $–CH_2–CH(COONa)–$) is a polymer widely used in consumer products. Acrylate polymers generally are considered to possess an anionic charge. While sodium neutralized acrylates are the most common form used in industry, there are also other salts available including potassium, lithium, and ammonium.

2.5.6 Applications

Acrylates and acrylic chemistry have a wide variety of industrial uses that include:

1. Sequestering agents in detergents. (By binding hard water elements such as Ca and Mg, the surfactants in detergents work more efficiently.)
2. Thickening agents.
3. Coatings.
4. Super absorbent polymers. These cross-linked acrylic polymers are used in baby diapers. Copolymer versions are used in agriculture and other specialty absorbent applications.

These cross-linked acrylic polymers are referred to as “Super Absorbents” and “Water Crystals”. The origins of super absorbent polymer chemistry trace back to the early 1960s when the US Department of Agriculture developed the first super absorbent polymer material. This chemical is featured in the Maximum Absorbency Garment used by NASA.

Sodium polyacrylate belongs to a family of water-loving or hydrophilic polymers. It has the ability to absorb up to 800 times its weight in distilled water. Sodium polyacrylate is a powder that takes the form of a coiled chain (see Fig. 2.18). There are two important groups that are found on the polymer chains, carbonyl (COOH) and sodium (Na). These two groups are important to the overall absorption potential of the polymer. When the polymer is in the presence of a liquid, the sodium dissociates from the carbonyl group creating two ions, carboxyl (COO^-) and sodium cation (Na^+). The carboxyl groups then begin to repel each other because they have the same negative charge. As a result of the repulsion between the like charges, the sodium polyacrylate chain uncoils or swells and forms a gel substance. The action of swelling allows more liquid to associate with the polymer chain. There are four major contributors to sodium polyacrylate’s ability to absorb liquids or swell. These contributors are hydrophilic chains, charge repulsion, osmosis, and cross-linked between chains. Ions in the polymer chain such as carboxyl groups (COO^-) and sodium (Na^+) attract water molecules, thus making the

polymer hydrophilic. Charge repulsion between carboxyl groups allow the polymer to uncoil and interact with more water molecules (See Fig. 2.17).

When these dry coiled molecules (see Fig. 2.18) are placed in water, hydrogen bonding with the HOH surrounding them causes them to unfold and extend their chains as shown in Fig. 2.19.

When the molecules straighten out, they increase the viscosity of the surrounding liquid. That is why several types of acrylates are used as thickeners.

Super absorbent chemistry requires two things: The addition of small cross-linking molecules between the polymer strands; and the partial neutralization of the carboxyl acid groups (–COOH) along the polymer backbone (–COO^-Na^+).

Water molecules are drawn into the network across a diffusion gradient which is formed by the sodium neutralization of the polymer backbone. The polymer chains

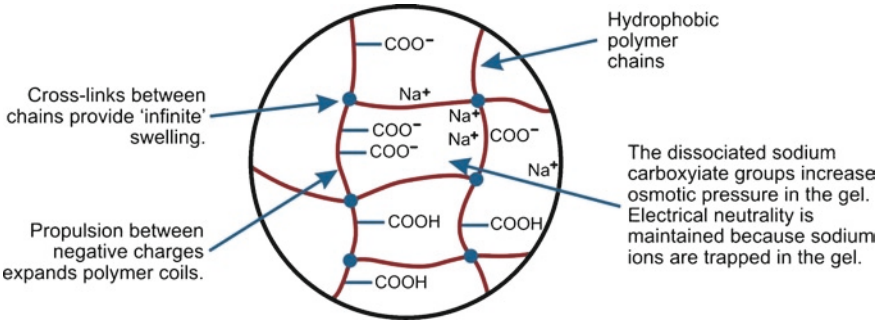


Fig. 2.17 Charge repulsion between carboxyl groups in cross-linked SAP

Fig. 2.18 Acrylate polymers – in a dry coiled state

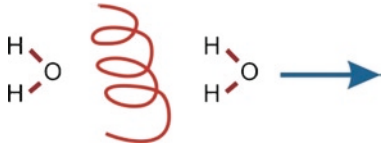


Fig. 2.19 Interaction of SAP with water

want to straighten but are constrained due to the cross-linking. Thus, the particles expand as water moves into the network (Fig. 2.20).

The water is tightly held in the network by hydrogen-bonding. Many soluble metals will also ion-exchange with the sodium along the polymer backbone and be bound.

A view of a crosslinks in super absorbent molecules is shown in Fig. 2.21 and the more practical polymer network in Fig. 2.22.

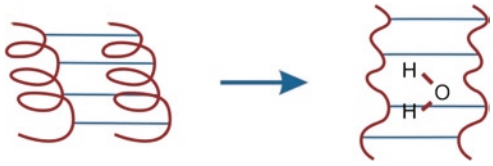


Fig. 2.20 Expansion between polymer chains with interaction with water

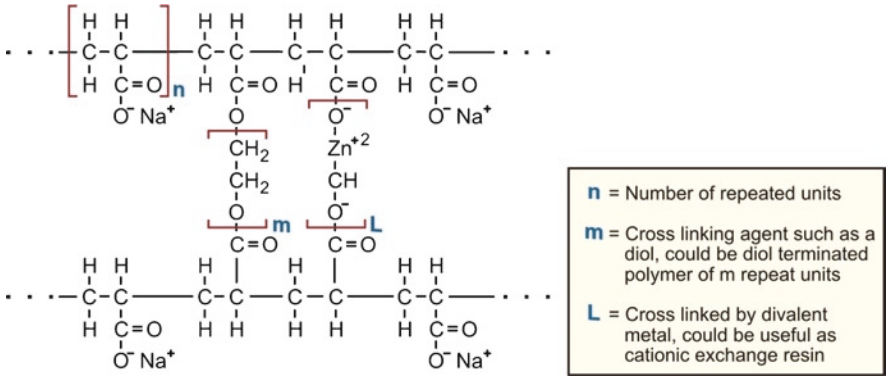


Fig. 2.21 Cross-linked polymer chains

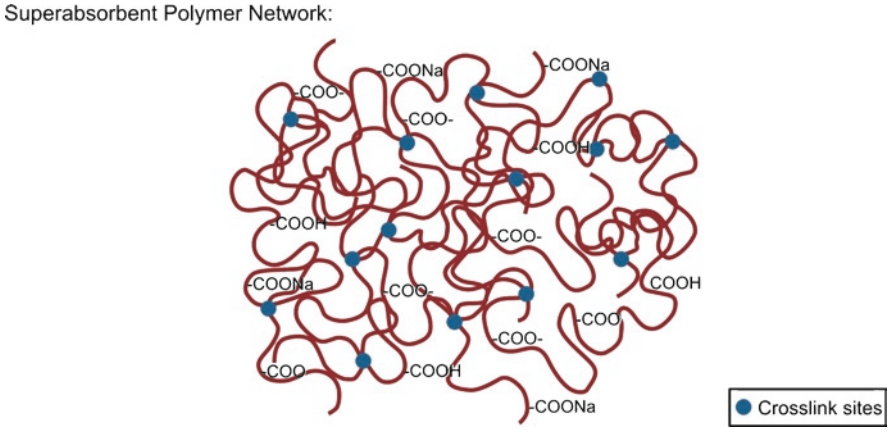


Fig. 2.22 View of superabsorbent polymer network with cross-linked sites (SAP)

2.5.7 Preparation

A super-hydrophilic absorbing polymer was synthesized from acrylic monomers and chemically crosslinked during the polymerization reaction to optimize barrier-dressing properties:

- Blood and water absorption
- Formation of a continuous barrier type film after absorbing fluids
- Protection of wounds from microbial attack
- Excellent adhesion to soft tissues
- Capable of pulverization to form fine particulate consistency for application
- Environmental stability and long shelf stability
- Low weight–volume

2.5.8 Reactive Mechanism

The reactive mechanism consists of three simultaneously occurring phenomena:

1. Absorbance of blood and infection from the wounded and hemorrhaging tissue,
2. Controlled solubility and ionic diffusion of the antimicrobial agent, such as, chlorhexidine, to the microorganisms (see Figs. 2.23 and 2.24), and
3. Free draining of the wound fluids and infections.

Tremendous advantages are accomplished by this method of treating tissue:

1. Environmental protection of wounded tissues,
2. Protection from microbial infection, and
3. Allows the body to heal itself.

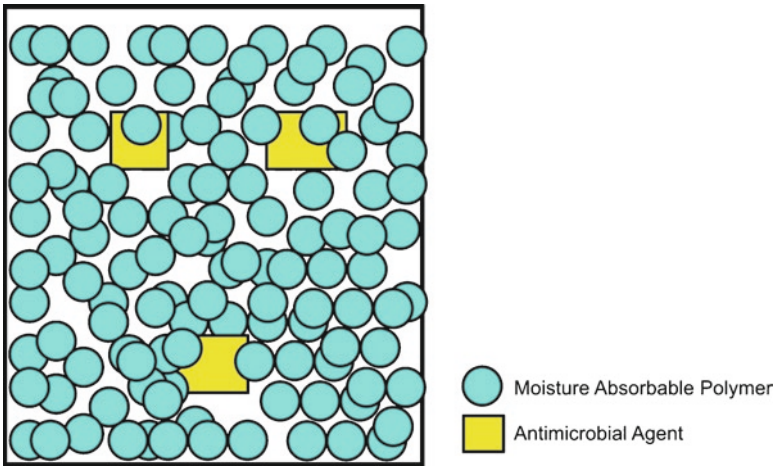


Fig. 2.23 Solid powder mixture of moisture absorbable polymer and antimicrobial agent

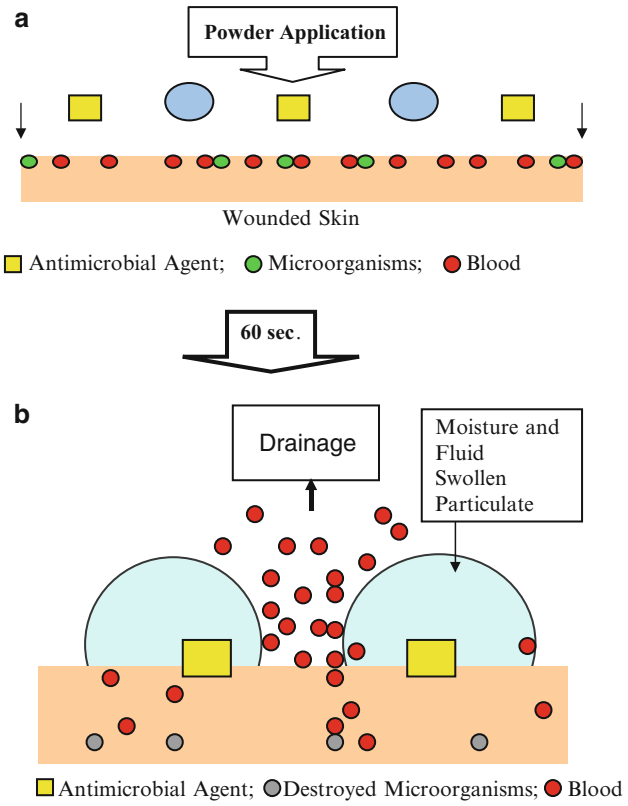


Fig. 2.24 Application of particulate applied absorbable skin barrier dressing

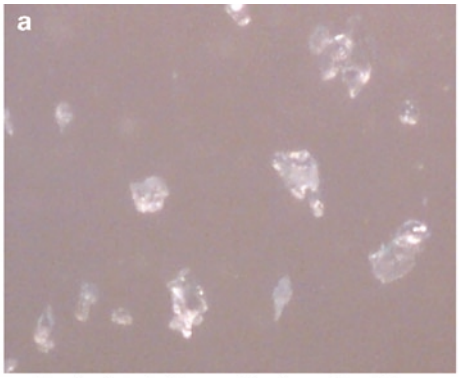
2.5.9 Dressing Preservative Agents

1. Chlorhexidine diacetate is partially soluble in water, and the solubility increases with temperature, which provides a particulate form for application, and
2. Chlorhexidine is most effective against microorganisms in the "solid" form compared to a solution preparation.

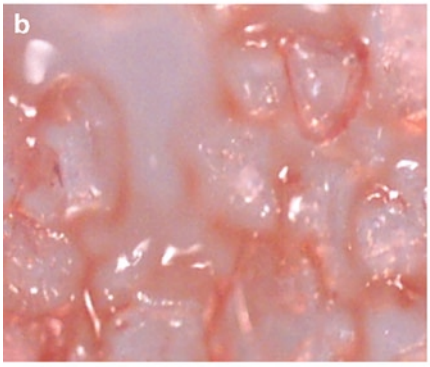
The images in Fig. 2.25 demonstrate the moisture/blood absorption of the absorbable polymer, and a particulate size (50–200 μm) was chosen to visibly observe and photograph the growth in size of the particulate. However, a 5–15 μm size would better coat the nonlinear surface of tissue.

2.5.10 Results of Testing Barrier Dressing on Excised Rats

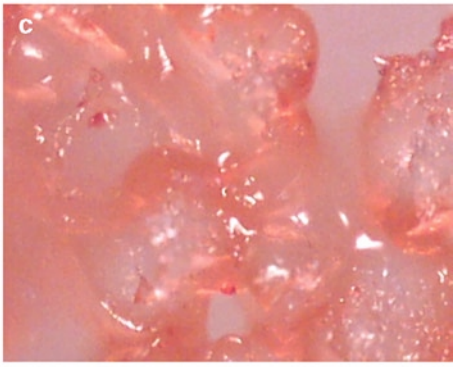
Rats were excised (dorsal skin) and a statistical test was initiated to determine the efficacy of the dressing. A contaminated, full thickness excision model was



Particulate before application, 60 x Mag.



60 Seconds after application, 10x mag



200 Sec. after application, 10x mag.

Fig. 2.25 Moisture absorbing particulate barrier dressing in porcine blood to demonstrate significant increase in particle volume

used where a 4×10 cm wound was treated with test dressings and challenged with approximately 10^7 CFU of *Pseudomonas aeruginosa*. Statistical comparison was made between experimental dressings and untreated controls. The dressing was sprinkled onto the excised rat tissue; it covered the entire excised area, absorbed fluids and bonded with the tissue to form a physical barrier as expected. The rats that were treated with the dressing and inoculated did not survive, and the reason was found to be that bacteria penetrated to the exposed tissue. The Spauge-Dawley rats in feeding bins are shown in Fig. 2.26. The exposed tissue after excision of a rat is shown in Fig. 2.27. The dry and wet individual particles of the barrier dressing are shown in Fig. 2.25. Excised rate skin is shown in Fig. 2.28 and in Fig. 2.29 having been sprinkled with the particulate dressing. The exposed rat tissue is shown in Fig. 2.30 having been covered with particulate dressing, some of the dressing is removed for inspection. A cross-sectional view from Fig. 2.30 is shown in Fig. 2.31, and a closer view is shown in Fig. 2.32.

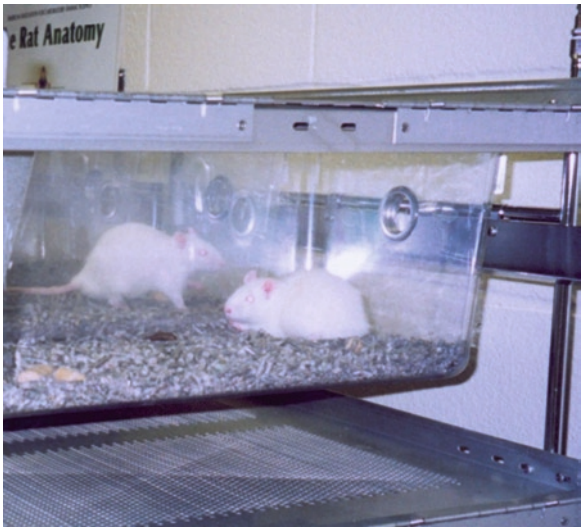


Fig. 2.26 Sprague–Dawley rats prior to excision

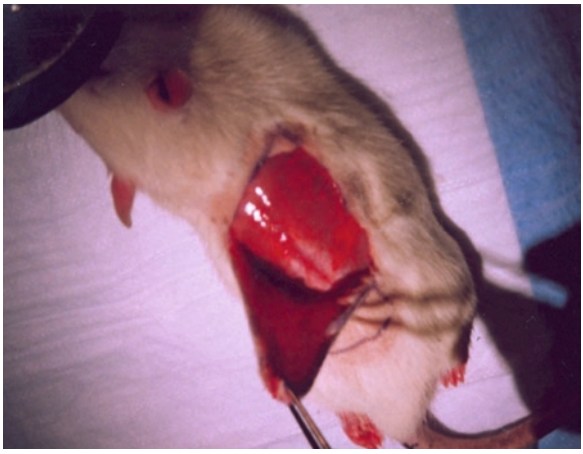


Fig. 2.27 Excision of rat

2.5.11 Reason for Failure to Provide Barrier Properties

Microscopic analysis of the dressing and necrotic rat tissue revealed that the particulate dressing was porous, consisted of 90% water and was vulnerable to microbial attack. The import of material, and therefore dressing composition, is analyzed in Sect. 2.7. However, it is important to realize that any barrier dressing for a wound

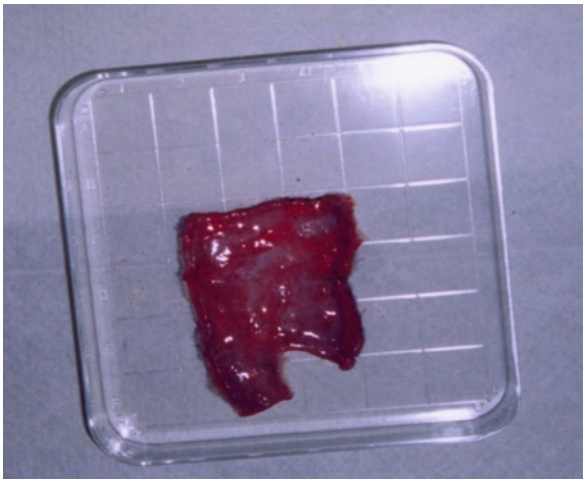


Fig. 2.28 Excised rat tissue in Petri dish, subcutaneous tissue exposed

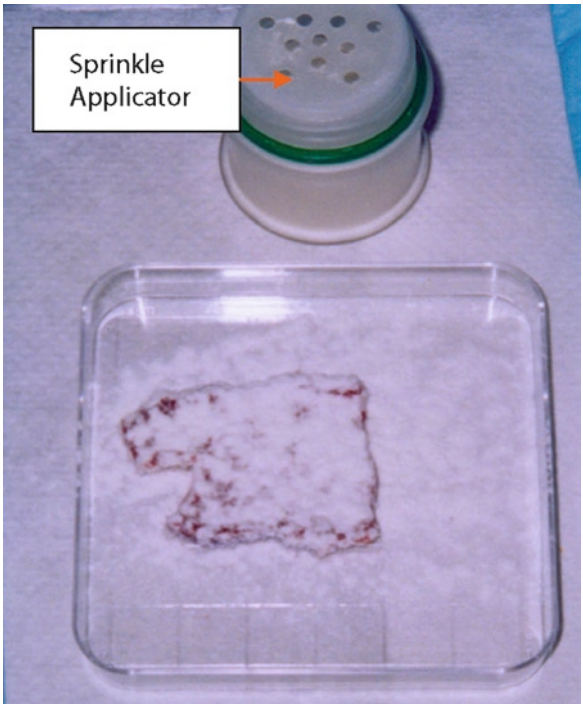


Fig. 2.29 Particulate dressing barrier applied to subcutaneous tissue in Fig. 2.28

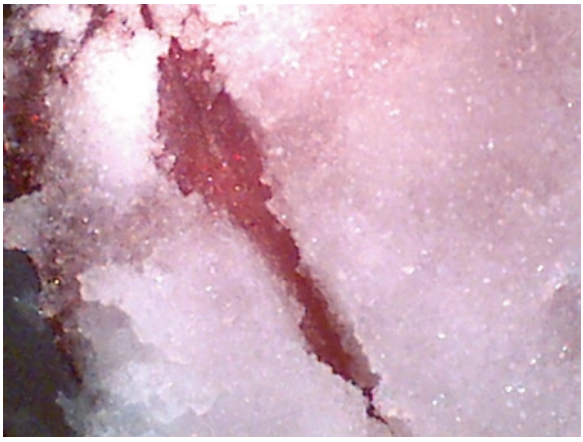


Fig. 2.30 Topological view of subcutaneous rat tissue coated with particulate barrier dressing after 60 s application (*cut section for inspection*)

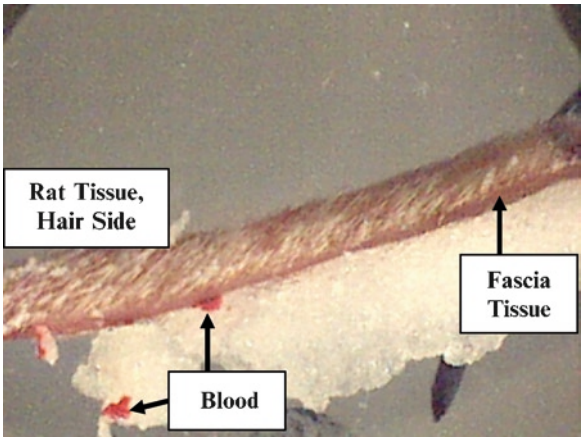


Fig. 2.31 Cross-sectional view of particulate dressing in Fig. 2.30 and demonstrating adhesion to tissue, ×10 magnification

must be impervious to water, nutrients, salts, and microorganisms as demonstrated in Fig. 2.33. The four 24 ml vials in the figure are filled with a collagen–water mixture to produce a solid and colloid type "gel." In addition, each vial A through D contains a decreasing amount of water, 95–50%. Two drops of FD&C Red Dye were placed over the surface of each gel, sealed, and allowed to stand for 48 h to observe the diffusion of dye through the thickness of the gel. It can be seen from this figure that the diffusion of the dye (3 cm/48 h) is not impeded by water concentration (and corresponding viscosity) through the gel. The dye components separate by rate of diffusion, and the yellow component is observed diffusing faster

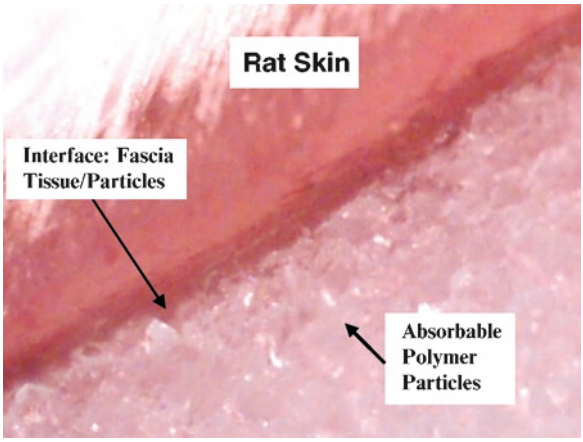


Fig. 2.32 Cross-sectional view (×60 magnification) of the bond between the rat tissue and particulate barrier dressing, red hue is due to reflections from blood

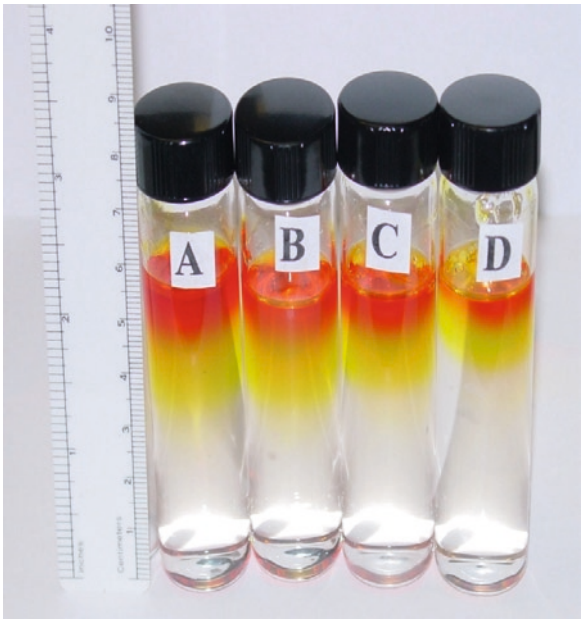


Fig. 2.33 Demonstration of the diffusion of FD&C Red Dye through increasing percent water in collagen gels over 48 h at 25°C: A – 95%, B – 85%, C – 75% and D – 65%

than the reddish component, which demonstrates that some materials diffuse faster than others. Also, the decreasing water concentration corresponds to increasing viscosity and "firmness" of the gel, but the diffusion of dye is only slightly influenced. Barrier dressings are only 50–150 μm in thickness and diffusion of nutrients

through these thicknesses happens in less than an hour. For this reason, dressings that are composed of water are not acceptable due to a high permeability that results in contamination of the wounded tissue, infection, and mortality.

The rate of diffusion (see Fig. 2.33) is controlled by the difference in concentration across the gel, or more conveniently, by the concentration gradient d*c*/d*x* where *c* is the concentration of the water in mass per unit volume of solution. The diffusion rate is found to be proportional to the cross-sectional area A. If the diffusion rate is written d*w*/d*t* and the mass of dye (*w*) transformed across the boundary per second (*t*), one has Fick's law of diffusion (Barrow 1973),

$$\mathrm{d}w/\mathrm{d}t = -D\,\mathrm{d}c/\mathrm{d}x$$

The proportionality constant *D* is called the diffusion coefficient, and the negative sign is introduced so that *D* will have a positive value. The diffusion coefficient can be recognized as the amount of solute (dye) that diffuses across a unit area in 1 s under the influence of a unit concentration gradient. The diffusion coefficient is characteristic for a given solvent (water) at a given temperature, of the diffusing tendency of the solute (dye) as demonstrated in Fig. 2.34, the dye can be observed to be diffusing from the reservoir (bottom) to the top of the collagen gel.

The range of bacteria diameter is 0.5–2.0 μm (Black 2002) with different aspect ratios and shapes compared to a simple sphere. Also, the surface chemistry of bacteria varies with type and environment, but the smallest microbes (viruses ~20 nm) do not obey transport or diffusion laws (molecular size) if moving through other substances such as water. Rather, some bacteria are able to regulate locomotion in aqueous solutions, but only for short distances. Therefore, bacteria may "eat" their way through a barrier more easily than they can physically move it. However, they take advantage of "cracks" and "pinholes" that often form in dressings, all the more incentive to provide an impenetrable barrier dressing for wounds.

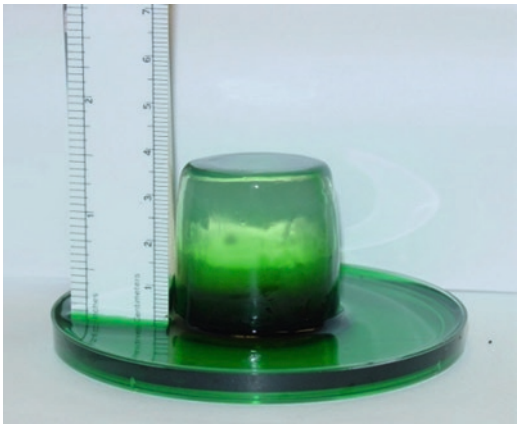


Fig. 2.34 Collagen placed in (90% water) placed in pool of water solution of FD&C Green Dye to observe the dye diffusing upward against gravity

The influence of noncovalent interactions (Mathews et al. 2000) between molecules determines the rate of diffusion as discussed, and they are hydrogen bonding, van der Waals repulsion, dispersion, and charge-, dipole-types. Water provides hydrogen bonding from water molecules, and nutrients with salts present sites on molecules (e.g., $–NH_2$ from collagen) for interaction with water (H^+ and OH^-). Gravity is not a consideration as demonstrated in Fig. 2.35 where the gel is placed in a pool of FD&C Green dye, and the dye diffuses upward to eventually reach a chemical equilibrium and a uniform color.

Proteus mirabilis bacteria was dispersed in agar before solidifying into a Petri dish, and colonization occurred which seems contradictory for an aerobic microorganism, but is understandable considering the "gel" structure is permeable to air, water vapor, and nutrients. The previous observation is further reason not to construct a barrier dressing from an aqueous gel, hydrogel or other similar material containing more than about 20% water where protection from bacteria is a critical requirement.

Finally, the particulate barrier did serve to absorb pools of blood (~150×), adhered to the injured tissue and provide physical abrasion properties that may eventually be a compliment or pretreatment to a wound instead of an environmentally protective barrier dressing. A demonstration of rapid blood absorption by the barrier dressing is shown in Figs. 2.35–2.38. Theoretically, only seconds would be required to absorb fluid from bleeding wounds and form a protective gelatinous layer over the wound.

The solidified porcine blood in Figs. 2.38 and 2.39 is the result of gelation of the blood using the barrier dressing material. The complete process required 30 s. Blood flow from a punctured vessel requires a pressure differential pressure (e.g., 0–300 mm Hg) to initiate flow, but an applied pressure or barrier over a punctured blood vessel will prevent flow as in the relationship in the following equation,

$$V_2 = V_1(P_2 - P_1)^{1/2}$$

where V_1 is the initial velocity of blood through a vessel, V_2 is the final velocity, P_1 is the initial pressure, and P_2 is the final pressure. The change due to a wound is

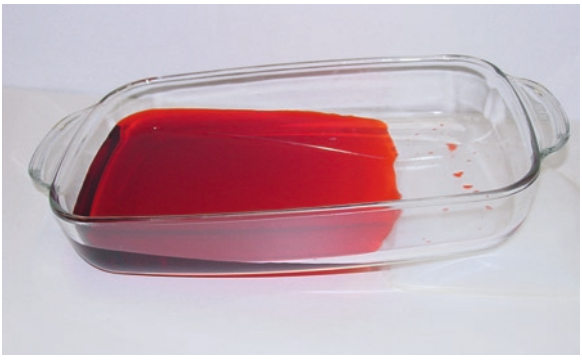


Fig. 2.35 Standing porcine blood in glass tray to simulate a bleeding wound

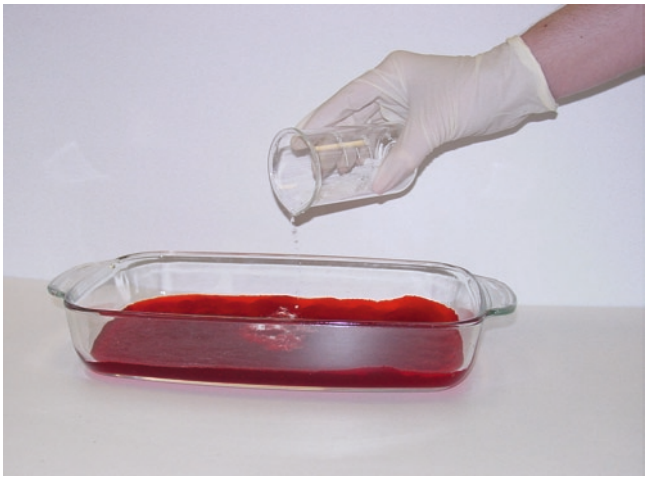


Fig. 2.36 Sprinkling of particulate dressing on blood

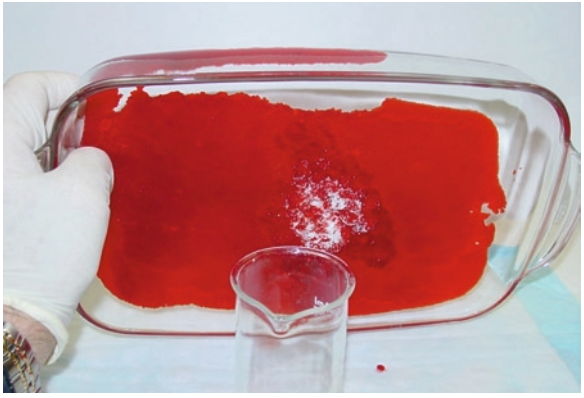


Fig. 2.37 Demonstration of efficient absorbance of porcine blood after 30 s by holding the tray vertically showing a solid nonfluid mass that adheres to the glass tray

related to the square root of the pressure differential multiplied by the initial velocity. The rate of blood flow is related to the rate that is explained as,

$$\text{Rate} = \text{A} \times \text{V}$$

where *A* is the cross-sectional area of the vessel and *V* is the velocity, and the rate is usually expressed in square centimeters per minute or other units.

The solid gel provides a "pressure barrier" to blood flow. The barrier dressing could be a valuable first step hemostatic agent under field conditions prior to advanced treatment.

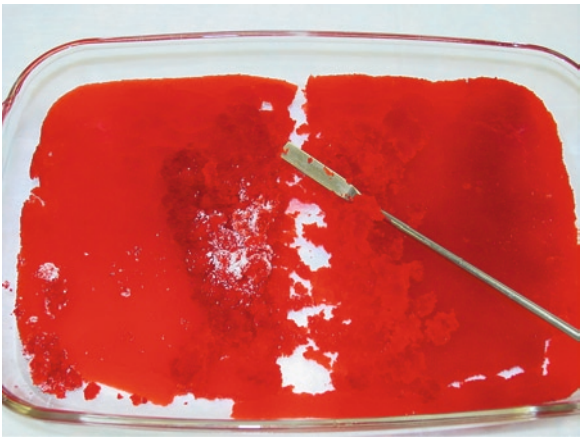


Fig. 2.38 Demonstration of absorbed porcine blood after cutting with a spatula to show the solid state of the combined dressing and blood

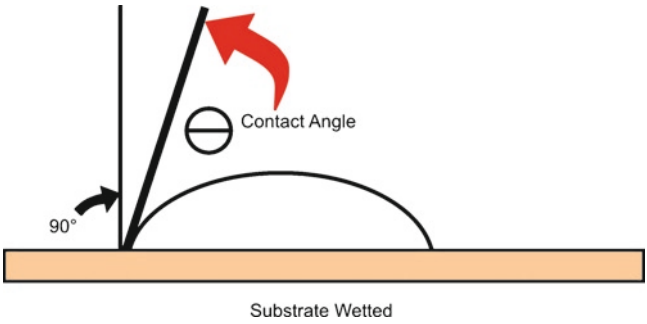


Fig. 2.39 Measurement of contact angle between drop of liquid and solid substrate

2.6 Physical Properties of Barrier Dressing Materials

2.6.1 Results of Testing Barrier Dressings on Agar Plates

This study consisted of applying coating or films of dressing material on agar plates to determine the "applicability" of wounded tissue which is moist and very hydrophilic, conditions that usually do not provide good properties for adhesive substrates (i.e., adherent).

2.6.2 *Surface Energy*

The importance of surface energy involves the wetting properties of a liquid when applied on a solid and, in this case, adhesives applied to tissue. The adhesive must spread on the surface to the very hydrophilic, irregular contoured and moist tissue in order to adhere to the tissue and provide a continuous barrier. A liquid must possess a surface energy less than that of the substrate to wet, spread, and adhere to the substrate, otherwise, it will "bead" and not form a bond with the substrate. For the reader's information, secondary bonds are the forces between adhesives and substrates including hydrogen.

A method of measuring surface energy is shown in Fig. 2.40 where a drop of a control liquid is placed on the surface of a test substrate and the contact angle is measured with a goniometer. A series of control liquids are applied to the substrate where each develops a contact angle corresponding to a surface energy. A plot of the percentage composition of water and ethanol mixtures versus surface energy at different temperatures is shown in Fig. 2.41. To determine the surface energy of any substrate, measure the contact angle of control liquids and plot the cosine of each contact angle versus surface energy and regress to cos $\theta = 1$; the intercept on the surface energy axis is the surface energy of the substrate as shown in Fig. 2.42.

A pertinent application of surface energy is the test of a water-soluble FD&C Green Dye on porcine tissue to determine the permeability of the dye through the tissue. If the surface energy of the dye is less than the tissue, then the dye will spread across the surface of the tissue which occurs in Fig. 2.42. Also, the dye is hydrophilic as is the tissue and the dye permeates the tissue. In the case of barrier dressing, it is desirable to wet or spread the liquid dressing on the tissue followed by solidifying and forming a continuous barrier (25–40 μm thickness). It is further

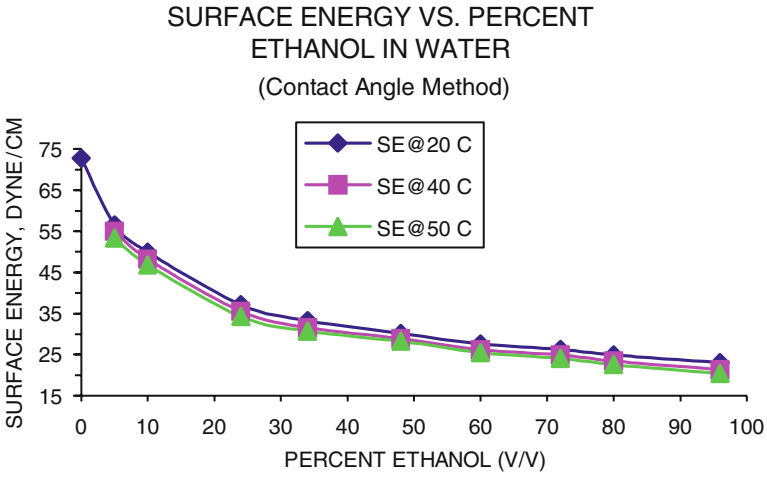


Fig. 2.40 Water and ethanol mixtures versus surface energy at 20, 40, and 50°C

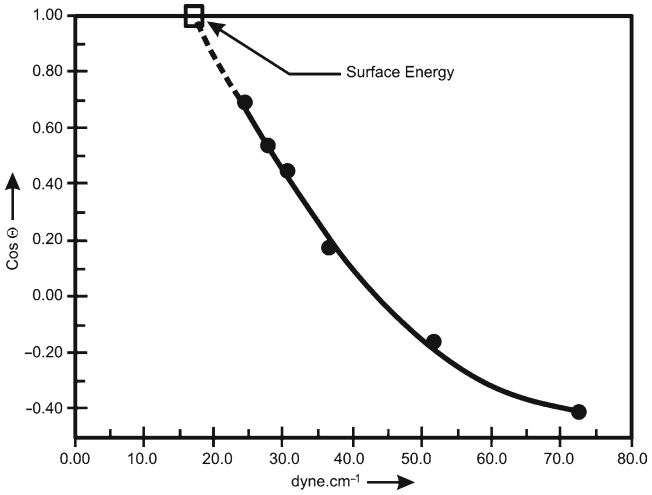


Fig. 2.41 Surface energy versus cos θ for the determination of surface energy of Teflon^{R}

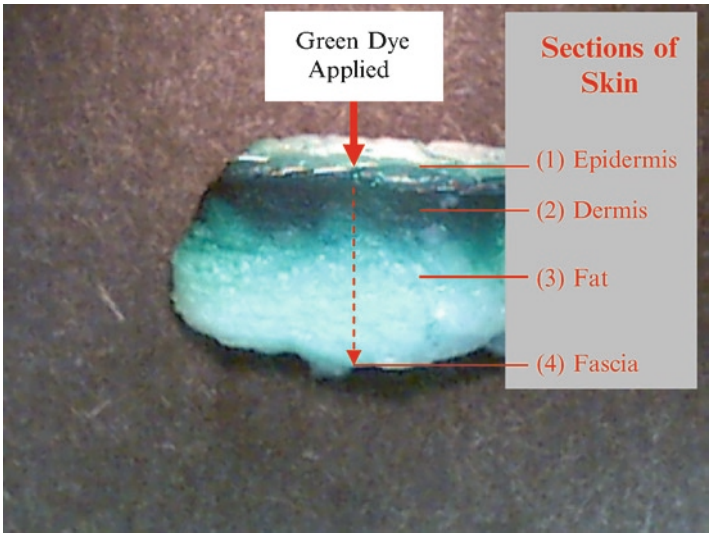


Fig. 2.42 Demonstration of the permeability of FD&C Green Dye through porcine skin (taken from shoulder)

desirable that the barrier possesses a low surface energy so that it will not be wetted by bacteria and/or be permeable. A list of dressing materials and corresponding surface energies are listed in Table 2.7.

A major problem with natural biocompatible materials (collegan, chitosa, etc.) used for tissue barriers is that they absorb water and are permeable to water soluble/

Table 2.7 Surface energies (20°C) of barrier dressings

Material	Surface energy dyne cm^{-1}	Contact angle (θ) 100% H_2O
Teflon	17.1	–
PVAC/P/C	22.0	80.5
Dermabond^R	23.0	89.5
PB/P/C	27.5	77.2
Polyethylene	31.0	–
Oleic acid	32.5	–
Water	72.8	–
Iron	1,650.0	–

Note 1: *PVAC/P/C* Polyvinyl acetate/plasticizer/chlorhexidine
Note 2: *PB/P/C* Polybutyral/plasticizer/chlorhexidine
Note 3: *P* Plasticizer (ethylene glycol dibenzoate)
Note 4: *C* Chlorhexidine diacetate
Note 5: *Dermabond^R* 2-Octyl-cyanoacrylate/plasticizer/preservative
Note 6: Teflon, polyethylene film, and water are included as a control surface(CRC Handbook, 52 ed.)

dispersible contamination and organisms. Some synthetic biocompatible materials as polyethylene glycol have a similar problem when used as tissue barriers. Any mateial that is to be used as a tissue barrier must be impermeable to water solutions/suspensions and organisms.

2.6.3 Water Vapor Transmission

The controlled rate of water loss from skin is important for wound healing and, therefore, any barrier dressing applied over injured skin or exposed tissue must not reduce the conditions for wound healing including the natural loss of water vapor and gases.

Visscher et al. (2001) reported the effects of semipermeable films and water vapor transport (WVT) on human skin following a standardized wound (81.8 $g\,m^{-2}\,h^{-1}$ WVT), induced by tape stripping, by measuring transepidermal water loss (TEWL), skin hydration, rate of moisture accumulations, and erythema. Wounds treated with semipermeable films (25.3–64.3 $g\,m^{-2}\,h^{-1}$ WTV) underwent more rapid recovery than wounds or wounds under complete occlusion (0.0–0.5 $g\,m^{-2}\,h^{-1}$). Barrier films that produced intermediate levels of skin hydration during recovery produced the highest barrier recovery and general healing. Sites exposed to highest levels of hydration showed the poorest recovery indicating that occlusion retards healing. The results supported the hypothesis that semipermeable wound dressings augment barrier repair and skin quality by providing an optimized water vapor gradient during the wound healing process. Mechanistically, superficial wounds can be considered in two groups: those in which the epidermis is penetrated or denuded, and those in which the epidermal barrier is damaged but not breached. In the former case, wound healing proceeds by epithelization with migration of cells from hair follicles, sweat-glands, and from around the edges of the wound. In the latter case,

repair is subtle and involves augmentation of epidermal DNA and lipid synthesis by mechanisms linked to the transepidermal water gradient. The ultimate result of both types of wound healing is the restoration of barrier integrity with formation of an intact stratum corneum epidermis. Since our evolution as aerobic organisms, we have become dependent on oxygen as a catalyst and energy source for many cellular functions including maintenance, metabolism, and repair. Oxygen has a significant role in wound healing, being essential to provide the additional energy source for the repairing process. Oxygen may, in fact, be the rate limiting step in early wound repair. Many other components, in addition to oxygen, are interrelated to provide the optimal environment for healing, including nutritional state, immune function, cardiopulmonary function, oxygen carrying capacity, blood flow, blood volume, temperature, and hormonal mediators. Tandara and Mustoe (2004) reported the interruption of blood flow leading to hypoxia immediately after damaging tissue, and the importance of oxygen in the wounded tissue to promote healing. Christophersen et al. (1991) reported the free radical mechanisms involved during the utilization of oxygen in tissue. The measurement of water vapor transmission is also indicative of oxygen gas transmission through the same membrane and contact with the wounded tissue. However, oxygen is delivered from the blood supply when wounds must be covered to prevent infection.

A method of measuring WVT for barrier dressing is the water vapor transmission cup shown in Fig. 2.43a. A plain cellulose paper sheet was coated with the barrier dressing, allowed to cure or dry for 24 h, followed by preparing a 25 cm^2 disc and placing the coated disc between rubber gaskets in the cup as shown in Fig. 2.43b. The assembled cup was weighed and placed in a constant temperature oven (Fig. 2.44) at 37°C for 24 h, after which the cup was reweighed to determine the loss of water through the barrier dressing in this case. The results of the measurements for different barrier materials are contained in Table 2.8.

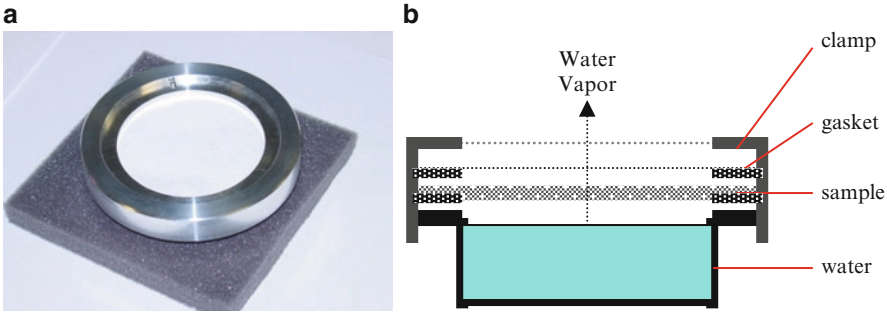


Fig. 2.43 (**a**) Water Vapor Transmission Cup (Perm Cup), ASTM D 1653-03, ISO 7783, and (**b**) Cross-sectional view of assembly comprising cup including water reservoir, clamp, and gasket

Fig. 2.44 Water vapor transmission cup in constant temperature oven, 37°C

Table 2.8 Water vapor transmission rate (WVTR) of barrier dressings at 37°C, ASTM D 1653-03

Dressing	g h^{-1}	WVTR (g $m^{-2} h^{-1}$)
Blank paper only	0.425	170.0
Poly(vinyl alcohol)	0.343	137.2
Poly(vinyl acetate)	0.158	63.3
Emulsion	0.115	46.0
Poly(vinyl butyrate-*co*-vinyl acetate-*co*-alcohol)	0.027	10.8
2-Octylcyano-acrylate; (Dermabond^{R})	0.026	10.4
Poly(ethylene) extruded film	8.33×10^{-4}	0.3

Note 1: Porous cellulose paper was used as blank substrate = 25 cm^2 = 2.5×10^{-3} m^{-2}

2.6.4 Dynamic Mechanical Analysis

The physical properties of barrier dressings were evaluated using the Seiko Model DMS 210 Dynamic Mechanical Analyzer Instrument (see Fig. 2.45). Referring to Fig. 2.46, dynamic mechanical analysis consists of oscillating (1 Hz) tensile force of a material in an environmentally (37°C) controlled chamber (see Fig. 2.47) to measure loss modulus (E″) and stored modulus (E′). Many materials including polymers and tissue are viscoelastic, meaning that they deform (stretch or pull) with applied force and return to their original shape with time. The effect is a function of the viscous property (E″) within the material that resists deformation and the elastic property (E′)

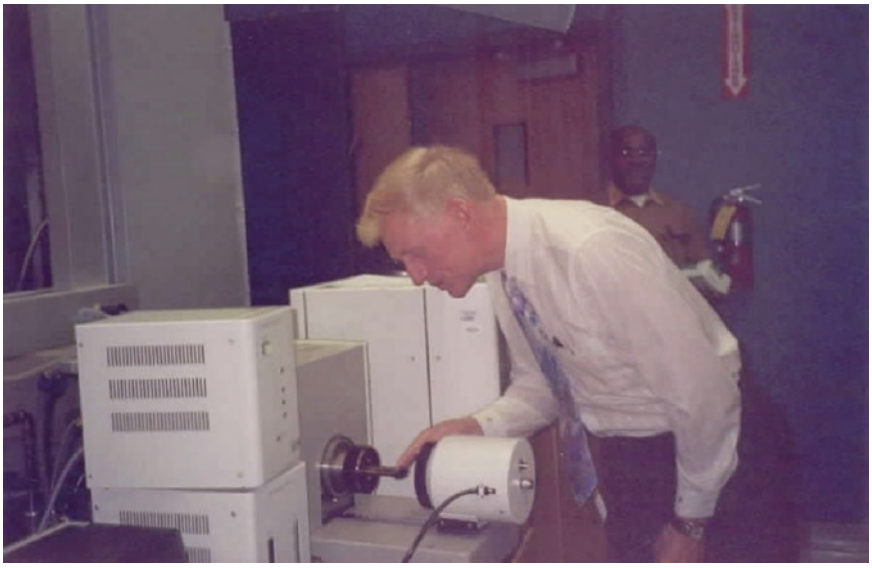


Fig. 2.45 Seiko model dynamic mechanical analyzer, the operator's finger is touching the test specimen between grips

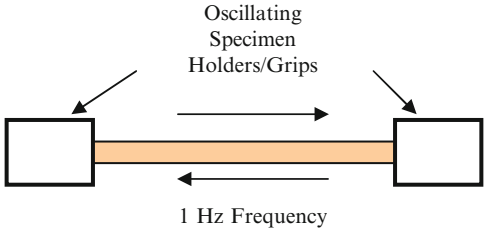


Fig. 2.46 Illustration of the oscillating forces applied to a specimen during testing

that allows the material to deform and return as shown in Fig. 2.48 for porcine skin. The relationship of modulus and pecent plasticizer in polyvinyl acetate is shown in Fig. 2.49 and the effect of percent plasticizer with glass transition temperature (brittleness) in Fig. 2.50. Plastizicers are useful for adjusting the viscoelastic properties to simulate those of skin. The number of rats that survived the innoculation with bacteria are shown in Fig. 2.51. The survivorship in all cases were improved with the treatment compared to no treatment. Porcine skin is more elastic than viscous because the tan θ is less than 1.0. Taking a common example, common window caulking is viscoelastic putty because it deforms to any shape, but possesses enough elasticity to "spring-back" and compress against surfaces and omit water from entering around its edges like a rubber gasket.

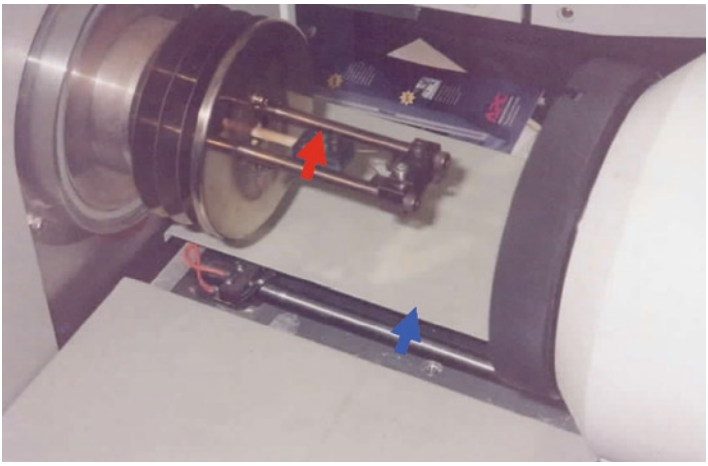


Fig. 2.47 View of DMA specimen holder, *red arrow* indicates specimen and *blue arrow* indicates environmental chamber that closes over the specimen grips during testing

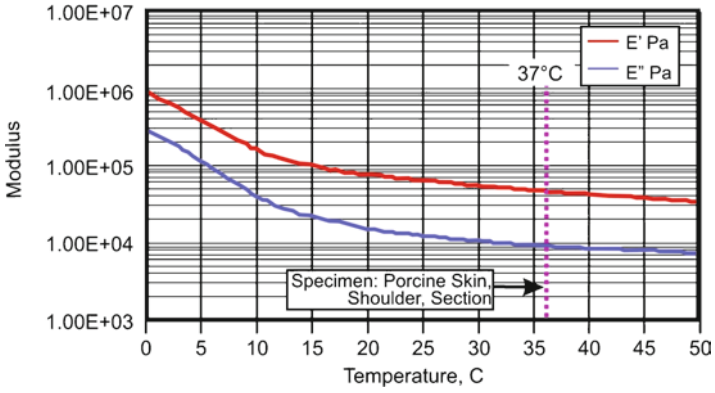


Fig. 2.48 Stored (E′) and loss (E″) modulus versus temperature of porcine skin, shoulder section

The data in Table 2.9 represents a composite list of pertinent thermal and viscoelastic properties for barrier dressing materials, and the following observations can be drawn these data. The tensile (pull) testing results of barrier dressing are listed in Table 2.10.

- Immersion in water lowers tan θ (viscosity/elasticity) for smaller tan $\theta \approx$ toughness
- T_g does not translate to embrittlement of the dressing, but a harder consistency
- Values from films formed over dry substrates produced lower tan θ values because water plasticizes or weakens the structure (i.e., reduces Young's modulus) of each material

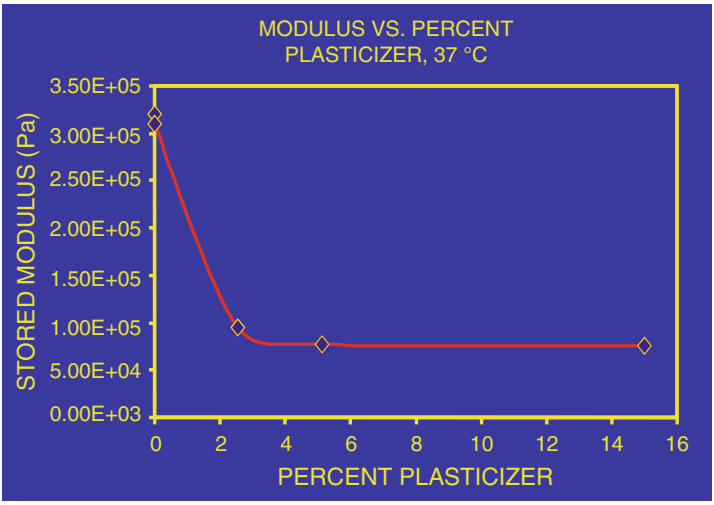


Fig. 2.49 Modulus versus percent plasticizer (dioctyl phthalate) at 37°C

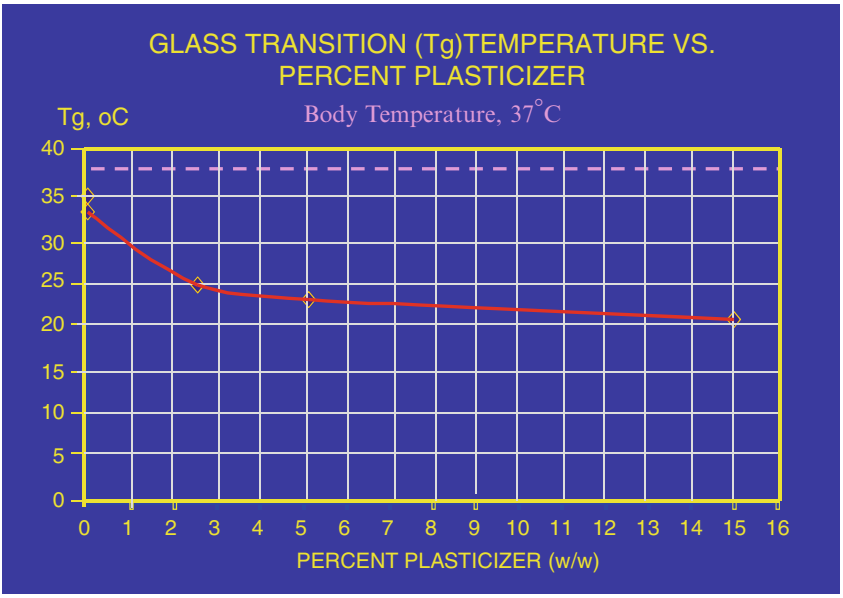


Fig. 2.50 Glass transition temperature versus percent plasticizer

- Porcine tissue possesses a tan θ of 0.20 or a ration of viscous/elastic property of 1:5, representing good extension and elastic properties. A material that adheres to the tissue must have a similar tan θ or it will not strain (stretch, pull, flex, etc.) with the tissue and crack until it loses adhesion and disbonds from the tissue, failing as a barrier dressing.

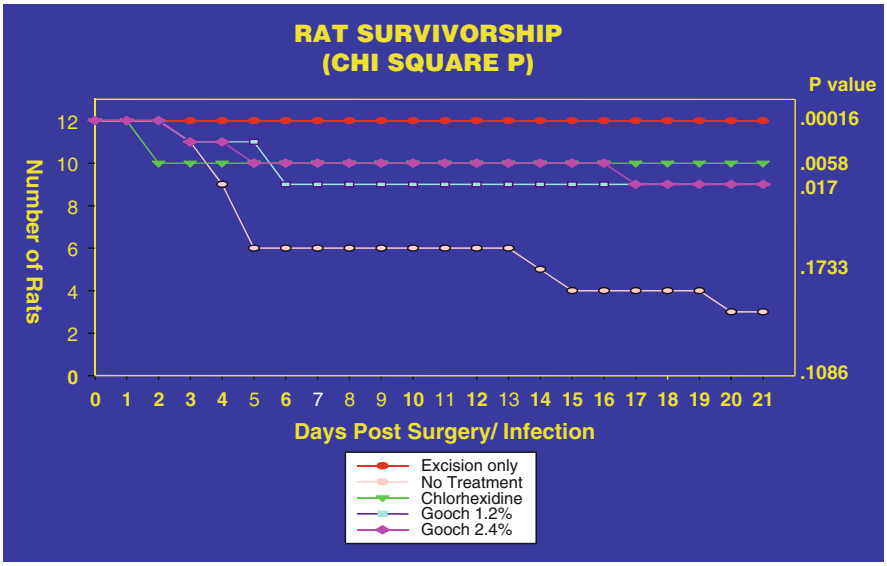


Fig. 2.51 Rat survivorship, chi square analysis

Table 2.9 Tan θ of liquid dressing films formed over agar

	Tan θ (E″/E′)			
Liquid dressing	0°C	25°C	37°C	T_g °C
1. PVAC/P/C -	0.05	0.06	1.3	33
2. “ ” Immersed in water 48 h before testing	0.06	2.02	8.7	33
3. PB/P/C	0.17	0.55	0.43	31
4. (3) “ ” Immersed in water 48 h before testing	0.42	0.76	0.68	31
5. 2-Octyl cyanoacrylate (Dermabond^R)	0.06	0.05	0.07	24
6. (5) “ ” Immersed in water 48 h before testing	0.13	0.14	0.07	24
7. Liquid Bandage^R	0.24	0.26	0.35	?
8. (7) Immersed in water 48 h in water				
9. Porcine skin (kept moist in saline solution)	–	0.19	0.20	0

Table 2.10 Results of tensile testing for barrier dressings

Material	kPa
Cyanoacrylate dermabond^R	408.8
PVAC/P/C	109.8
PB/P/C	87.6
Acrylate emulsion	20.1
GIT#19 Nonaqueous suspension	50.5

2.6.5 Adhesion to Tissue

The Standard Test Method for Strength Properties of Tissue Adhesive in T-Peel by Tension Loading, ASTM F 2256-03, was not employed for testing barrier dressings; only porcine tissue was available in 15 cm strips that possessed thick hair on the epidermis side and a thick fat layer on the underside that was not conducive to testing. Removal of the fat layer to isolate the dermis will be necessary before testing by the "T-Peel" method.

The barrier dressings were tested in tensile mode, adhered at ends, as discussed in Sect. 2.3. These tests provided good relative adhesive values (kPa) for comparing the barrier dressings for their ability to adhere to tissue.

The applicator in Fig. 2.52 is the liquid spray type that applies the liquid barrier to the wound without touching the tissue. The thickness of the barrier film can be adjusted by reapplication of the sprayed liquid barrier, and "drying to touch"occur with 15-20 seconds. Testing the barrier film on rats requires excision of the skin (dorsol section) that begins with mapping a section for excision in Fig. 2.53 and application, the exposed wet and soft tissue in shown Fig. 2.54 followed by spraying the liquid barrier to the excised tissue, and the dried protective barrier film is firmly in place on the excised rate skin in Fig. 2.55. The application of the liquid barrier was successfully applied over a wet excised tissue.

2.6.6 Removability of Barrier Dressing from Wounds

The convenient removal of liquid or particulate applied dressing is desirable after a wound has healed sufficiently, a new application is required or other reason the clinician may have. A convenient hand-held device was developed for this purpose consisting of reverse-side pressure-sensitive adhesive tape applied on supply and take-up

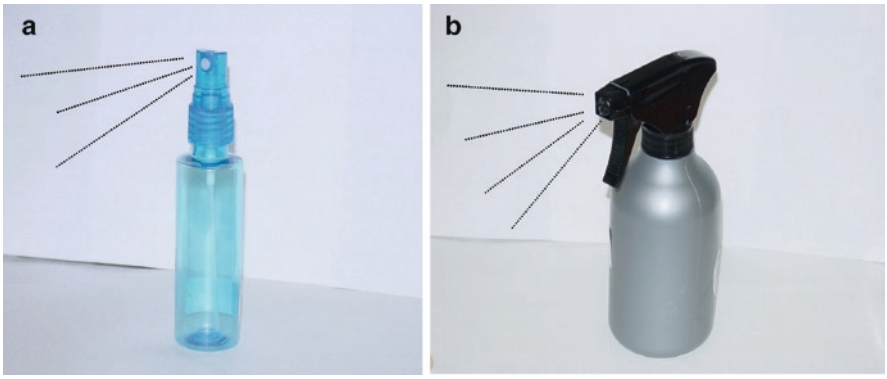


Fig. 2.52 Spray on applicators of barrier dressing

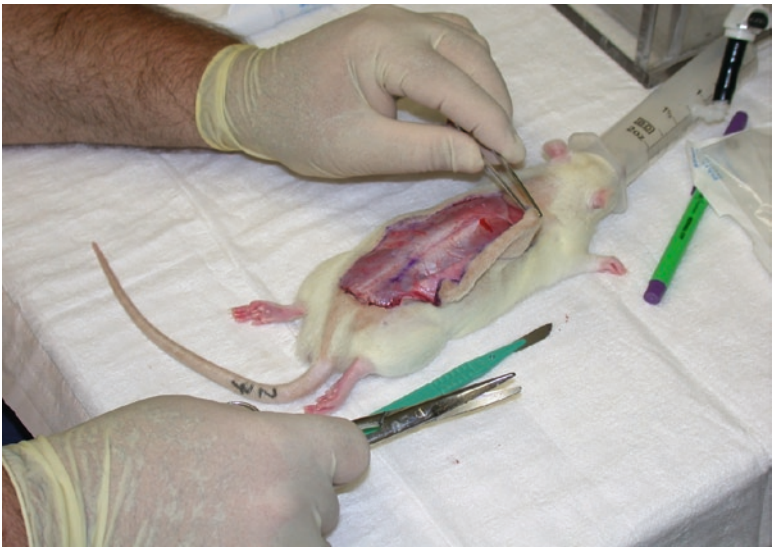


Fig. 2.53 Excised tissue

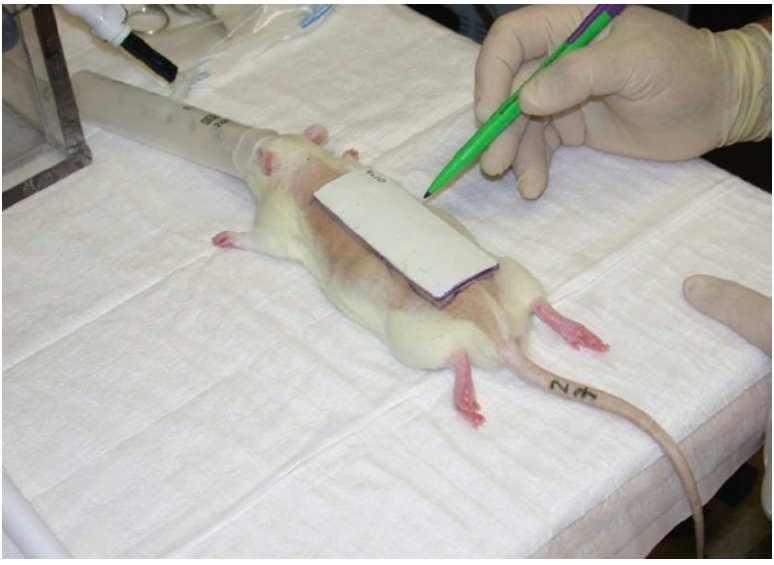


Fig. 2.54 Mapping of dorsal excision pattern

spools shown in Fig. 2.56. The combination of adhesion and upward directed torque makes this device successful for removing all liquid-applied dressings.

In addition, the removed dressing is not reexposed to the tissue, but rolled-up on the take-up spool. Tissue can be precleaned and prepared for a dressing by using this device (see Fig. 2.56).

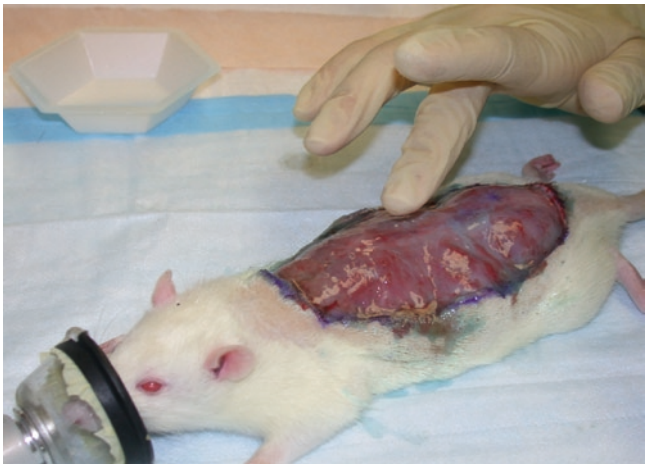


Fig. 2.55 Formation of flexible, adhesive, and durable barrier film

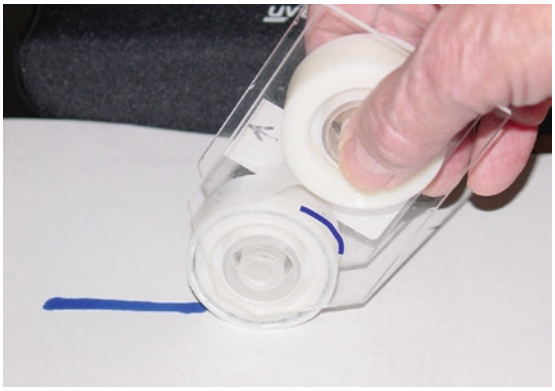


Fig. 2.56 Demonstration of barrier dressing removal (*blue*) by reverse-side pressure-sensitive tape and roller; note that the dressing remains on the tape and protects the substrate from further exposure to the dressing after removal; the tape can be safely disposed after use

The construction of the case is acrylic or other clear plastic material that will allow inspection of the remaining tape on the spool in order that the operator will know when to replace it.

2.7 Bacteria Permeability of Dressings

The liquid dressing (polymer/solvent) was designed to be placed over soft tissue, dried by solvent evaporation to form a coherent barrier film as shown in Fig. 2.57. The rate of drying is dependent on the vapor pressure of the solvent as demonstrated in Fig. 2.58.

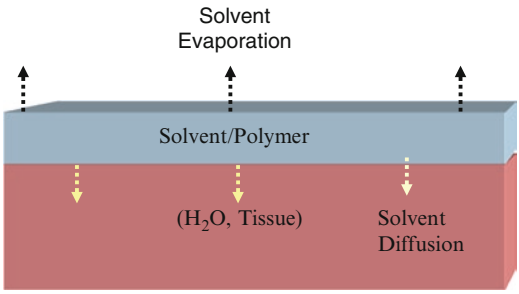


Fig. 2.57 Mechanism of barrier application and solvent evaporation

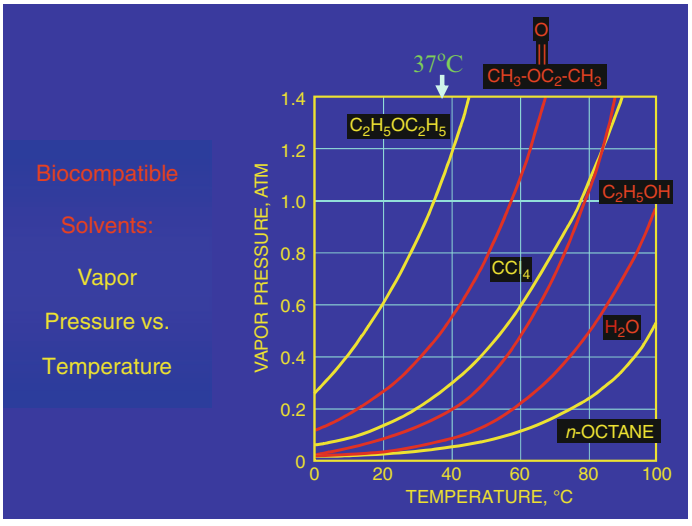


Fig. 2.58 Biocompatible solvents (Acetone, ethanol and water): vapor pressure and temperature

The permeability of dressing materials to bacteria was of paramount interest considering the primary objective of a barrier dressing is to prevent infection of wounds from exposure to microorganisms. The study was designed to determine the growth or penetration of bacteria applied over plain agar (with nutrients) followed by topical application of bacteria and incubation at 37°C. Body temperature was chosen for the incubation condition to simulate the wounded tissue of a patient; and aerobic bacteria were selected for common and probable contact in natural environments (sand, dirt, water, etc.). Anaerobic bacteria were not tested because they were available for testing in this laboratory. However, the *Staphylococcus* genus is a facultative anaerobe that utilizes oxygen when it is present (aerobic) and become anaerobic when it is absent. Gerhardt et al. (2001) explained that aerobic bacteria colonize in a liquid (e.g., flask culture), if two criteria are met: (1) Gas exchange, oxygen and carbon dioxide, through the liquid and (2) the attainable liquid surface area available for oxygen transport.

The barrier dressings materials were selected for biocompatibility and physical properties discussed in Sect. 2.6. Each dressing was formulated with additive materials to achieve desired properties rather than existing as a single chemical compound:

- Acrylic emulsion – Acrylic monomers and hydrophobes polymerized via mini-emulsion (miniEP) polymerization (Gooch 2002) in an aqueous medium.
- Poly(vinyl acetate vinyl alcohol) – A water plasticized polyvinyl acetate with minor hydrolysis to form vinyl alcohol sites to a molecular weight of ~50,000 g/mole; plasticized with mono/diethylene glycol dibenzoate. This material was dissolved in ethanol for application purposes.
- Poly(vinyl alcohol) – A water-soluble copolymer of ~150,000 g/mole polymer from 99% hydrolysis of polyvinyl acetate.
- Poly(butyral-*co*-vinyl acetate-*co*-vinyl alcohol) – A terpolymer with greater water resistance than polyvinyl acetate, but good adhesion to tissue when applied in ethanol solution.
- Polycyanoacrylate – Dermabond^{R} product consisting of 2-ocyl-cyanocrylate plasticized and stabilized for antimicrobial growth.
- Agar – Plain growth medium with nutrients to support bacteria growth.

The above dressing materials were all-biocompatible, but comprised a range of physical properties that were observed for permeability and growth of bacteria. Agar is about 95% water and provided a hydrophilic substrate similar to tissue for barrier (coating or film type layer) compared to a dry or lipophilic substrate. A measured volume of each bacterium inoculum was placed over a dried barrier dressing in individual Petri dishes that contained agar growth medium followed by incubation at 37°C for 7 days. The results of these studies are listed in Table 2.11.

- Acrylic emulsion – The emulsion consisted of suspended crosslinked (gel) particles that are not water-soluble and form a film upon evaporation of the aqueous phase. However, the water did not evaporate quickly enough to form a continuous film on agar because agar is 95% water, and it continuously provided moisture that prevented film formation. The result was a porous barrier, but a continuous film was later obtained by dissolving dried emulsion solids in ethanol.
- Polyvinyl acetate-vinyl alcohol – This plasticized polymer formulation is insoluble in water and was dissolved in ethanol and quickly (~5 min) formed

Table 2.11 Barrier dressing material and bacteria permeability

Dressing material	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Escherichia coli*	*Staphylococcus aureus*
Acrylic emulsion	+	+	+	+
Poly (vinyl acetate – vinyl alcohol)	–	–	–	–
Poly (vinyl alcohol)	+	+	+	+
Polyvinyl butyral	–	–	–	–
Polycyanoacrylate	–	–	–	–
Agar gel	+	+	+	+

Note: (+) Bacteria growth was observed on surface of the barrier dressing material, and (–) indicated no growth

a continuous film and impenetrable barrier, but is permeable to water vapor.

- Polyvinyl alcohol – This polymer was dissolved in water and did not form a continuous film, and the hydrophilic property prevented the formation of a barrier film. This emulsion consists of emulsified particles that must fuse during evaporation of water and form a film, but cannot do so if the water phase remains which is what happened in this case.
- Polyvinyl butyral – A nonvapor or liquid permeable plasticized polymer formulation with additive materials that forms a continuous film from an ethanol solution (~5 min).
- Polycyanocrylate – 2-Octyl-cyanoacrylate plasticized and self-stabilized is the slower, but slightly more flexible (octyl- vs. ethyl-substituent group) ionically curing adhesive that is catalyzed by weak bases including water. The liquid and nonsolvated Dermabond^{R} product forms a continuous film in ~3–5 min that is continuous and very adherent to tissue.
- Agar – The standard alginate (5%) in water formulation for filling Petri dishes. This material is a "gel" or solid resin in water material to represent a nonpolymeric material through which nutrients diffuse to colonize bacteria.

From Table 2.11 and the discussion in Sect. 2.6, it is apparent that only moisture impenetrable materials are suitable for barrier dressings. In other words, nothing, except, water vapor, may be transmitted through the barrier dressing. Nutrients, it will be shown below (see Fig. 2.59), are included in this theory because they diffuse the barrier to nourish the bacteria (aerobic in this study) on the surface and colonize. As observed from these experiments, advanced bacterial growth is eventually continuous through the thicknesses of barrier and agar.

The example of *Staphylococcus aureus* on polyvinyl acetate and plain agar (see Fig. 2.60) best shows the efficacy of a barrier to resist or support bacteria growth.

The observations in Sect. 2.6.1 clearly apply to the barrier effects in Fig. 2.62 especially with regard to the moisture or water vapor permeability. The permeability of water vapor for polyvinyl alcohol is unacceptable compared to acceptable levels in polyvinyl acetate, polybutyral and cyanoacrylate. A high level of moisture

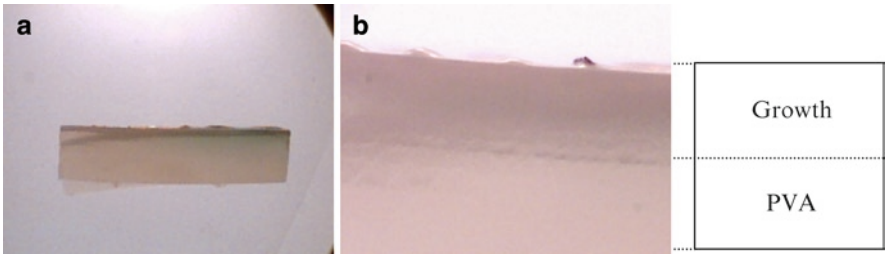


Fig. 2.59 Cross-sectional views of back-illuminated polyvinyl alcohol coated over agar showing growth of *Staphylococcus aureus;* the view in (**a**) (×10 magnification) shows the complete thickness and view in (**b**) (×60 magnification) shows in the bacteria growth, polyvinyl alcohol and growth through the polyvinyl alcohol layer and into the agar

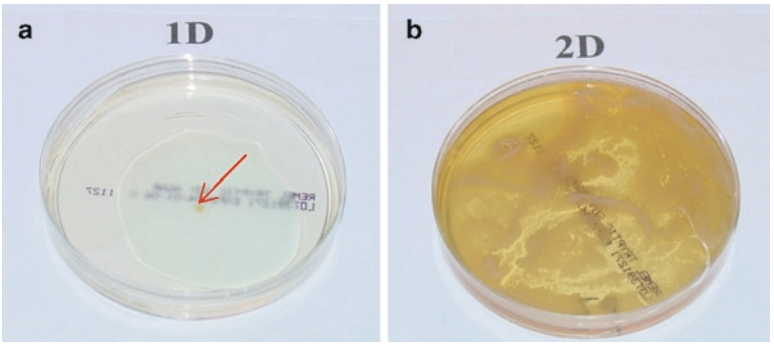


Fig. 2.60 Topological views of formulated polyvinyl acetate over agar (**a**) and plain agar (**b**) in Petri dishes after having been inoculated with *Staphylococcus aureus* and incubated for 7 days at 37°C; note the original yellow drop of bacteria suspension in (**a**) and the prolific growth in (**b**) which demonstrates the barrier effect

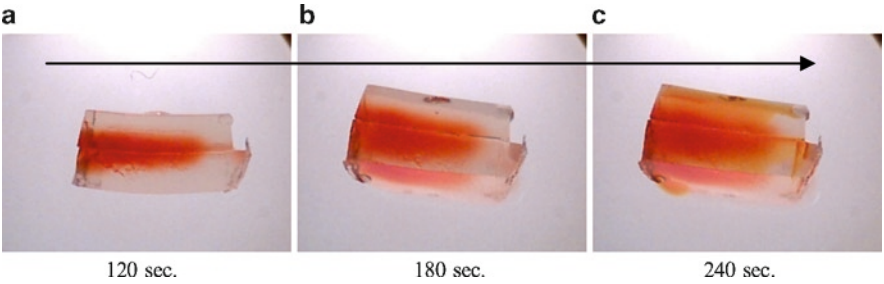


Fig. 2.61 Cross-sectional views of a single drop of a water-dispersible red dye between sections of plain agar from Petri dishes to demonstrate the natural diffusion of nutrients through gels containing major fractions of water

permeability results in a liquid-like medium that allows the diffusion of nutrients as if in liquid water.

Diffusion of water-dispersed nutrients in solid gels as agar or wound dressings is demonstrated in Fig. 2.61. A single drop of water dispersible FD&C Red Dye (McCormick Co.) was placed between two sections of plain agar and the rapid advance of dye via diffusion can be observed in the series of figures. The observations from Fig. 2.62 are that nutrients, same as the red dye, diffuse a "gel" that consists primarily of water. The forces involved in diffusion are well known including secondary molecular forces and osmotic pressure that tends to maintain a concentration equilibrium of dispersed (soluble-nonionic) particles. In other words, particles as nutrients or ions as salts tend to establish equilibrium throughout an aqueous medium, and when bacteria removes by digesting a fraction of those particles, the equilibrium is shifted toward the low concentration to reestablish equilibrium. More specifically, the nutrients and salts will travel to the low concentration as the bacteria removes nutrients and salts. Within the body and its tissues, the term "homeostatic" is more applicable for maintaining equilibrium for different chemical compositions.

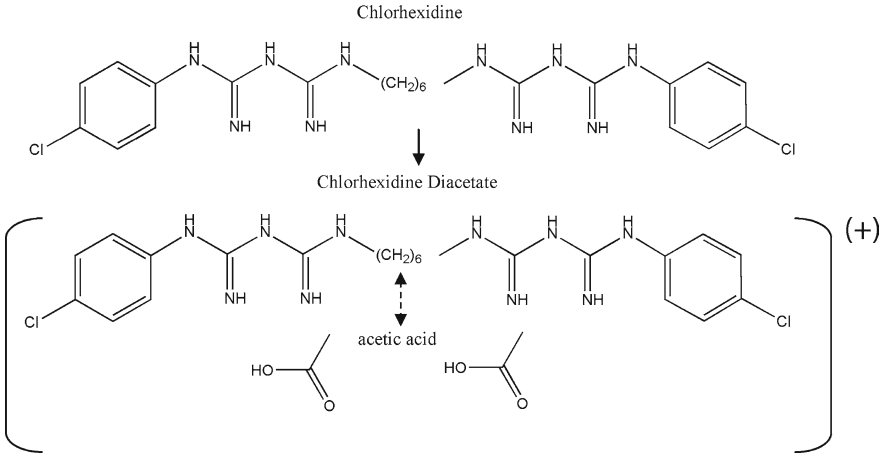


Fig. 2.62 Chlorhexidine and its reaction with acetic acid to form chlorhexidine diacetate (complex molecular structure), where the acetic acid and chlorhexidine base are in equilibrium (+ charge), when dissolved, they subsequently dissociate in water

2.8 Preservative and Antimicrobial Agents

In a microorganism prone environment, it is preferable to protect a dressing with a preservative such as chlorhexidine to prevent the contamination of the dressing. It is necessary to consider the solubility of an agent in the liquid phase of the dressing during the selection of an antimicrobial agent. The formulated polyvinyl acetate and polybutyral materials were dissolved in ethanol that is also a solvent for chlorhexidine diacetate. The information (Block, 2001) in Table 2.12 provides solubilities of chlorhexidine compounds in different solvents.

From inspection of Table 2.12, it is apparent that only chlorhexidine diacetate is sufficiently soluble in ethanol, so the liquid applied barrier dressing consisting of polymers dissolved in ethanol must be preserved by chlorhexidine diacetate, because water is not an option. The concentration of this agent is another consideration, but the range of options is clear from the above table.

The concentrations of chlorhexidine diacetate for antimicrobial growth and sporicidal activity are listed in Table 2.13. The levels of chlorhexidine concentration for bacteriostatic compared to bacteriocidal are about twofold and a similar relationship for spores. Chlorhexidine diacetate is an effective general topical antibacterial agent directed mainly toward gram-positive and gram-negative bacteria; it is inactive against bacterial spores except elevated temperatures, and acid fast-bacilli are inhibited but not killed by aqueous solutions. The infectivity of some lipophilic viruses [e.g., influenza virus, herpes virus, human immunodeficiency virus (HIV)] is rapidly inactivated by chlorhexidine, although aqueous solutions are not active against the small protein-coat viruses.

Table 2.12 Solubility of chlorhexidine in solvents

Chlorhexidine compound	Water	Ethanol at	2-Propanone (20°C)	Dichloromethane
Base	0.008	<0.001	<0.001	<0.001
D-Gluconate	20.2	<0.001	<0.001	<0.001
Diacetate	1.9	5.1	<0.001	<0.001
Dihydrochloride	0.06	<0.001	<0.001	<0.001
Dinitrate	0.03	<0.001	<0.001	<0.001
Carbonate	0.02	<0.001	<0.001	<0.001
Sulfate	0.01	<0.001	<0.001	<0.001
Hydroxide	0.001	<0.001	<0.001	<0.001

Sources: *Principles and Practices of Disinfection, Preservation, and Sterilization*, S. S. Block, ed., Blackwell Scientific Publications, St. Louis, 1982; Disinfection, *Sterilization, and Preservation*, S. S. Block, ed., 5th ed., Lippincott Williams & Wilkins, New York, 2001, pp. 321

Table 2.13 Bactericidal and sporicidal concentrations of disinfectants

Antibacterial agent	Bactericidal	Sporicidal (%wt/vol)	Bacteriostatic	Sporostatic
Chlorhexidine diacetate	0.0002	>0.05	0.0001	0.0001
Cetylpyridium chloride	0.002	>0.05	0.0005–0.01	0.00025
Phenol	0.05	>5	0.2	0.2

Source: Disinfection, sterilization, and preservation (2001)

Yeasts (including *Candida albicans*) and dermatophytes are usually sensitive, although chlorhexidine fungicidal action is subject to species variation, as are other agents. The effective concentrations of chlorhexidine agents for bactericidal, bacteriostatic, sporicidal, and sporostatic organisms are listed in Table 2.13.

Chlorhexidine is 1,6-di(4-chlorophenyl-diguanido)hexane, molecular weight 505.46 g/mole, a cationic bisbiguanide of the formula is shown in Fig. 2.62 and it becomes the diacetate when reacted with two-moles of acetic acid.

Chlorhexidine is a strong base (Lewis acid-base theory) because it reacts with acids to form salts of the RX_2 type, and it is practically insoluble in water (<0.008% wt/vol at 20°C). The water solubility of the different salts varies widely as demonstrated in Table 2.13. Chlorhexidine is moderately surface-active (a net + chare over its surface) and forms micelles (molecular aggregates form colloidal particles) in solution; the critical micellar concentration of the acetate is 0.01% wt/vol at 25°C (Heard and Ashworth 1969). Aqueous solutions of chlorhexidine are most stable within the pH range of 5–8, and above pH 8.0 chlorhexidine is precipitated because conditions for a base (>pH 7) reaction are present.

The positive charge is due to the formation of the guanidinum cation on the chlorhexidine molecule (Fig. 2.63) that is formed by tautomerism (Morrison and Boyd 1992) and shown Figs. 2.64 and 2.66.

The mechanism of antibacterial action was reviewed by Woodcock (1988). At relatively low concentrations, the action of chlorhexidine is bacteriostatic, and at higher concentrations, it is rapidly bacteriocidal, with actual levels varying somewhat from species to species. The lethal process consists of a series of related

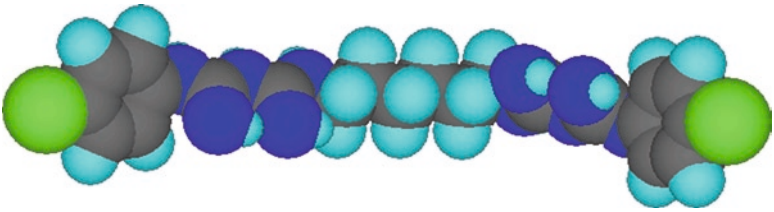


Fig. 2.63 Three-dimensional molecular model of chlorhexidine, 1,6-di(4-chlorophenyl-diguanido) hexane, molecule weight 505.46: *gray* – carbon, *blue* – hydrogen, *dark blue* – nitrogen, and *green* – chlorine

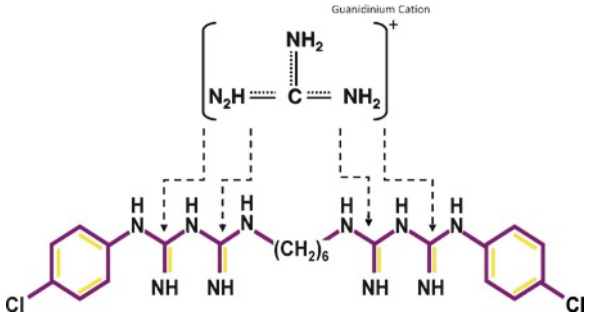


Fig. 2.64 Guanidinium cation as represented in chlorhexidine molecule, and one form of tautomerism (a total of ten tautomers) to form the cation in equilibrium with other tautomer arrangements (calculated total of 10)

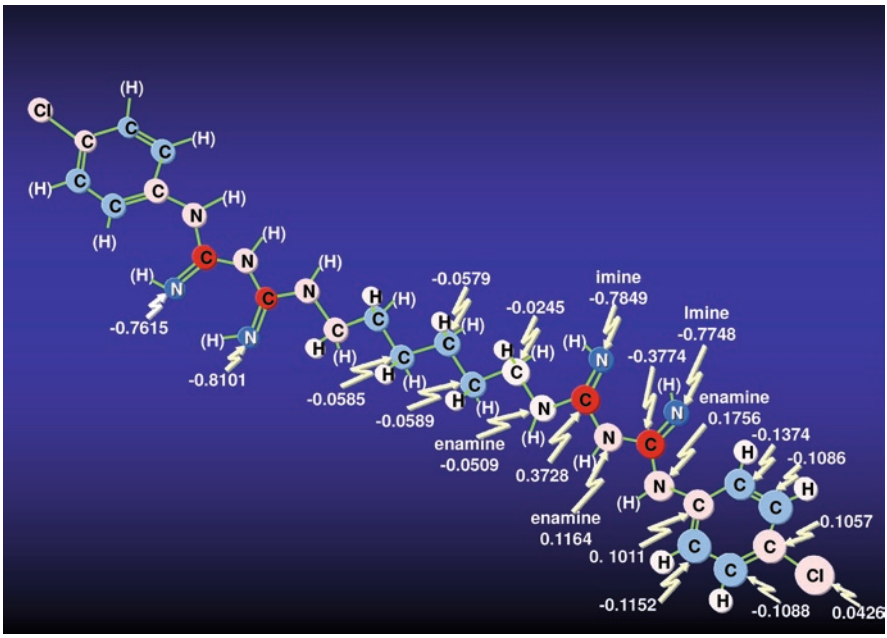


Fig. 2.65 Chlorhexidine partial charges (J/mole) assigned to chemical units from extended Huckle molecular orbital theory (Rauck, 2001; Smith and March, 2001)

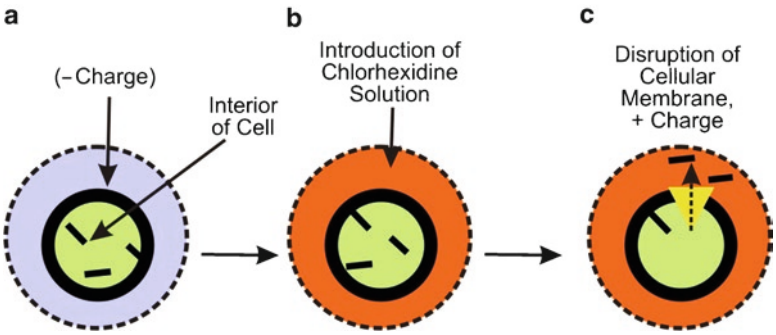


Fig. 2.66 The mechanism of antibacterial activity of chlorhexidine diacetate on a bacteria cell: (**a**) Natural cell with surrounding negative surface charge (*gray*), (**b**) introduction of chlorhexidine diacetate, pH < 7, surrounding positive charge (*red*) and (**c**) disruption of cell membrane

cytologic and physiologic changes, some of which are reversible, that culminate in the death of the cell. The sequence is thought to be as follows:

1. Rapid attraction toward the bacterial cell
2. Specific and strong adsorption to certain phosphate-containing compounds on the bacterial surface
3. Overcoming the bacterial cell wall exclusion mechanisms
4. Attraction toward the cytoplasmic membrane
5. Leakage of low-molecular weight cytoplasmic components, such as potassium ions, and inhibition of certain membrane-bond enzymes, such as adenosyl triphosphatase
6. Precipitation of the cytoplasm by the formation of complexes with phosphated entities, such as adenosine triphosphate and nucleic acids

Chlorhexidine is 1,6-di(4-chlorophenyl-diguanido)hexane, molecular weight 505.46 g/mole, a cationic bisbiguanide of the formula in shown in Fig. 2.64 and it becomes the diacetate when reacted with two-moles of acetic acid.

Characteristically, a bacterial cell is negatively charged, the nature of the ionogenic groups varying with bacterial species. It has been shown that given sufficient chlorhexidine, the surface charge of the bacterial cell is rapidly neutralized and then reversed. The degree of charge reversal is proportional to the chlorhexidine concentration and reaches a stable equilibrium within 5 min. The rapid electrostatic attraction of the cationic chlorhexidine molecules and the negatively charged bacterial cell undoubtedly contributes to the rapid rate of kill associated with chlorhexidine, although surface charge reversal is secondary to cell death. Electron microscopy and assay for characteristic outer membrane components, such as 2-keto-3-deoxyoctonate (KDO), demonstrate that sublethal concentrations of chlorhexidine bring about changes in the outer membrane integrity of gram-negative cells. An efflux of divalent cations, especially calcium ions, occurs prior to or during such outer-membrane changes. Chlorhexidine molecules are thought to compete for the negative sites on the peptidoglycan, thereby displacing metallic cations.

Local skin irritation reactions occasionally are reported with the use of chlorhexidine. Long-term experience demonstrated an extremely low incidence of sensitization reactions. There have been isolated reports of generation allergic reactions, and in the most severe cases shock has occurred (Kimura et a1. 1994).

Shelf stability of a product is of critical importance because it is affected by long-term antimicrobial and antioxidizing activity enhanced by preservative agents compared to only antimicrobial agents that are added to dressings for thwarting infection in a wound. Preferably, a preservative can be added to a product that possesses broad-spectrum antimicrobial activity that will allow the product to maintain activity before reaching a planned expiration date, and prevent infection when applied to wounds.

2.9 Optimal Properties of Barrier Dressings

The properties of human tissue were studied before evaluating noninflammatory response of injured tissue to contact with barrier dressings to protect wounds, and pertinent physical properties are contained in Appendix A 2.6.1. The following properties are those deduced from the experiments and tests.

- Impermeable to nutrients for supporting growth of aerobic microorganisms and impenetrable to anaerobic microorganisms
- Water vapor and gas semipermeable:
- Permeability – g $m^{-2} h^{-1}$;
- Water vapor transmission rate (WVTR) – g m^{-2} per 24 h period, at 37°C
- Standard water vapor transmission rate – g $m^{-2} h^{-1}$.
- Flexible, modulus – tan θ:
- Adhesion – Pa:
- Shelf stability, months:
- Low weight and cube:
- Convenient application:
- Fast set-up, curing and drying:
- Low cost:
- Nonflammable to low flammable:

Formulations of 2-octyl cyanoacrylate (Liquid Bandage^{R}), polyvinyl acetate, polybutryal were successful in rat studies, however, a spray-on bandage is recommended for ease-of-use and, especially for one-hand application such as with a "pump-type spray" bottle. Aerosol cans are under pressure and may not be allowed to be carried on aircraft.

The following formulations are recommended for testing on excised rates:

- Siloxane-based formulation:
  - Hexamethyldisiloxane (solvent)
  - Polyphenylmetholsiloxane
  - Preservative agent

- Acrylate terpolymer:
  - Poly(methacrylate-isobutene-moniisopropylmaleate)
  - Ethyl acrylate
  - Pentane

Both of these formulations can be applied to tissue by pouring or spraying on wounds. Both formulations are particularly conducive for pressure-spray or pump-spray applications. They both form continuous impermeable films over moist tissue, are water-proof after application, have long shelf stability, and are biocompatible. The siloxane-based material appears to have no "stinging" effect on excised tissue, and it does possess a very low surface energy that is difficult for environmental contamination and bacteria to attach. These materials are inexpensive compared to cyanoacrylates.

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Chapter 3
Tissue Adhesives

3.1 Introduction

A complete discussion on adhesives and sealants for medical applications and sutureless surgery is not possible in this report, but the author recommends a review of the proceedings from the *Surgical Applications of Tissue Sealants and Adhesives* presented in New Orleans on October 6–7, 2001 (see *References Section*). In addition, the "Tissue Sealant and Adhesive 6th, 7 and 8th Annual Conferences" sponsored by the Cambridge Healthtech Institute are excellent sources of information.

Tissue adhesives bind to protein, collagen, and other components of moist skin, etc., by noncovalent forces that are listed in Fig. 3.1. The hydrogen bond is the strongest among these series, and its mechanism is illustrated in Fig. 3.1. Different types of hydrogen bonds are listed in Fig. 3.2. The relative strengths of hydrogen bonds are shown in Fig. 3.3. Hydrogen bonding is a donor–acceptor interaction specifically involving hydrogen atoms (Jeffery, 1197). Hydrogen bonds are formed when the electronegativity, as defined by Pauling (1939), of A in an A–H covalent bond is such to withdraw electrons and leave the proton partially unshielded. To interact with this donor A–H bond, the acceptor B must have one lone pair of electrons or polarizable π electrons. The importance of the noncovalent bond in the human anatomy has been more appreciated with the advancement of modern chemistry and biology owing to a better understanding of orbital theory or organic chemistry (Rauck 2001).

The interactions of components of soft tissue within themselves as shown in Figs. 3.4 and 3.5 are indicative of the noncovalent bonds that must be formed between synthetic polymeric wound dressings and soft tissue in order to form a bond between tissues surfaces.

J.W. Gooch, *Biocompatible Polymeric Materials and Tourniquets for Wounds*,
Topics in Applied Chemistry, DOI 10.1007/978-1-4419-6586-8_3,

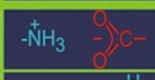
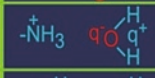
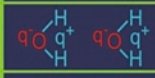
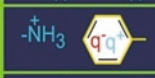
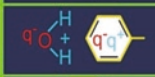
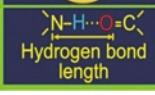
Interaction	Example	Relation of Energy to Distance (r)
(a) Charge-charge Longest-range force, nondirectional		$1/r$
(b) Charge-dipole Depends on orientation of dipole		$1/r^2$
(c) Dipole-dipole Depends on mutual orientation of dipoles		$1/r^3$
(d) Charge-induced dipole Depends on polarizability of molecule in which dipole is induced		$1/r^4$
(e) Dipole-induced dipole Depends on polarizability of molecule in which dipole is induced		$1/r^5$
(f) Dispersion Involves mutual synchronization of fluctuating charges		$1/r^6$
(g) van der Waals repulsion Occurs when outer electron orbitals overlap		$1/r^{12}$
(h) Hydrogen bond Charge attraction + partial covalent bond		Length of bond fixed

Fig. 3.1 Noncovalent bonding interactions, models, examples and dependence of energy on distance (*r*)

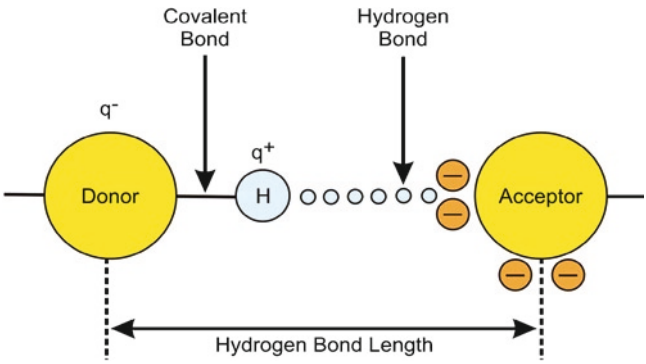
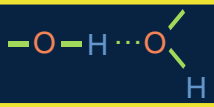
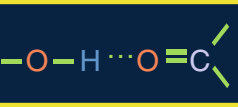
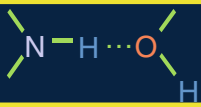
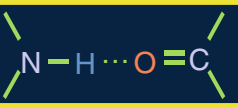
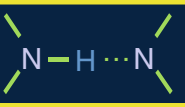
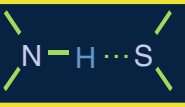


Fig. 3.2 Hydrogen bond (*dotted line*), donor, and acceptor, the H-bond exists only between the donor and acceptor with no covalent properties but about 0.1 the strength of a covalent bond

3.2 Photopolymerizable Adhesives

3.2.1 Soft Gels

Miki et al. (2000) used a photopolymerized liquid applied material for sealing corneal perforations. The novel photocrosslinkable methacrylated hyaluronic

Donor---Acceptor	Bond Length* (nm)	Comment
	0.28	H bond formed in water
	0.28	Bonding of water to other molecules often involves these
	0.29	
	0.29	Very important in protein and nucleic acid structures
	0.31	
	0.37	Relative rare; weaker than above

* Defined as distance from center of donor atom to center of acceptor atom.
For example, in the N –H O = C <bond it is the N – O distance.

Fig. 3.3 Hydrogen bonds and lengths showing the different types of hydrogen bonds, bond lengths and examples

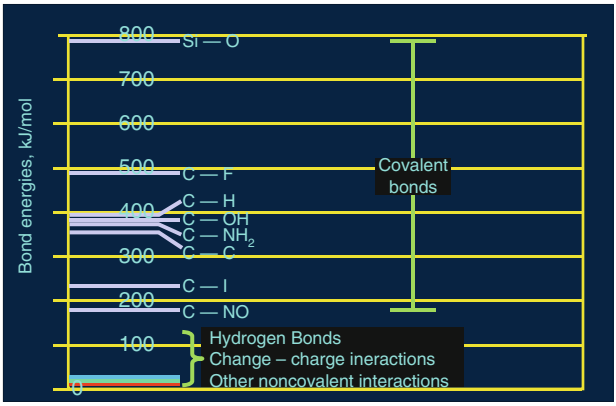


Fig. 3.4 Energies of covalent and noncovalent bonds

acid polymer sealed 37 of 38 experimental corneal perforations in rabbits. Corneal gluing with cyanoacrylate (cures on contact with surface) is an increasingly popular treatment, although this procedure is not approved for clinical use

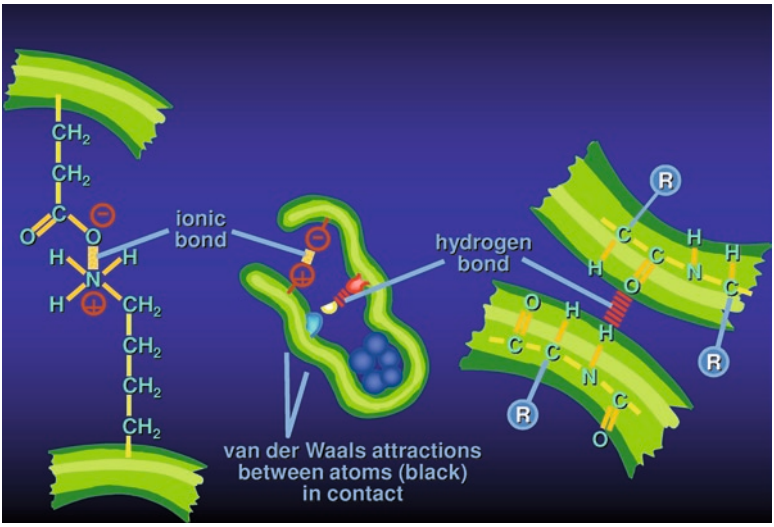


Fig. 3.5 Three types of noncovalent bonds that help protein fold: Hydrogen, ionic and van der Waals

in the USA. Cyanoacrylate is not smooth or malleable, and it exhibits a hard tissue-scratching surface and requires the patient to use a soft contact lens for comfort. Another surgical limitation when using cyanoacrylate is the immediate polymerization on a wet or moist corneal surface, and that property reduces the time allocated but increases the difficulty in applying the glue to a precise location in a well-controlled manner.

Smeds and Grinstall (2001) discussed an in situ polymerization technique for tissue engineering. Two photocrosslinkable polysaccharides composed of alginate and hyaluronan were initiated with visible radiation (photolysis) to form soft, flexible, and viscoelastic hydrogels. The degree of methacrylate modification (necessary to form sites for polymerization) affected mechanical properties such as swelling, compression, and creep compliance. Significant swelling (water) was observed up to 14-times original dry weight. The hyaluronan-based hydrogel is stronger and more resilient than the corresponding alginate gel. Scanning electron microscopy (SEM) and atomic force microscope (AFM) studies on both hydrogels show smooth and uniform surfaces at the macroscopic level with salient features observed only on the nanometer scale. Rapid polymerization in a minimally invasive manner indicates that these hydrogels are relevant for biomedical applications such as sealing wounds and reconstructing soft tissues.

Stable hydrogels can be formed by photocrosslinking the methacrylated polysaccharides by either argon ion laser (514 nm) or ultraviolet (245 nm) in the presence of a photoinitiating system. These hydrogels appear to be candidates for the sutureless closing of wounds. For example, two-sections of porcine skin are shown in Fig. 3.6, and their end-surfaces are wetted with an adhesive and

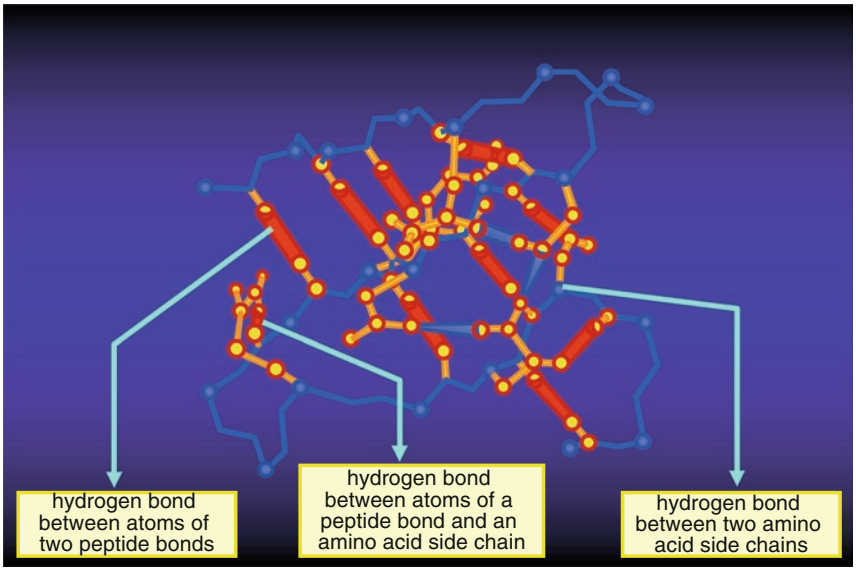


Fig. 3.6 Hydrogen bonds in a protein molecule: Peptide–peptide, peptide–amino acid and amino acid–amino acid

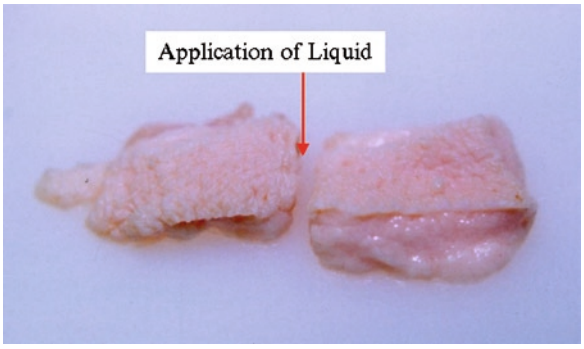


Fig. 3.7 Sections of porcine skin sectioned prior to bonding

with a lamp (preferably 100–1,999 mW/cm^2 and 400–510 nm spectral range) as shown in Fig. 3.7. Fiber optic-focused mercury lamps and lasers are most efficient with radiation and cure adhesives fastest. The bonded sections of porcine skin are shown in Fig. 3.8 and porcine aorta in Fig. 3.9. The tissue in either case can be bonded with adhesive applied to matting-surfaces or around the matting-surfaces, and the latter option provides greater opportunity for healing between surfaces without the interference of a barrier between tissues that the cured-adhesive material provides.

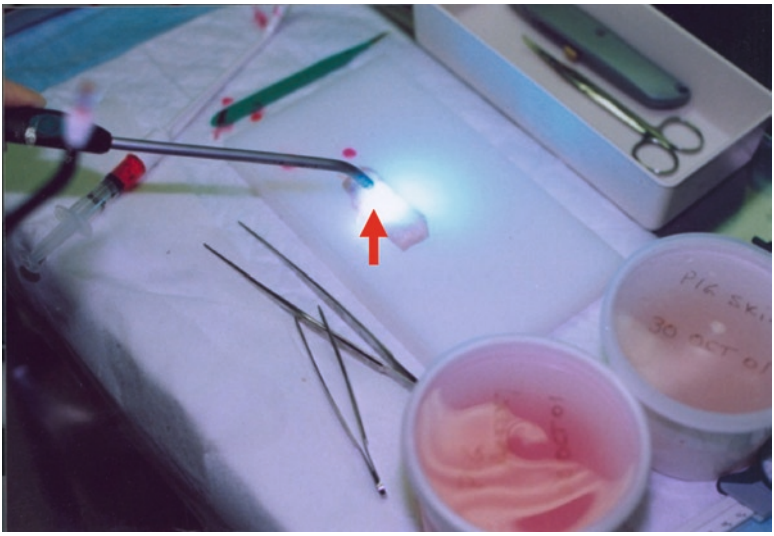


Fig. 3.8 Sections of porcine skin bonded with liquid photopolymerizable adhesive (*red arrow* showing optical fiber white light source)

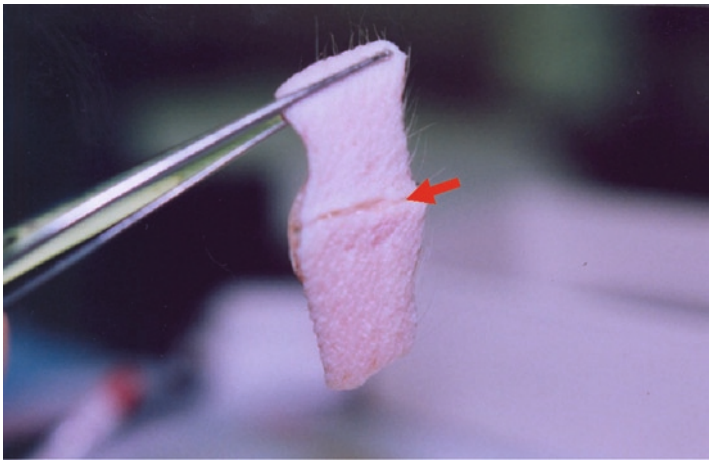


Fig. 3.9 Connected sections of porcine skin demonstrating strong photopolymerized bond (*red arrow* shows adhesive bond)

3.2.2 Hard Gels

Muggli et al. (1998) and others discussed the polymerization behavior of a new class of dimethacrylated anhydride monomers that react to form highly cross-linked degradable networks using different photoinitiation schemes. Polymerizations occurred in seconds to minutes depending on the initiating conditions, and conversions in excess

of 0.95 were achievable. A photobleaching visible light initiating system was used to improve the depth of cure for the production of polymers with appreciable dimensions. One potential application for the proposed multifunctional monomers is in vivo curing of high-strength, degradable polymers for fracture fixation or filling of trabecular bone defects. The polymers from this method are hard and relatively strong compared to soft compliant gels.

3.3 Multicomponent Bonding Adhesives

3.3.1 Albumin/Glutaraldehyde Adhesive

An albumin-gluteraldehyde tissue adhesive, commericially known as BioGLue and commerically manufactured by CryoLife, Inc., is a sealant for bronchial anastomoses and parenchyma lesions. The glue is a two-component (noncatalyzed) liquid applied adhesive that is mixed in a small handgun while traveling through a kinetic mixer (swirling while subdividing the flow). The replacement or support of sutures by gluing procedures has been investigated for many years. The fibrin glue, used most frequently today, did not warrant reliable sealing. Cyanoacrylate and gelatin-resorcin-formaldehyde adhesives have not been accepted generally in clinical practice because of problematic histocompatibility. However, a nontoxic, durable, and easy-to-use adhesive has not been available for lung surgery. Mixed bred sheep were used for the experiments. The results of the study supported the use of BioGlue^{R}, which was found to be a durable and easy-to-use adjunctive method of sealing bronchial anastomosis and lung parenchyma defects with minimal secondary healing disruptions such as granuloma formation. The results indicate that the use of the adhesive in human pulmonary surgery should be effective.

Gundry et al. (2000) discussed heart surgery utilizing BioGlue to seal a coronary anastomosis with high bursting strength. The experiments were performed on bovine hearts from a slaughterhouse and on live goats. The glue was applied on the outside of the tissue surfaces (in thick applications) and contact areas between tissues were purposely avoided. All goat hearts were weaned uneventfully from cardiopulmonary bypass. Generally, the author reported that the results were encouraging.

The adhesive material is in “bulk” and also described as a “wad” because it is not a thin-bead type of adhesive, but rather a bulky and thick application that absorbs into the body over a period of time.

3.3.2 Fibrin Glue

In addition to the standard fibrin cements derived from natural fibrogen, there is also a product manufactured by AuraZyme Pharmaceuticals referred to as FIBRX pho-

topolymerizable adhesive that was originally for hemostasis purposes. The active ingredient is fibrogen that photopolymerizes enzymatically. Increasing the percentage of active ingredient in this hydrogel increases its strength for bonding tissue.

3.4 Single-Component Bonding Adhesives

3.4.1 Hydrogels

Park and Park (1996) described a hydrogel as a three-dimensional network of hydrophilic polymers in which a large amount of water is present. In general, the amount of water is at least 20% of the total weight of the hydrogel structure. If water composes more than 95% of the total weight, then the hydrogel is called super-absorbent. The most characteristic property of hydrogel is that it swells in the presence of water and shrinks in its absence. The extent of swelling is determined by the nature (mainly hydrophilicity) of polymer chains and the crosslink density. Crosslink density is the number of chemical bonds between polymer chains expressed in molecular weight between crosslinks (e.g., 550 grams/crosslink) and other expressions as well. The lower molecular weight between crosslinks corresponds to higher crosslink density and a more rigid structure. If a hydrogel material is dried, the swollen network of the hydrogel will shrink during drying. Thus, the dried hydrogels (xerogels) become much smaller in size than when swollen with water. Hydrogels can retain their shape through several cycles of alternate shrinking and swelling although they can also degrade. Broadly interpreted, other hydrophilic solvents can be substituted for water such as polyethylene glycol.

Hydrogels and biodegradable polymers for bioapplications were discussed extensively by Ottenbrite et al. (1994). The involvement of a large number of scientists resulted in a better understanding of the physiochemical properties of hydrogels and development of new types of hydrogels. Organic hydrogels were physically characterized by Addad (1996) to explain swellability and reactions to minute's changes in temperature, etc.

The above photopolymerizable adhesives can be *broadly* included within the glass of hydrogels even if the hydrophilic components are a material such as polyethylene glycol substituted for water.

3.4.2 Dendrimers

Sepe (1998) discussed polyether-ester dendrimers synthesized from glycerol and lactic acid as globular monodisperse polymers composed of branched repeating units emitting from a central core. The key constituents of the polymer are combined with reiterative reaction steps from simple and abundant starting materials.

These dendrimers expand the repertoire of polymers available for study. Current investigations are primarily limited to linear polymers that possess ill-defined solution structures and fewer hydroxyl groups for further modification. The introduction of biocompatible building blocks (e.g., glycerol and lactic acid) augments the favorable and already known physical properties of dendrimers. These properties are likely to facilitate the design of new materials for specific biomedical and tissue engineering applications.

3.4.3 Cyanoacrylate

A comprehensive technical discussion on the chemistry and history of cyanoacrylate is contained in Chap. 2. Barrier Dressings, Sect. 2.4 Cyanoacrylate.

Cyanoacrylate has been used for sealing corneal perforations, but it is not smooth or malleable, and it exhibits a hard tissue-scratching surface and requires the patient to use a soft contact lens for comfort. Another surgical limitation when using cyanoacrylate is the immediate polymerization on a wet or moist corneal surface, and that property reduces the time allocated for and increases the difficulty in applying the glue to a precise location in a well-controlled manner.

Cyanoacrylate is not usable for in-dwelling applications, because the by-products of decomposition within the body are harmful to healthy tissue. Therefore, this material, although useful for sealing skin incisions with sutures, is not a viable candidate for repairing internal organs or vessels.

Ethicon, Inc., (Somerville, NJ) vendors Dermabond Topical Skin Adhesive for closing surface or skin type wounds. This material is not intended for internal use. Closure Medical Corporation manufactures the actual adhesive. Ethicon, Inc. is a Johnson & Johnson company. Dermabond is chemically "2-octylcyanoacrylate" and it cures on contact with water, ethanol and others (i.e., addition polymerization and catalysis by weak bases). For internal tissues, the excessive moisture can overcure cyanoacrylate by saturating its surface with moisture, cause disbondment from the substrate, and render it useless as an adhesive. The cured polymer possesses a hard and brittle consistency.

The modification of cyanoacrylate with other polymers and/or plasticizers provided more flexible adhesives, but the lack of bond and disbondment from excessive moisture continued to "float off" the adhesive without making firm contact with the tissue.

3.4.4 Emulsion Polymerized Gel Adhesives

Miniemulsions are prepared by emulsifying monomers and polymerization initiation agents in a single batch process (nonfeed) to form suspended particles of nanometer size (Gooch et al. 2000). Precrosslinked or gel adhesives have been prepared via miniemulsions by emulsifying hydrophilic acrylic monomers and crosslinking agents to

form nanometer size aqueous suspended particles of soft gels. The emulsion can be applied in a thin film (<20 μm) to form consistent tacky films that form a strong flexible bond on contact with an opposite surface treated with the same emulsion. Drying the aqueous phase of the emulsion can be accelerated with a warm stream of dry air. Also, drying can be accelerated with the addition of ethanol (or other alcohols) to increase the vapor pressure and rate of drying. The advantage of the gel-contact adhesive is the easy application of an aqueous fluid, air-drying, and immediate noncatalyzed formation of a strong bond between tissues. Another advantage is the ability to incorporate antimicrobial agents into the emulsified particles in situ along with other beneficial agents. These emulsions can be foamed to occupy large volumes.

Miniemulsions have the ability to accommodate many different types of monomers and additives (e.g., antimicrobial agents) including condensation-reaction materials and free-radical reaction monomers; biodegradable materials may be incorporated. An additional advantage of this type of adhesive is a strong bond without 100% coverage of tissue thereby allowing tissue-to-tissue contact for faster healing. However, excessive water prevented the particles in the emulsion from coalescing and forming a continuous film, therefore, wet surfaces are not ideal adherents for this type of adhesive.

3.5 Review of Sutureless Adhesives from Literature

The following Table 3.1 represents a summary of information from a review of literature.

3.6 Testing the Bonding Properties on Soft Tissue

Missing from the literature are standardized and comparative physical strength data for adhesives in neat form or as applied to the skin. The most comprehensive examples of the stress–strain relationships of excised human skin are appended (Appendix 2.6.1.); three axes of any material are demonstrated below, where *X*- and *Y*-axes are lateral (perpendicular to each other), but in the same plane, whereas the *Z* direction is not in the same plane, but perpendicular to *X*- and *Y*-axes as demonstrated in Fig. 3.10.

The three-dimensional geometric axes shown in Fig. 3.11 are important during testing for defining the orientation of a material (e.g., uniaxial or biaxial) and the corresponding direction of force (push or pull) with strength. The method of measuring the strength of the bond by tensile testing where both ends of the bonded sections are pulled in jaws – is shown by the instrument in Fig. 3.9. In other words, the direction or pull or applied force will have a different effect according to where it is applied, for example, on the skin, etc. The end-to-end bonding of skin and vessels (Figs. 3.11) are useful because, conventionally, tissues are sutured in the near- or full-thickness mode of skin, and adhesives can be applied to overlap parts of the bonded areas where necessary to strengthen specific areas where necessary.

Table 3.1 Comparison of sutureless bonding adhesives from literature

Adhesive	Advantages	Disadvantages
Photopolymerized hyaluronic acid (HA-MA) (polysaccharides)	Visible light cure Flexible/elastic	Requires several minutes to set and longer to completely cure
Photopolymerized Polyethylene Glycol	Flexible/elastic Cures < 60 s	Requires storing at –20°C
Photopolymerized anhydrides	Visible light cure	Requires several minutes to set and longer to completely cure
	Strong structure	Rigid/inflexible
Fibrin Glue	Cures on mixing	Requires 2+ min to set
Photopolymerized Fibrogen adhesives/sealants	Visible light cure Hermastatic	10 s to set, 60 s to cure
Porcine collagen/polyglutamic acid	Similar to fibrin, but absorbs into body faster	Requires 2+ min to set
Albumin/glutaraldehyde	Cures by mixing Sets in 30-60 s	Requires large amounts of adhesive for bonding and not for contact with healing tissue
Cyanoacrylate	Cures in 30–60 s	Incompatible materials Rigid/inflexible Disbondment with excessive moisture
Emulsified gel-contact	Cures 2–4 min; Medium strength/flexible	Cure should be faster; questionable biocompatibility

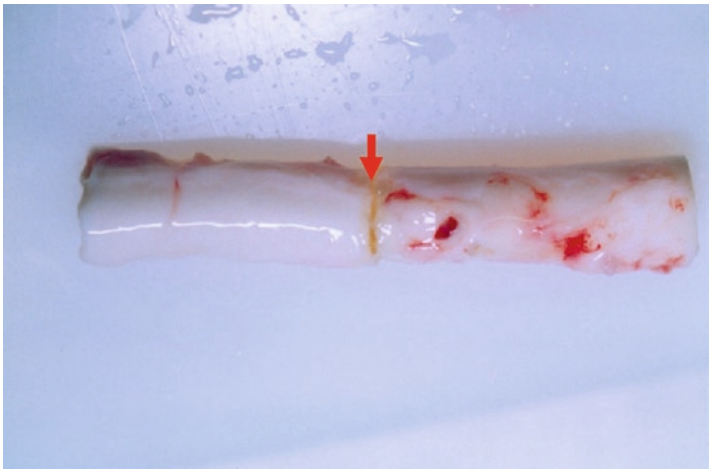


Fig. 3.10 Porcine aorta sections bonded with adhesive cured with polymerization (*red arrow* shows adhesive bond (see Fig. 3.13).

Theoretically, sutures can be eliminated (or a minimum of sutures) and a perfect bond can be provided by adhesives within 60 s to provide sufficient bond-strength while preventing the leakage of blood from vessels or skin, whereas sutures require a period of healing before a perfect bond is achieved. The bond can be seamlessly formed to withstand a hydrostatic pressure test comparable to human blood pressure within the same curing time (~60 s).

The following standard test methods for evaluating and comparing adhesives were employed: Tensile test using an LRX Material Testing Machine (Fig. 3.12) or equivalent for the purpose of evaluating adhesive materials, including a tensile (pull) test and shear (peel) test. Also, it will be important to observe and report the strength at break compared to animal tissue without adhesive.

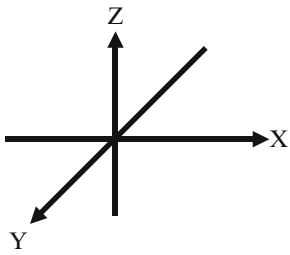


Fig. 3.11 Three-dimensional planes of axes of a material

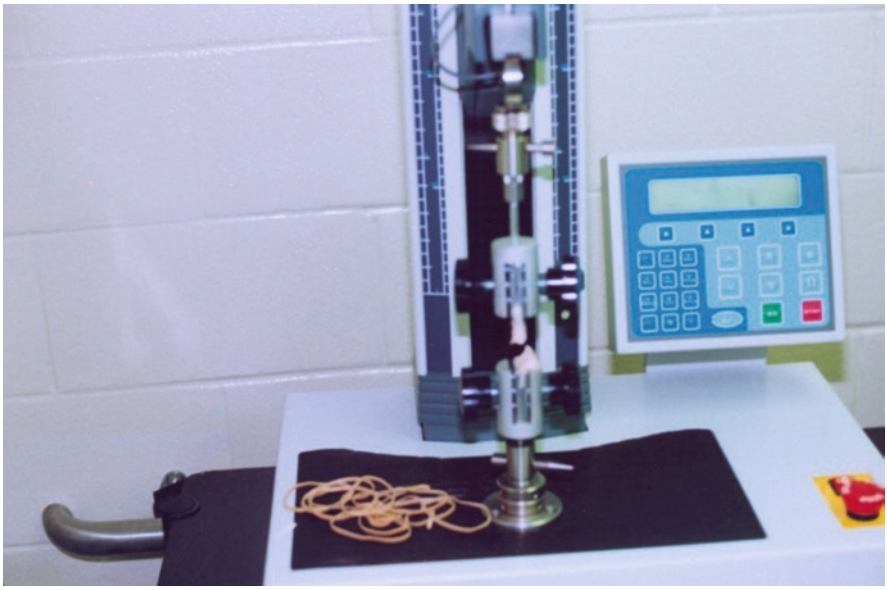


Fig. 3.12 LRX materials testing machine®

Fig. 3.13 Operator using white light (509 nm) lamp to establish end-to-end bonding of two sections of porcine skin

Important parameters for tensile testing include:

- Rate of applied force or rate of travel of grippers holding the test specimen. (10 mm/min cited by Sekine 2001).
- Temperature of the specimen and testing environment (body temperature = 98.6°F or 37°C)
- Cross-sectional area of the test specimen
- Overall length of specimen between testing grips

The stressed or pulled specimen bonded with photopolymerized adhesive failed as shown in Fig. 3.14 within the bonded area as expected, because porcine skin breaks at a tensile strength several times greater than the adhesive. The thickness and placement of adhesive is importantbecause a barrier between the injured tissue surfaces impedes the healing process. The adhesive is most preferably placed in a very thin layer on matting tissue surfaces and more substantively on exterior surfaces around the bond.

A typical "stress versus strain" relationship is shown in Fig. 3.15. The breakpoint is the stress at a strain at which the material fails.

The following stress–strain curve is an example of a "pull" test where the specimen is stressed until it ruptures.

The strength of the material is calculated as:

$$\text{Strength} = \text{Force}/\text{Area}$$

$$\text{Strain} = (\text{Extension during test}/\text{Initial extension}) \times 100$$

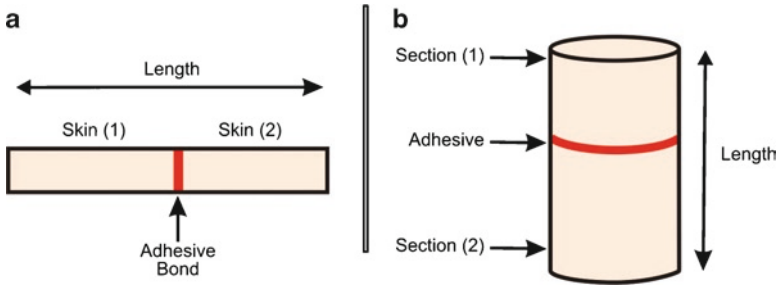


Fig. 3.14 Basic types of models for testing adhesives (**a**) end-to-end skin flap, and (**b**) end-to-end blood vessel

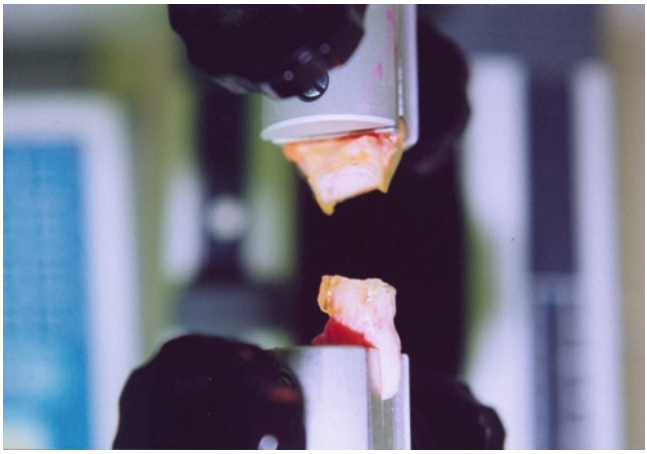


Fig. 3.15 Tensile test of "end to end" bonded porcine aorta at break

Another important factor for tensile testing is cyclic testing or "applying force and releasing (or relaxing) force" on a test specimen to evaluate its ability to endure cyclic fatigue. A typical "stress vs. strain" relationship is shown in Fig. 3.16 and a cyclic stress relationship in Fig. 3.17.

This test may be useful for advanced tests where the bond must endure repeated deformation with some associated stresses including the following.

(a) Tests comprising direct application of adhesives to animal specimens for purpose of evaluating biocompatibility between adhesives and animal tissue. These shall the following observation:
(b) A peel test will provide the most relevant strength data for evaluating the physical characteristics of the material with regard to the protective barrier for burned tissue.

Test methods will logically include tensile testing to measure strength, and hydrostatic testing at a pressure with a liquid to test for leaks. Pressures not exceeding 300 mm Hg pressure (internal heart fluid pressure) could be employed for this purpose.

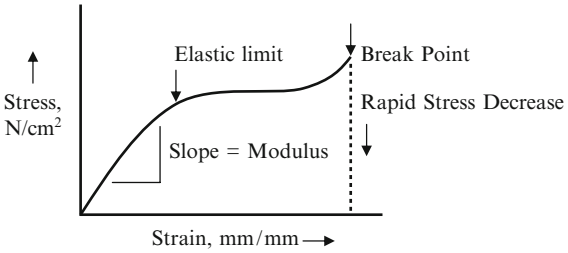


Fig. 3.16 Stress versus stain, elastic limit and break point

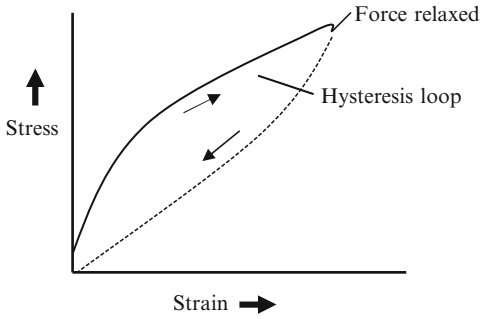


Fig. 3.17 Standard stress versus strain curve for testing materials

3.7 Summary of Tissue Bonding Adhesives

The criteria for sutureless adhesives are still being developed. Although the criteria are not well defined, the following Table 3.2 embodies certain essential properties that will continue to apply until *in vivo* testing is complete. These criteria are offered as a template for collecting data after application of adhesives in animals, and the data listed in Table 3.3 are indications of the stress-strain relationships to be expected in humans.

Leading candidates from the Sects. 3.2–3.5 will be selected and will be evaluated in an orderly form to characterize their properties followed by application to animals for the evaluation of biocompatibility and effectiveness for closing wounds without sutures.

Commercial Photopolymerizable Adhesives:

- Genzyme Focal-Seal (Polyethylene glygol-lactide)
- Crylolife FIBRX (fibrogen)

Developing Photopolymeriable Adhesive Adhesives:

- Duke University (polyhyaluronic acid)
- Harvard University (not tested)

Table 3.2 Suggested criteria for sutureless adhesives

Property	Value
Curing	
Time to set, seconds	–
Time to reach 100% strength, seconds	–
Physical	
Tensile strength, Pa	–
Modulus, Pa	–
Cyclic fatigue, Hz/Cycles	–
Tear/peel strength, Pa	–
Biodegradability (In Vivo)	
Time to degrade, hours	–
Time to absorb into body, hours	–

Table 3.3 Tensile testing (pull only) results of commercial and developing adhesives

Adhesive	Tensile strength (kPa)	Extension (%)
Genzyme, Inc., Focal-Seal		
Porcine skin	75.8	703.7
Porcine aorta	161.0	148.8
Adhesive alone	299.2	1,237.5
Cryolife, Inc., FIBRX		
Porcine skin	5.2	10.8
Porcine aorta	127.8	81.7
Cryolife, Inc., BioGlue		
Porcine skin	37.3	8.9
Porcine aorta	380.5	43.3
Neat adhesive	703.5	21.0
Ethicon, Inc., Dermabond (2-cyanoacrylate) (for comparative purposes only)		
Porcine skin	408.8	42.9
Porcine aorta	431.2	13.9
Duke University		
Porcine skin	151.5	89.6
Porcine aorta	116.7	35.5

Fortunately, there exist many candidates for closing wounds using different types of adhesives. However, little information is available for a comprehensive and comparative evaluation of adhesives for sutureless bonding of tissue. The literature mentions some testing on animals which is significant, but little physical testing information such as tensile strength; fatigue cycling or tear/peel resistance is mentioned. It is suggested that this be done. As shown in the above table, the first comparative data (one sample per measurement) on these materials have been collected although they are incomplete. At least ten samples should be tested for physical properties as shown above whereas only one sample was tested per measurement due to constraints of time and resources.

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Chapter 4
Antibacterial Aqueous Emulsions

4.1 Introduction

Researchers at the Center for Biologic Nanotechnology, University of Michigan, Ann Arbor Michigan published and patented results of project MDA 972-1-007 of the Unconventional Pathogen Countermeasures Program, Defense Advanced Research Project Agency. The introduction of biological agents to our working and living environment has forced an examination of preventative and decontamination methods (Mobley 1995). The project involved antibacterial aqueous nanoemulsions to treat bacteria-infected people and disinfect contaminated surfaces such as vehicles and medical equipment exposed to anthrax spores. Literature and patents were reviewed, materials and equipment were procured, and emulsions were prepared and evaluated for antibacterial activity. The antibacterial emulsion technology could possibly be useful if it is reproducible. Regarding emulsion technology, the term "emulsion" is the industry standard, and "nanoemulsion" pertains to emulsified particle less than a micron in diameter (e.g., typical of paint latex particle size), but greater than a nanometer. Actually, particle diameters for industrial emulsions are usually in the nanometer range.

An emulsion consists of lipophilic particles dispersed in an aqueous phase and stabilized with a surfactant as shown in Fig. 4.1. The surfactant is a molecule or a polymerized molecule that possesses hydrophilic and lipophilic groups usually at the opposite end of the molecule. The hydrophilic group is soluble in the aqueous phase and stabilizes the particle in the aqueous medium while the lipophilic group is soluble in the lipid or oil phase, that is, in the following cases, lipophilic or hydrophobic.

Individual materials discussed in patents were tested separately to determine their antibacterial activities along with known control materials such as chlorhexidine derivatives. Attempts were made to compare prior art to any novel developments in the US patents and recently published literature.

Fatty acids were not discussed in the US patents or literature for the purpose of forming nanoemulsions, but they were included in the study for comparative purposes and because of their low viscosities compared to ester-oils that consist of three fatty acids molecules per oil molecule. The lower viscosity liquids are easier to shear in a homogenizer

J.W. Gooch, *Biocompatible Polymeric Materials and Tourniquets for Wounds*,
Topics in Applied Chemistry, DOI 10.1007/978-1-4419-6586-8_4,

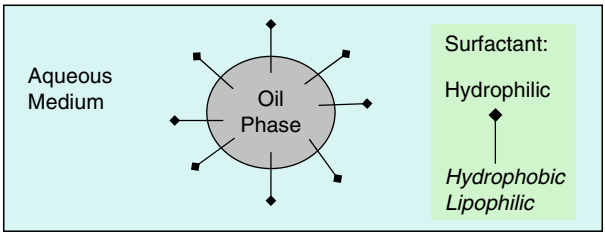

Fig. 4.1 Emulsified particle demonstrating hydrophilic and hydrophobic end groups of surfactant

if the surfactant is stabilized and, therefore, produce smaller diameter particles. Reducing the diameter of particles corresponds to longer shelf-stability of emulsions. The composition of oils is listed in Table 4.1. The composition of vegetable and marine oils is discussed next. Oils, fats, tallows, and other names for vegetable, animal, and marine oils consist of three molecules of fatty acids to one molecule of trifunctional glycerol to form a single triglyceride molecule. The structures of common fatty acids are show in Table 4.2.

A major consideration when using a water-soluble antibacterial agent, such as water soluble Sulfamylon^{R} in solid form, is osmolality (Segel 1976, Guyton 1986) which causes excessive shifts in osmotic pressure due to aqueous-soluble materials applied to tissues resulting in the destruction of tissue cells. In the following experiments, different concentrations of Sulfamylon^{R} and other agents were sometimes tested above practical limits for the application to tissue for the purpose of studying antibacterial activities. Sulfamylon^{R} is a known antibacterial agent used as a control in this study.

The objectives of the following research were as follows:

- Review information from antibacterial emulsions reported in patents and literature
- Reproduce emulsions purported to be antibacterial
- Test reproduce emulsions for antibacterial activity and reproduce, if possible
- Report experimental results

4.1.1 Literature

The following brief literature review of bacteria cellular structure lists just some author's reports from the comprehensive published literature on findings that are pertinent to the present work.

- The detailed structures of bacteria (and spores), viruses and fungi were discussed by Black (2002), and the spore structures were discussed by Russell (1990).

Table 4.1 Fatty acid composition of vegetable and marine oils (Gooch 1997)

	Number of carbons	Double bonds	Tung	Olitica	Dehydrated castor	Fish	Linseed	Safflower	Soya	Talls oil acids	Cottonseed	Coconut
Oleic	18	1	8	6	9	10	22	13	25	46	24	
Linoleic	18	2	4		82	15	16	75	51	41	40	7
Linolenic	13	3	3				52	1	9	3		2
Eleostearic	18	3	80									
Licanic	18	3		78								
Ricinoleic	18	1			9							
Palmitoleic	16	1										
Arachidonic	20	4				30						
Clupanedinic	22	5				25						
Stearic	18	0	1	5	2	2	4	4	4	3	4	6
Palmitic	16	0	8	7		12	6	6	11	5	29	11
Myristic	14	0				6					1	18
Lauric	12	0										44
Capric	10	0										6
Caprylic	8	0										6
Iodine Value							170–190	140–150	120–140	128–138	99–138	7–10
Viscosity – Gardner Holdt							A	A	A	A	A	A
Lb/gal							7.76	7.70	7.70	7.53	7.63	7.68
Color, Gardner							11	10	10	4	8	5
Acid value							4	4	3	194	1	2
Saponification value							190	190	190	196	190	250

Table 4.2 Fatty acids, IUPAC name and sources (Gooch 1997)

Common name	Carbonatoms	Doublebonds	Scientific name	Oil/fat sources (Triglyceride)
Butyric acid	4	0	Butanoic acid	Butterfat
Caproic acid	6	0	Hexanoic acid	Butterfat
Caprylic acid	8	0	Octanoic acid	Coconut oil
Capric acid	10	0	Decanoic acid	Coconut oil
Lauric acid	12	0	Dodecanoic acid	Coconut oil
Myristic acid	14	0	Tetradecanoic acid	Palm kernel oil
Palmitic acid	16	0	Hexadecanoic acid	Palm oil
Palmitoleic acid	16	1	9-Hexadecenoic acid	Animal fats
Stearic acid	18	0	Octadecanoic acid	Animal fats
Oleic acid	18	1	*cis*-9-Octadecenoic acid	Olive oil
Ricinoleic acid	18	1	Hydroxy-9-*cis*-octadecenoic acid	Castor oil
Vaccenic acid	18	1	Octadec-*trans*-11-enoic acid	Butterfat
Linoleic acid	18	2	*cis,cis*-9,12-octadecadienoic acid	Grape seed oil
Linolenic acid (Alpha)	18	3	*cis,cis,cis*-9,12,15-octadecatrienoic	Flaxseed (linseed) oil
Linolenic acid (Gamma)	18	3	*cis,cis,cis*- 6,9,12-octadecatrienoic acid	Borage oil
Arachidic acid	20	0	Icosanoic acid	Peanut oil, fish oil
Gadoleic acid	20	1	*cis*-9-eicosenoic acid	Fish oil
Arachidonic acid (AA)	20	4	*cis,cis,cis,cis*-5,8,11, 14-eicosatetraenoic acid	Liver fats
Eicosapentaenoic acid	20	5	*cis,cis,cis,cis,cis*-5,8,11,14,17-eicosapentaenoic acid	Fish oil
Behenic acid	22	0	Docosanoic acid	Rapeseed oil
Erucic acid	22	1	*cis*-Docos-13-enoic acid	Rapeseed oil
Docosahexaenoic acid	22	6	*cis,cis,cis,cis,cis*-7,10,13,16, 19-docosapentaenoic acid	Fish oil
Lignoceric acid	24	0	Tetracosanoic acid	Small amounts in most fats

- McDonnell and Russell (1999) and Block (2001) comprehensively listed the many different methods of preventing infections.
- Russell (1990) reported the outer cellular membrane of the bacteria cell, which is understood as well as the more complex structure of the spore membrane – agents that are bacteriostatic and bacteriocide as well as sporostatic and sporicidal.
- Leive (1973) offered theories on the function of the gram-negative envelope.
- Helenius and Simmons (1975) discussed the solubilization of bacteria cell membranes with detergents or surfactants, and Nixdorff (1978) discussed the interaction of lipopolysaccharides with detergents and the possible role in the detergent resistance of the outer membranes of gram-negative bacteria.
- Tagesson and Edling (1984) reported the information on the surface-active agents and additives and their effects on the integrity of rat intestinal mucosa.
- Howard (1985) discussed the toxicity of antimicrobial agents.
- Foster and Johnstone (1990) offered a theory for the mechanism of germination for the spore.
- Rutala (1996) prepared a comprehensive set of guidelines for sporicidal agents to combat bacterial spores and in 1996 provided guidelines for the selection of topical and other antimicrobial agents.
- Howard (1985) provided a review of the toxicity of topical antimicrobial agents and their harmful effects.
- Mobley (1995) gave insight into the future by reviewing biological warfare in the 20th century and lessons from past wars.
- McDonnell and Russell (1999) provided a review of antiseptics and disinfectants for activity, action, and resistance of microorganisms.

4.1.2 Patents

Patents have been granted for innovations involving the preparation and activities of broad-spectrum antimicrobial emulsions from 1977 (Sippos) to 2000 (Baker). All of these patents claim antibacterial activity, but all involve additives in the non-aqueous phase of the emulsion that are known to be antibacterial alone and before emulsification. Wide spectrum applications for these nanoemulsions have been claimed with positive results for bacteria, fungi, and viruses. The term "nanoemulsion" is used in US patents discussed below, but the generic term for the product of an emulsification (Gooch 2002, 1980) of a liquid within a liquid is an "emulsion." United States patents 6,015,832 and 5,547,677 were examined and formulations in key claim statements were reproduced, and tested using standard methods for effectiveness. Additional patents listed in the reference section were reviewed as part of this study.

The following patent literature was studied to support the investigation:

- US Patent 6,015,832 (2000), Baker, J. R.
- US Patent 5,547,677 (1996), Wright, D. C.

- US Patent 4,636, 525 (1987), Ochiai, M. K.
- US Patent 4,450,573 (1985), Neurath, M. K.
- US Patent 4,197,318 (1980), Sipos, T. J.
- US Patent 4,147, 663 (1979), Rutledge, T. F.
- US Patent 4,020,183 (1977), Asculai, S. S.
- US Patent 4,006,218 (1977), Sipos, T. J.

4.1.3 Preparation of Emsulsions

The methods of preparing aqueous emulsions are outlined in the referenced literature and US patents. For comparative purposes, the information, formulations, and procedures were followed precisely.

The Silverson Homogenizer (Fig. 4.2) was initially employed to prepare oil in water emulsions. Shearing the particles using the Fisher Dismembrator (Fig. 4.3) reduced the average particle diameters in each emulsion further. The APV-Homogenizer was discovered to be the most efficient method of reducing the average particle size of an emulsion to the narrowest distribution. The Silverson and Fisher machines use zonal method of imparting energy where the emulsion particles are recirculated during shearing, providing a range of particles sizes until they are rendered uniform with constant shearing energy. Whereas, the APC-Homogenizer is a "flow-through" type machine where a continuous stream flows through a cavitation zone and into

Fig. 4.2 Silverson Homogenizer machine used for shearing liquid in water to form emulsions

Fig. 4.3 Fisher Scientific Sonic Digital Dismembrator, controller on left and horn on right

a separate vessel without mixing with other particles. This method is most effective because "re-shearing" particles can cause them to impinge, coalesce, and form larger particles, which is a process of diminishing returns. The Brookfield Viscometer (Model DV-E) is a revolving spindle in a liquid type instrument (Fig. 4.4), and it was employed to measure the viscosities of materials as oil and fatty acids used in the formulations of emulsions.

4.2 Testing Plan: Standard Sporicidal and Antibacterial Methods

4.2.1 Kirby-Bauer Plate Test (Disk Sensitivity Method)

The Kirby-Bauer technique (Black 2002) was used to test for antimicrobial activity of the product. The procedure follows:

- In the morning, BHI (brains-heart infusion) nutrient broth was inoculated with bacteria, from an overnight culture or agar, to be used within 4 h. Within that incubation time, Mueller–Hinton (MH) plates were allowed to reach room temperature.
- Tubes containing 9 ml each of agar (1.5% wt/wt) were placed in a boiling bath to liquefy, and held at 55°C to prevent solidification.
- After 4 h, the agar tubes were inoculated with 1 μl volumes bacteria cultures prepared earlier, and the agar was immediately poured onto an MH plate. The

Fig. 4.4 The Brookfield Viscometer (Model DV-E) is a revolving spindle in a liquid type instrument

plate was rotated to spread the overlay evenly and allowed to dry for 5 min. This step was repeated for a duplicate plate.

- A test-product was placed in each no. 6 well plate. Discs (6 mm dia.) prepared from porous filter paper were placed in the test-product for a standard time (1 min) followed by transferring to a designated section of the agar/overlay plate. After 5 min, the plate was placed in an incubator (37°C) in the upside-down position.
- After 24 h, the zone of inhibition (no bacterial growth) was measured and compared to standards for the specific bacteria utilized for the test.
- Bacteria cultured for these tests were: *Proteus mirabilis*, *Pseudomonas aeruginosa* (59-1244/lux), *and Staphylococcus aureus* (29213 or ATCC^{R} 25923). Luminescent bacteria are abbreviated as "lux."

4.2.2 Baker Suspension Test (Adapted)

The Baker suspension test (Hamouda and Baker 2000) was used to test the antimicrobial activity of a product in a liquid suspension. The procedure follows:

- A 20 µl volume of overnight bacteria-culture was placed in 2 ml of BHI broth and was grown for a minimum of 5 h in a 37°C incubator.
- A 200 µl volume of 5 h growth of BHI broth was placed into microcentrifuge tubes (one tube for test-product tested).
- A 200 µl volume of the test-product was placed in a microcentrifuge tube and was inverted two to three times to mix.
- BHI broth was used instead of a product as the control in a separate tube.
- All tubes were placed in a shaking-incubator for 15 min at 37°C.
- In a no. 96 well plate, place180 µl of BHI in six wells, and repeat for each test-product.
- After 15 min incubation, transfer 20 µl volume of the bacteria/test-product into the first well of the no. 96 plate and continue down the plate with 1:10 dilutions. Repeat for all bacteria test-products and control, and follow with colony counts.
- Deduct the CFU/ml and use the control to calculate the percent kill of bacteria.
- Bacteria cultured for these tests were: *P. mirabilis*, *P. aeruginosa* (59-1244/lux), *and S. aureus* (29213 or ATCC^{R} 25923).

4.3 Results of Testing

4.3.1 Kirby-Bauer Plates (Disk Sensitivity) Method

The formulations in US Patent 6,015, 832 (Baker 2000) were reproduced and tested together with additive materials to the emulsified particles described in this patent:

- Tributyl phosphate, $(CH_3CH_2CH_2CH_2O)PO$ (Sigma-Aldrich Corp.)
- Cetylpyridinium chloride Monohydrate, $C_{21}H_{38}ClN.H_2O$ (Sigma-Aldrich Corp.)
- Dimethyldioctacylammonium bromide, $[CH_3(CH_2)_{17}]_2N(Br)(CH_3)_2$ (Sigma-Aldrich Corp.)
- Ethylenediaminetetracetic acid, $C_{10}H_{16}N_2O_8$ (Sigma-Aldrich Corp.)

It is interesting interest to note that all of the above compounds (properties appended) dissociate in aqueous solutions to produce cations (+), and this phenomenon has been observed to provide antibacterial activity (Seymour 2001).

Controls were employed to compare emulsions and additives mentioned in the patents to standards and known antibacterial agents such as 6% chlorhexidine diacetate in 70% isopropanol (wt/vol). Nonionic surfactants have no antibacterial activity

Table 4.3 Additives and activities of antibacterial nanoemulsions in US Patents, Kirby-Bauer plate method

	Proteus mirabilis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		59-1244/lux	ATCC[R] 25913
Material	Average diameter of zone, mm		
Tributyl phosphate (disc)	(6)	(6)	(12)
Cetylpyridinium chloride monohydrate	(6)	(6)	(6)
Dimethyldioctacylassmmonium bromide (solid)	(6)	(6)	(6)
Ethylenediamine-tetramineacetic acid (solid)	(31)	(22)	(29)
Control:Chlorhexidine, standard 6% chlorhexidine diacetate in 70% isopropanol disc (disc)	(14)	(15)	(24)

Note 1: 6 mm is diameter of test disk
Note 2: Acceptable zone diameter is greater than or equal to control disc
Note 3: Zone diameter measured with Fisher–Lilly Antibiotic Zone Reader II
Note 4: Solids were placed on discs in equivalent masses with a spatula and measured from middle of solid
Note 5: ATCC[R] is a registered trademark for American Type Culture Collection

and were not tested except as a combined component in the emulsions. Standard bacteria cultures were employed to test the activity of the agents:

- *P. mirabilis*
- *P. aeruginosa* 59-1244/lux
- *S. aureus* ATCCR 25913

The results of testing of all of the above materials with standard cultures of bacteria are appended Fig. A4.1, and the accompanying blank Petri dish is for visual inspection of test materials and their identification on the Petri dishes. The antibacterial activity of each material is contained in Table 4.1. Ethylenediaminetetraacetic acid had consistent activity, tributyl phosphate exhibited activity against *S. aureus*, and the control (chlorhexidine diacetate) performed consistently against all bacteria.

Referring to US Patent 6, 015, 832 (Table 1), an emulsion was prepared from the disclosure and the activity tested against *P. ageruinosa* and *Escherichia coli* bacteria. The results are reported in Table 4.2 from plate tests and an image of each agar plate is appended (Fig. A4.1). The emulsions were prepared again for reproducibility, and the tests were repeated (without dilution) in Table 4.3 with similar results.

4.3.2 Baker Suspension (Dilution) Method

The nanoemulsions in US. Patent 6,015, 832 (Tables 1 and 2 in patent) were tested for antimicrobial activity, using the same bacteria above, together with oleic fatty acid and caprylic fatty acid. The average particle size of the nanoemulsions in (US. Patent 6,015, 832) were smaller than reported in the patent, and

the author theorized that the viscosity (57.8 cP) of the lipophilic soy oil-medium was too large and substituting lower viscosity fatty acids, oleic (28.7 cP) and caprylic (8.5 cP), would decrease the particle size of emulsions. The particle size decreased, but not to the lower average diameters reported in the patent. The Baker Suspension Method (procedure appended) was employed to test the activity of the liquid agents in liquid media. The results of the suspension tests are reported in Table 4.4, and only caprylic acid had significant antibacterial activity.

Emulsions were prepared from caprylic and olive oil to evaluate the effect of caprylic acid as an antibacterial agent in an emulsion. Pure caprylic acid did not form a stable emulsion, but did so when dissolved in olive oil, and was stable in excess of 7 days. All other steps to emulsify the liquids were the same, and the results are shown in Table 4.5. Antibacterial activity is significant at 1% caprylic acid, but acceptable antibacterial activity is achieved after

Table 4.4 Activity of nanoemulsions, US Patent 6,015,832, Kirby-Bauer plate method

	Proteus mirabilis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		59-1244/lux	ATCC^{R} 25923
Material	Average diameter of zone, mm		
US Patent 6,015,832 Nanoemulsion			
1:1	6	6	11.5
1:50	8.5	6	8.5
1:1000	6	6	11.8
Control			
5%S, 15%C	15	18	22.5
5%S, 10%C	16.5	19.25	22

Note 1: S is propanol solution (wt/vol)
Note 2: Tween^{R} surfactants (see Reference Section) were used consistently in the formulations for preparation of these emulsions

Table 4.5 Activity of nanoemulsions, US Patent 6,015,832 and US Patent 5,547,677, Kirby-Bauer plate method

	Proteus mirabilis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		59-1244/lux	ATCC^{R} 29213
Material	Average diameter of zone, mm		
US Patent 6,015,832	6.0	6.0	14.8
US Patent 5,547,677	6.0	6.0	9.5
Controls			
10% Chlorhexidine diacetate	25.25	190	24.25
20% Chlorhexidine digluconate	17.75	20.25	25.5

Note 1: Experimental data are appended (Fig. A4.4)
Note 2: The standard disk diameter is 6 mm, an acceptable experimental value (zone of inhibition) is greater than the disc and can also be compared to chlorhexidine controls that are known to be effective antibacterial agents

5% caprylic acid in an olive oil emulsion. However, a more stable emulsion was desired, because the emulsions below separated after 14 days. Lower average particle diameters were required to increase the stability of the same emulsions.

4.4 Novel Antimicrobial Emulsions and Particulate Formulations

4.4.1 Emulsification of Fatty Acids

As expected, the emulsified fatty acids chosen for their low viscosities did correspond to lower average particle size, but the emulsions consisting of only nonionic surfactant and caprylic acid was antimicrobial though not very stable (separation in 24 h). Because none of the surfactants are antimicrobial, the caprylic acid (octadecanoic acid) was deducted to be the active agent. On the basis of these unexpected observations, emulsions were prepared from mixtures of caprylic and oleic acids that were stable in excess of days at 5–15% wt/wt caprylic in oleic acid. The nonsoluble and nonionic oleic acid serves to control the exposure of slightly soluble caprylic acid on water and the effect on pH surrounding the emulsified particle, while allowing the caprylic acid to disassociate in water to form a weak acidic zone and positive charge around the emulsified particle, and this process is demonstrated in Fig. 4.5.

It is important to point out that although the caprylic acid/oleic acid emulsion demonstrated small, but measurable, antimicrobial activity in the liquid phase (Baker Suspension Test), and the Kirby-Bauer Plate test showed marginal antimicrobial

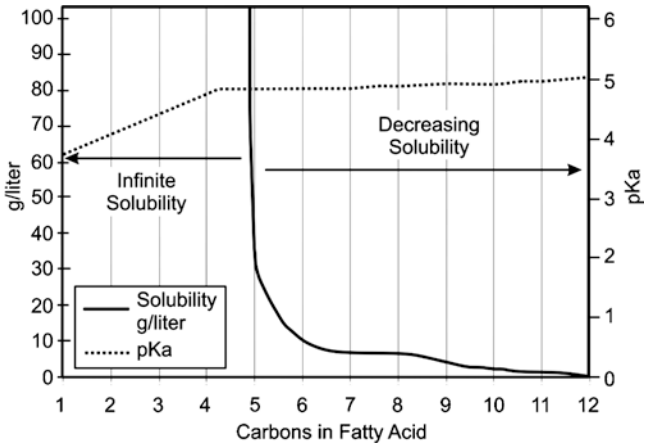

Fig. 4.5 Aqueous solubility, pKa versus fatty acids

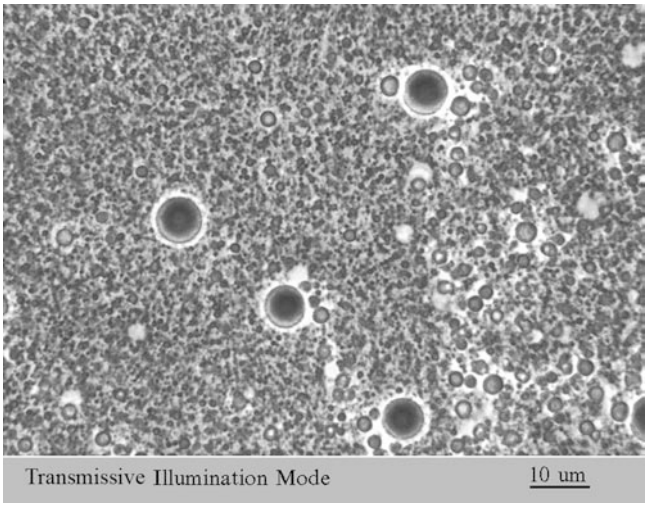

Fig. 4.6 Photomicrograph of oleic/caprylic fatty acid aqueous emulsions showing particle size distribution

activity. This means that the agent is most effective in liquid form when it can saturate a surface (e.g., a rinse or wash solution).

A superior method of emulsifying the above emulsions to the most narrow particle size distribution is by flowing a preemulsion (prepared using the Silverson Homogenizer) through the APV Products – Homogenizer that shears particles by utilizing a mechanical piston-cylinder "total flow-through" cavitation device. The flow-through emulsification mechanism allows a stream of preemulsion to be sheared simultaneously compared to a zonal shearing with a turbine or ultrasonic probe. A photomicrograph of an APV Products homogenized oleic acid emulsion (35% wt/wt) is shown in the Fig. 4.6, which demonstrates the range of particle sizes that are inherent in this emulsification process. The volume–weight and number–weight distributions and average particle diameters are provided in Fig. 4.7.

4.4.2 Emulsified Fatty Acids and Antimicrobial Activity

Stable aqueous emulsions were prepared consisting of vegetable oils and fatty acids, nonionic surfactants and deionized water, and prehomogenized with the Fisher Sonic Dismembrator and completed with the Silverson Homogenizer (emulsifier turbine).

Three fatty acids comprise an oil and are usually referred to as just "acids." Caprylic acid (octanoic acid or 1-heptanecarboxylic acid) is the most antimicrobial emulsified fatty acid compared to capric and oleic (higher in molecular weight compared to caprylic acid). Oleic acid (*cis*-9-octadecenoic) and caprylic acid are natural fatty acids found in vegetable and marine oils. The oleic acid or similar low

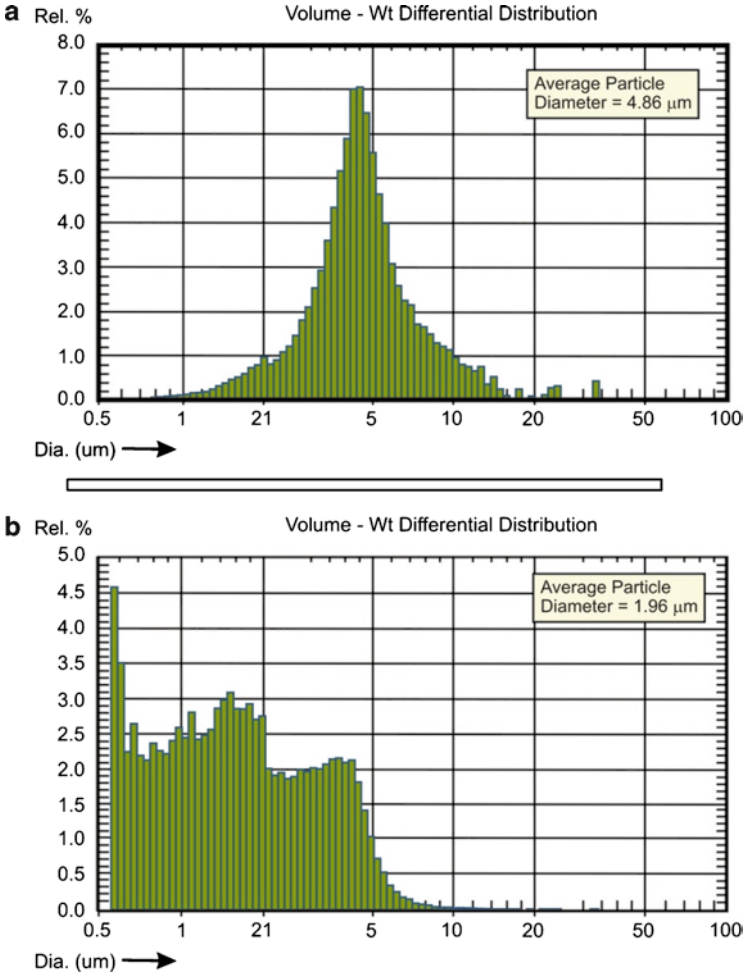

Fig. 4.7 Particle distribution of caprylic/oleic fatty acids by (**a**) volume–weight and (**b**) number–weight

viscosity fatty acid is necessary to reduce the particle diameters with shearing forces to prepare a stable emulsion utilizing caprylic acid (not a stable emulsion in pure form) that is slightly soluble in water. Caprylic acid's slight solubility (0.7 g/100 g H_2O) in water explains its proclivity to provide a cationic or positive charge around the emulsified oleic acid particle, in which caprylic acid is dissolved; and provide a slow-lease from the emulsified particle to provide antimicrobial activity. This observation is supported by testing data in Table 4.6 and is new and unreported in the literature to our knowledge.

Any factor that stabilizes the anion more than it stabilizes the acid should increase the acidity; any factor that makes the anion less stable should decrease acidity. The chain of CH_2 groups and single CH_3 group donates electrons and make

Table 4.6 Activity of nanoemulsions, US Patent 6,015,832, Baker Suspension and Kirby-Bauer plate methods

	Proteus mirabilis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		59-1244/lux	ATCC^{R} 29213
Material	CFU/ml (Average diameter of zone, mm)		
Oleic acid (28.7 cP)	14.5×10^{6}(6)	13.0×10^{6}(6)	8.0×10^{5}(6)
US Patent 6,015,832 (Table 3) (Baker)	20.5×10^{6} (6)	17.0×10^{6}(6)	22.0×10^{5}(6)
US Patent 6,015,832 (Table 1) (Baker)	10.5×10^{6} (6)	14.0×10^{6}(6)	17.0×10^{5}(8.5)
Caprylic Acid (8.5 cP) (100%)	0.0 (7.2)	0.0(7)	0.0(9.5)
Control (BHI Broth)	13.0×10^{6}(15)	17.5×10^{6}(15)	30.0×10^{5}(25.5)

Note 1: Kirby-Bauer Test – acceptable zone diameter (of inhibition) is greater than the control disk of 6 mm
Note 2: cP, centipoise viscosity (water = 1.0 cP at STP)
Note 3: Suspension test – acceptable CFU/ml is less than control
Note 4: The acronym CFU/ml indicates "colonies forming units per milliliter."

the anion less stable and decreases acidity. So, the longer the carbon chain, the less acidic the fatty acid. This is why caprylic acid is more acidic than capric and oleic, and more capable of providing cations (H^{+}).

The K_a (acidity constant) of caprylic acid is 4.5×10^{-5}, and it is slightly soluble in water. Solubility in water is an indication of acidity as observed from the above reaction between fatty acid and water, and a list of solubilities follow in Table 4.7. As the molecular weight increases, the solubility decreases due to the electron-donating groups ($-CH_3$ and $-CH_2$). Other acids in Table 4.7 are more soluble than caprylic, but too great solubility (greater K_a value) would force the acid into the aqueous phase (pulse activity) and deplete the emulsified particle of acid to quickly be an effective antibacterial agent. The longer-range activity would best be accomplished with a less soluble acid in the particle. Also, caprylic is a liquid and soluble in vegetable oil or the less viscous oleic acid. Oil solubility decreases with water solubility as determined through experimentation. The desired activity and success from the caprylic/oleic acid emulsion is due to a compromised selection of materials based and their properties.

The solubility of a carboxylic fatty acid is determined by its dissociation in water at a temperature (e.g., 25°C) and molecular configuration. The solubilities of carboxylic acids, which include fatty acids, are listed in Table 4.7. The melting point (M.P.) of each acid determines whether it is a liquid or solid at 25°C, and all of these acids have high boiling points (B.P.). All of these acids greater than the molecular weight of caprylic are solid at room temperature except oleic and linoleic due to their *cis*-configurations (bow-shape) at the unsaturated carbons which prevents them from becoming lattice crystalline structures. The oleic acid and its *cis*-configuration can be seen in Fig. 4.8. The linear structure of the capric acid allows it to become part of a lattice structure and a semisolid at room temperatures. There are *trans*-configurations in fatty acids that are also linear in structure, and they usually exist as solid materials.

Table 4.7 Activities of caprylic fatty acid/olive oil aqueous emulsions, Baker suspension method

		Pseudomonas aeruginosa	*Staphylococcus aureus*
		59-1244/lux	ATCC^{R} 29213
Material	*Proteus mirabilis*	CFU/ml	
Olive oil (78.2 cP)	3.2×10^{8}	1.5×10^{9}	1.17×10^{8}
1% Caprylic acid/24% Olive oil	1.9×10^{6}	8.5×10^{7}	1.0×10^{7}
5% Caprylic FA/21% Olive oil	0	0	0
10% Caprylic FA/90% Olive oil	0	0	0
Control BHI broth	5.7×10^{8}	9.1×10^{8}	4.6×10^{7}

Note 1: Formulation: 2.5% Tween^{R} 20, 72.5% sterile water and 25% caprylic/olive oil (wt/wt)
Note 2: The fatty acids were obtained from the Acme-Hardesty Company
Note 3: Acceptable CFU value for antimicrobial activity is equal to or less than the control

Referring to Table 4.8, there are differences between the two homogenizers and types of particle size analysis. However, an independent study (Gooch 2002) showed that emulsification with an APV Homogenizer (3,500 psig) produced an emulsion with a mean particle size of 0.688/1.128 μm (number/volume–weight geometric mean).

Hamouda et al. (2000) claimed 400–800 nm, 0.688 μm = 688 nm, so the emulsification procedure provided particle sizes that are roughly comparable to each other. The differences in particle size are believed to be due to the differences in instruments. However, the APV Homogenizer provided a much more narrow particle size "distribution" which produced a much more "stable" emulsion. The optimal pressure appears to be 6,000 psig. The activity of oil and fatty acid emulsions are listed in Table 4.9. The carboxylic acids are listed in Table 4.10 with regard to their melting and boiling temperatures and water solubilities. The fatty acid and oils for preparing emulsions with particle size and shearing pressure are listed in Table 4.11, and the particle size decreases with increasing pressure.

Caprylic/oleic fatty acid emulsion was found to be significantly antifungal using the Kirby-Bauer Plate Method. The diameter of antifungal zones was acceptable. The multispecies of organisms were grown on agar plates from "cultured cheese" over a period of a week at 25°C. Smaller colonies of bacteria could be observed growing in on the fungal growth areas on the agar plate. This series of test were not extended to the narrow feasibility scope of the study.

4.4.3 Mechanisms of Antibacterial Action

The mechanism of action of chlorhexidine and related biguanides was reviewed by researchers and reported in Block (2001). At relatively low concentrations, the action of chlorhexidine is bacteriostatic, and at higher concentrations, it is rapidly bactericidal, with the actual levels varying somewhat from species to species.

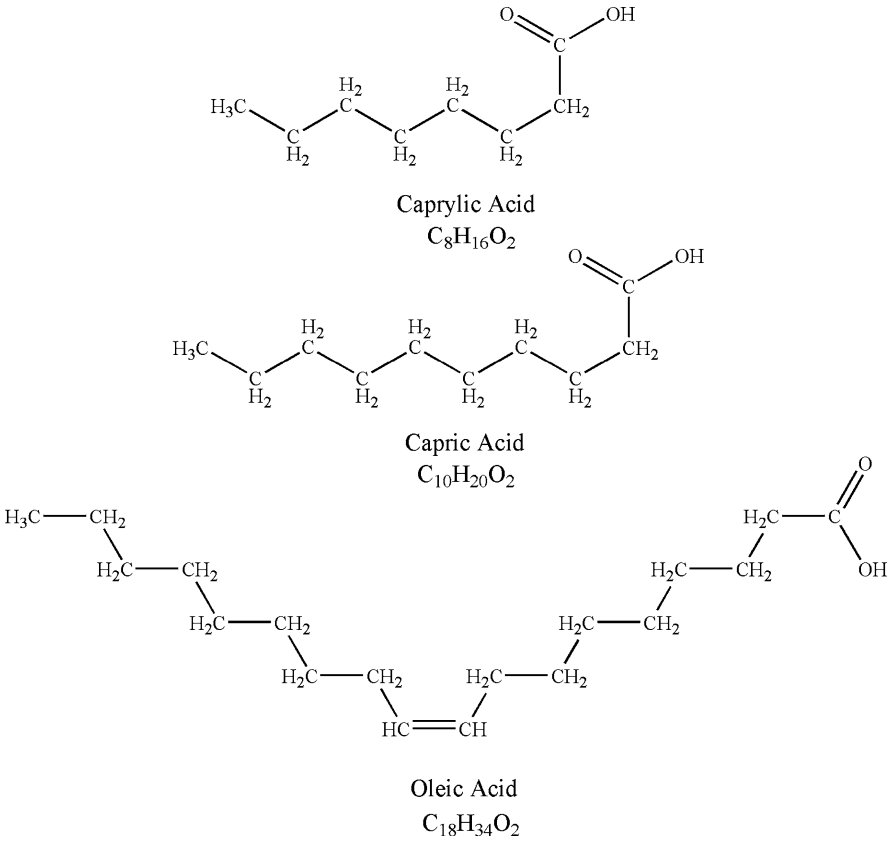

Fig. 4.8 Molecular structures of caprylic, capric and oleic (9-*cis*) fatty acids with atomic arrangements

Table 4.8 Fatty acids and viscosity

Carbons	Fatty acid	Viscosity (cP)
C4	Butyric (Butanoic acid)	1.5
C5	Valeric (Pentanoic acid)	5.1
C6	Caproic (Hexanoic acid)	6.5
C8	Caprylic (Octanoic acid)	7.5
C10	Capric (Decanoic acid)	9.3
C18	Oleic (*cis*-9-Octadecanoic acid)	27.3
C18	Lenoleic (*cis*, *cis*-9, 12-Octadecadienoic acid)	24.5
C18	Linolenic (*cis,cis,cis*-9, 12, 15-Octadecatrienoic acid)	21.2

The lethal process consists of a series of related cytologic and physiologic changes, some of which are reversible, that culminate in the death of the cell. The sequence is thought to be as follows: (1) rapid attraction toward the bacterial cell; (2) specific and strong adsorption to certain phosphate-containing compounds on

Table 4.9 Activity of oil and fatty acid emulsions, Baker suspension method

Material	*Proteus mirabilis*	*Pseudomonas aeruginosa* 59-1244/lux CFU/ml	*Staphylococcus aureus* ATCC^{R} 29213
Soybean oil (57.8 cP)	$8{,}105 \times 10^5$	9.0×10^5	32.0×10^4
Oleic fatty acid (28.7 cP)	83.5×10^5	9.5×10^5	47.5×10^4
Capric fatty acid (solid)	93.0×10^5	12.5×10^5	47.5×10^4
Control (BHI broth)	56.0×10^5	11.5×10^5	47.5×10^4

Note 1: All emulsions were prepared by the same formulation and methods
Note 2: Acceptable CFU/ml value for antimicrobial activity is equal to or less than the control
Note 3: *cP* centipoise viscosity (mPa)

Table 4.10 Carboxylic acids and solubility in water (Morrison and Boyd 1992)

Acid	Formula	M.P. (°C)	B.P. (°C)	Solubility (g/L H_2O)
Formic	HCOOH	8	100.5	∞
Acetic	CH_3COOH	16.6	118	∞
Butyric	$CH_3(CH2)_2COOH$	−6	164	∞
Valeric	$CH_3(CH2)_3COOH$	−34	187	37
Caproic	$CH_3(CH2)_4COOH$	−3	205	10
Caprylic	$CH_3(CH2)_6COOH$	16	239	7
Capric	$CH_3(CH2)_8COOH$	31	269	2
Lauric	$CH_3(CH2)_{10}COOH$	44	225	0
Stearic	$CH_3(CH2)_{16}COOH$	70	287	0
Oleic	*cis*-9Octadecenoic	16	223	0
Lenoleic	*cis, cis*-9,12-Octadecadienoic	−5	230	0

the bacterial surface; (3) overcoming the bacterial cell wall exclusion mechanisms; (4) attraction toward the cytoplasmic membrane; (5) leakage of low-molecular weight cytoplasmic components, such as potassium ions, and the inhibition of certain membrane-bound enzymes, such as adenosyl triphosphatase; (6) precipitation of the cytoplasm by the formation of complexes with phosphated entities, such as adenosine triphosphate and nucleic acids.

Characteristically, a bacterial cell is negatively charged, the nature of the ionogenic groups varying with bacterial species. It has been shown that, given sufficient chlorhexidine, the surface charge of the bacterial cell is rapidly neutralized and then reversed. The degree of charge reversal is proportional to the chlorhexidine concentration and reaches a stable equilibrium within 5 min. The rapid electrostatic attraction of the cationic chlorhexidine molecules and the negatively charged bacterial cell undoubtedly contributes to the rapid rate of kill associated with chlorhexidine, although surface charge reversal is secondary to cell death. Electron microscopy and assay for characteristic outermembrane components, such as 2-keto-3-deoxyoctonate

Table 4.11 Particle size study of aqueous emulsions

Mean particle size[d], μm (number/volume)		
Emulsion	Silverson	AVP Homogenizer
Oleic[a]		1.97/5.18[b] (2,000 psig)
Soybean oil[b]		0.92/3.63[b] (2,000 psig)
		0.80/1.77[b] (4,000 psig)
		0.80/1.68[b] (6,000 psig)
		0.84/3.16[b] (8,000 psig)
Soybean oil[b]diluted 1:1	3.36[b]	
Soybean oil[b]diluted 1:50	3.38[b]	
Soybean oil[b]diluted 1:100	3.38[b]	
Baker (Table 1) US Patent 6,015,832	0.639[a]	1.96/4.86[c]
Baker (Table 2) US Patent 6,015,832		1.91/4.23[c]
Wright (Table 1) US Patent 5,547,677	0.276[a]	
Wright (Table 2) US Patent 5,547,677	0.289	

[a]Oleic acid obtained from the Acme-Hardesty Company
[b]Soybean oil obtained from Sigma-Aldrich Corporation
[c]LS13 320 Particle Size Analyzer (Beckman-Coulter Co.), by volume–weight
[d]Model 770 AccuSizer (Particle Sizing Systems, Inc.), by volume–weight and number weight

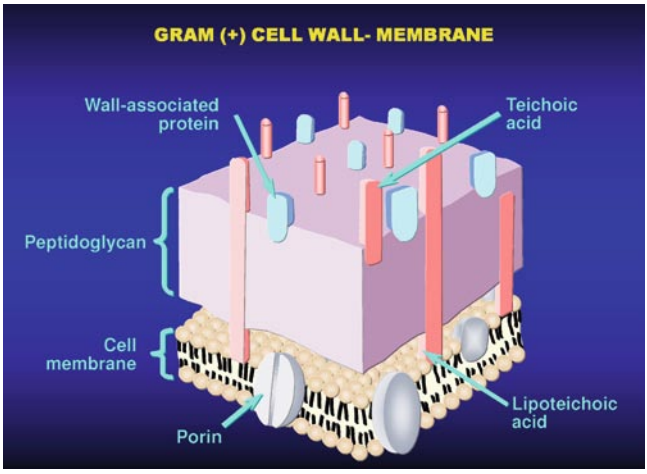

Fig. 4.9 Illustration of the gram positive cell wall and membrane

(KDO), demonstrate that sublethal concentrations of chlorhexidine bring about changes in the outer membrane integrity of gram-negative cells. An efflux of divalent cations, especially calcium ions, occurs prior to or during such outer-membrane changes. The Chlorhexidine molecules are thought to compete for the negative sites on the peptidoglycan, thereby displacing metallic cations (Figs. 4.9–4.14).

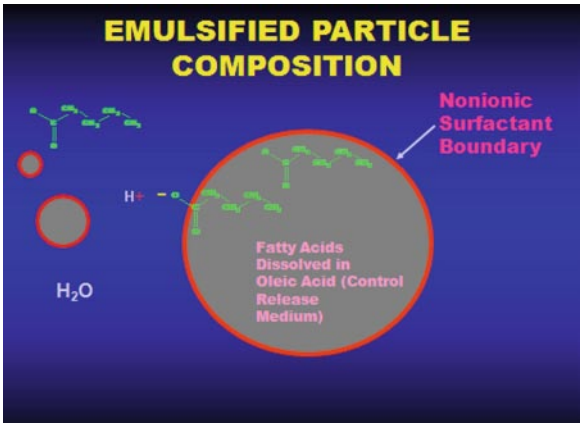

Fig. 4.10 Emulsified particle in an aqueous medium consisting of a fatty acid in a water insoluble medium, oleic acid, and showing the interaction with water at the surface of the particle

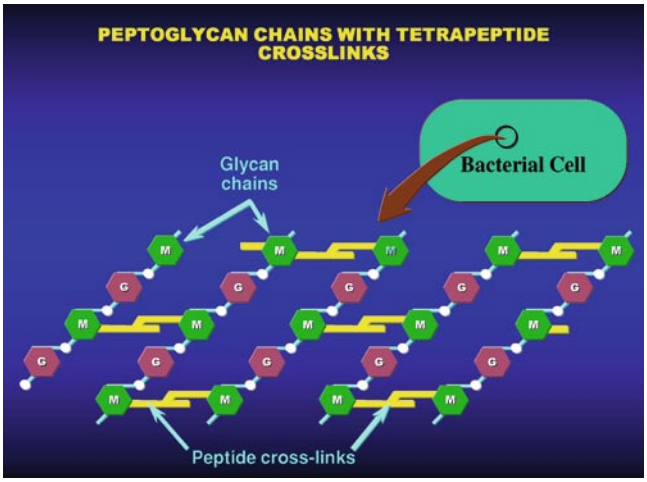

Fig. 4.11 Illustration of the typical cell wall structure showing a rigid and porous network of peptoglycan chains immobilized with tetratpeptide crosslinks. Observe the fatty acid (pentanoic) easily penetrating the cross-linked structure

Sodium octanoate is the water soluble sodium salt of octanoic (caprylic) acid, but it has no antibacterial or antifungal activity as tested by the same procedures described above, and the results are reported in Table 4.12.

4.4.4 Urea Hydrogen Peroxide

Solid and aqueous dissolved urea hydrogen peroxide (UHP) was theorized to be capable of providing antimicrobial activity in aqueous as well as particulate applied

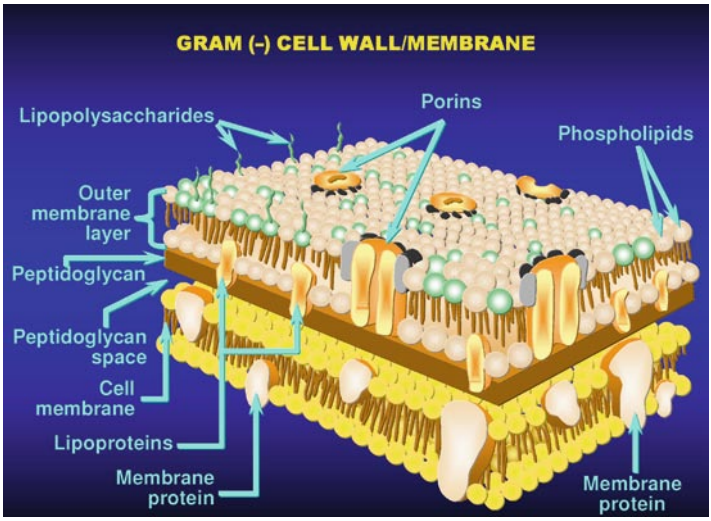

Fig. 4.12 Composition of typical gram negative cell wall and membrane

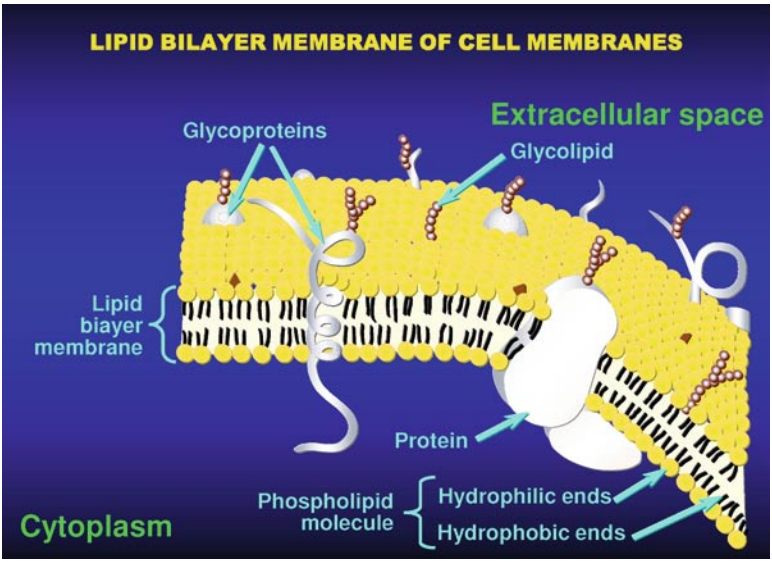

Fig. 4.13 Illustration of the bilayer structure of cell membranes showing phospholipid molecules and their opposite hydrophilic and hydrophobic sections

forms to tissue. Experiments were designed to test the antimicrobial activity by evaluating these agents with the Baker Suspension Methods. The results are contained in Table 4.13.

The levels of urea hydrogen peroxide that provide acceptable levels of antibacterial activity also create unacceptable osmolality levels. Antibacterial activities

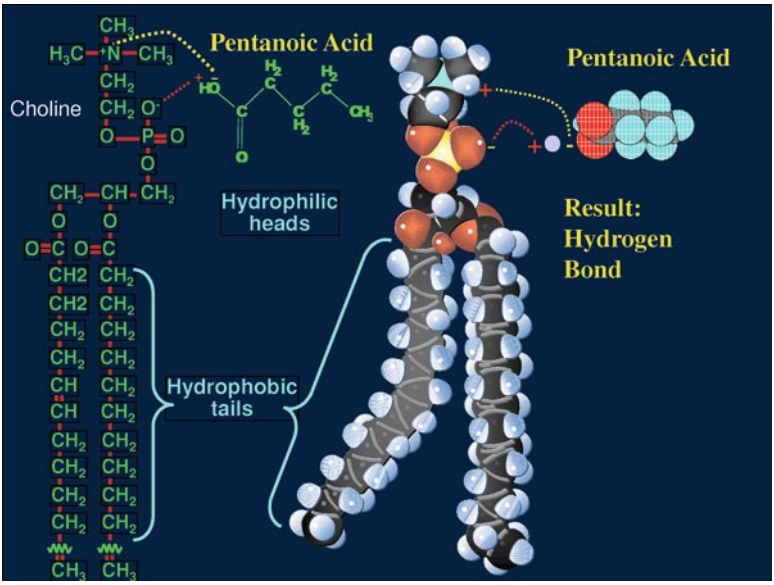

Fig. 4.14 An example of a reaction of fatty acid with phospholipid that results in the destruction of cell membrane

Table 4.12 Antifungal activity of caprylic acid and sodium octanoate

Material	Zones of inhibition, mm
Caprylic acid 1 (100%)	38.0
Caprylic acid 2 (50%)	34.0
Caprylic acid 3 (10%)	9.0
Caprylic acid 4 (5%)	6.8
Caprylic acid 5 (1%)	6.0
Sodium octanoate 1 (100%)	9.8
Sodium octanoate 2 (50%)	8.7
Sodium octanoate 3 (10%)	8.4
Sodium octanoate 4 (5%)	6.0
Sodium octanoate 5 (1%)	6.0

Note 1: 10 µl of each material above, except sodium octanoate, NaCA 1, as pipetted onto a 6 mm paper disc. For NaCA 1, a small amount of the powder was placed directly onto the agar

Note 2: Fungi spores from an active culture were suspended in 1 ml sterile 0.85 NaCl, then lawned on 150 mm Mueller–Hinton agar plates with a sterile cotton swab

of peroxides have been reported in the literature (Block 2001) as having limited antibacterial activities, and UHP appears to be typical of similar materials without special properties.

Table 4.13 Activities of urea hydrogen peroxide (UHP) and polyvinyl alcohol (PVA)

	Proteus mirabilis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
		59-1244/lux	29213
Material		CFU/ml	
2% UHP/98% PVA	159.5×10^{9}	7.45×10^{8}	
3% UHP/97% PVA	9.50×10^{8}	8.95×10^{8}	
4% UHP/96% PVA	6.0×10^{8}	8.85×10^{8}	
5% UHP/95% PVA	2.95×10^{8}	6.3×10^{8}	4.3×10^{7}
10% UHP/90% PVA	3.85×10^{8}	6.3×10^{8}	3.6×10^{7}
20% UHP/80% PVA	2.4×10^{8}	6.55×10^{8}	1.8×10^{7}
100% UHP/0% PVA	52×10^{7}	7.5×10^{7}	–
PVA (100%)	2.45×10^{8}	8.5×10^{8}	7.4×10^{7}
PVA/Sulfamylon^{R}, 90/10% (wt/wt)	7.5×10^{9}	1.0×10^{9}	7.5×10^{8}
Control: (BHI Broth)	5.55×10^{8}	1.20×10^{9}	5.6×10^{7}

Note 1: PVA and UHP were mixed in dry solid form
Note 2: PVA and Sulfamylon^{R} were mixed in dry form
Note 3: Acceptable CFU/ml values for antimicrobial activity is equal to or less than the control

4.5 Summary

The tests performed on antibacterial nanoemulsions (Baker and Wright patents) prepared in ISR laboratories were not successful as reported in patents and literature. The additive materials that were mentioned as being responsible for inducing acidic, cationic, or positive charges around emulsified oil particles in nanoemulsions were effective antibacterial agents alone or dissolved in water or solvents. The only activity observed from the aforementioned nanoemulsions was in the dilution (suspension) phase that is a process of mixing the nanoemulsion with a liquid suspension of bacteria, but the decrease in growth compared to the control was not acceptable. Each formulation of the Baker and Wright patents were reproduced and tested without the antibacterial activity reported in the patents. From these observations, there is neither anything new nor effective in this technology as bacteriocidal or sporicidal agents from experimental observations.

The exercise of preparing emulsions using oils-mixed additives is well described in Emulsification and Polymerization of Alkyd Resins (Gooch 1980, 2002), although without the addition of acidic or cationic agents for the purpose of inhibiting or destroying bacteria.

Materials that induce cationic or positive charges to retard growth of bacteria and fungi have been identified for decades and some have been applied for centuries. Caprylic fatty acid is known for its antifungal activity for processing food products (Hilgren and Salverda 2000), but its antibacterial/antifungal activity from an emulsion has not been reported in the literature or patent office. These emulsions are inexpensive,

colorless, nonoxidizing, and very removable while providing a broad spectrum of antimicrobial activity.

Caproic acid should be included in future work for emulsions prepared for bactericidal/fungicidal agents because it is slightly more soluble than caprylic, 1.0 g/100 g compared to 0.7 g/100 g H_2O, and it is a liquid. Theoretically, a combination of the two acids would be synergetic for control-release purposes.

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Tween 20, Chemical Classification – Ethoxylated Fatty Acids, Chemical Description – Polyoxyethylene (20) Sorbitan Monolaurate, Hydrophile-Lipophile Balance (HLB) 16.7, Uniqema, 1000 Uniqema Blvd., New Castle, Delaware 19720.
Viscometer, Model DV-E Digital, Brookfield Engineering Laboratories, Inc., 11 Commerce Boulevard, Middleboro, Maryland 02346.
Wright, D. C., US Patent 5,547,677 (1996).

Chapter 5
One-Hand Operated and Automatic Tourniquet

5.1 Introduction

On the battlefield, a properly applied tourniquet can be an extremely effective means of controlling severe extremity wound hemorrhage. However, a great deal of confusion exists among soldiers, medics, and military medical officers on a number of tourniquet-related issues. What is an appropriate combat tourniquet? When is it appropriate to use a tourniquet? When and by whom should a tourniquet be removed? Under what conditions should a tourniquet not be released or removed? What are the most effective ways to increase limb salvage while using a tourniquet?

The technical definition of a tourniquet is any device that is used to prevent blood from flowing through blood vessels below the placement of the tourniquet on either upper or lower limbs. A tourniquet prevents excessive loss of blood from a limb wound with the expectation of saving a life. On the battlefield, the tourniquet in one form or another has been used to control excessive hemorrhaging on nonvital extremities since the Roman Empire days, where a rope or cloth strap was used for tourniqueting a soldier's limb that has suffered a wound. The use of tourniquets has always been as controversial as it has been successful. This controversy results, as will be described later, as much from mistakes made in the application and release of the tourniquet as it does from the primitive design of the most commonly used tourniquets of today.

The first use and description of the modern battlefield tourniquet occurred during the 1500s and 1600s by German and French surgeons. The initial use was to control the excessive blood flow that accompanied limb amputations, both traumatic and surgical. The first form described was a tight cord tied around the limb above the point of amputation to stop the blood flow and loss. This form was modified by the insertion of a rod, usually a wooden stick or shaft, under the cord. The rod was twisted to tighten the cord or strap in order to stop the blood flow. This style of tourniquet became known as the Spanish windlass or windlass tourniquet. In 2001, the U.S. Army identified through testing a device which became known as the one-handed tourniquet. Later testing conducted in 2004 identified candidate commercial devices and rated the applicability to battlefield trauma. Two were selected and recommended for battlefield use. The *C-A-T Tourniquet*®, (Fig. 5.1) was modified

J.W. Gooch, *Biocompatible Polymeric Materials and Tourniquets for Wounds*,
Topics in Applied Chemistry, DOI 10.1007/978-1-4419-6586-8_5,

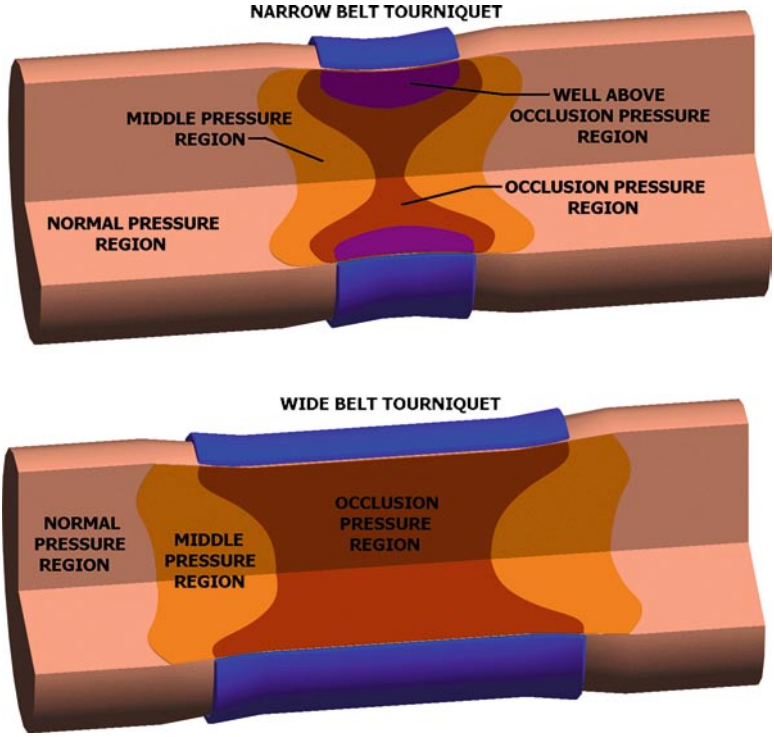

Fig. 5.1 Tourniquet edge-effect pressure for narrow and wide belt tourniquets

by the insertion of a rod, usually a wooden stick or shaft, under the cord. As can be seen, the initial mechanism (a twist-rod) is still used with only minimal improvements. These improvements include:

- A flat piece of material directly under the twist section to reduce the pinching and skin damage caused by the twisting of the strap.
- 2A strap buckle to allow quick fastening and adjustment of the strap before twisting.
- A twist-rod retention strap or clip to prevent the twist-rod from inappropriately untwisting.

Because of the fear of tourniquet-related limb damage, tourniquets are almost never used in civilian trauma cases and have been discouraged since World War II by many militaries due to the amputations suffered by soldiers when tourniquets were left on too long or over tightened. This practice was kept in place even though many if not most trauma specialists believed that if properly used, tourniquets saved lives. It was not until modern battlefield casualty studies were made that the need for tourniquets was reevaluated.

Studies of battlefield trauma and death in Vietnam, Somalia, Iraq, and Afghanistan showed two major changes in battlefield casualties that changed the

perception of the tourniquet in trauma treatment on the battlefield. The first was the increase in the percentage of limb injuries. This is attributed to the effective use of body armor, which reduced the number of abdominal injuries due to direct fire and shrapnel. The second was the increased capability of medical staff to evaluate the cause of death in individuals who died on the battlefield. In previous wars and conflicts, much of the statistics on battlefield deaths were derived solely from the wounded and injured that were transported to field medical stations. These factors led to a rise in the number of preventable deaths attributed to limb injuries accompanied by hemorrhaging. In fact, studies of Army experience found that as much as 10% of battlefield deaths in Vietnam and Somalia were caused by excessive extremity wound bleeding, and if a tourniquet had been used, the death of the service member could most likely have been prevented. These more recent studies led to a new assessment of the efficacy of the role of the tourniquet in battlefield trauma response.

Analysis of the data from the current conflicts in Afghanistan and Iraq shows that the increased frequency of field tourniquets in treating traumatic limb injuries has contributed to lower mortality due to battlefield injuries. This was accomplished with extremely low rates of tourniquet-induced complications. Contributing to the low rate of complications was the personnel training in the treatment of extremity injuries. The analysis further indicated that all battlefield personnel should carry tourniquets and that they should be carried directly on their person and not stowed in their vehicle, back packs, or other equipment bags.

The majority consensus of current battlefield surgeons is that if properly used, tourniquets save lives on the battlefield without increasing the limb loss numbers. This is supported by clinical research into the safety of the pneumatic tourniquet used in surgery. This type of tourniquet, which is routinely used in surgery, is considered safe for applications of up to 2 h. In addition, more recent studies and surveys into the use of tourniquets in battlefield trauma show that few if any limb losses of tourniqueted limbs were due to tourniquet application. On the modern battlefield, the time from injury to the field hospital is generally less than 2 h. Many medical personnel have acknowledged that applications in excess of 2 h may result in muscle and nerve damage which generally increases progressively with tourniquet application time. In one study that included many cases where the tourniquet was applied for longer than 2 h, complications were found to be present: however, none resulted in loss of the limb. It is not understood after what length of time limb loss will occur, in fact 4–6 h times have been reported in the WWII literature without limb loss.

Although tourniquets are presently used to occlude blood flow for clinical, emergency, or combat use, automatically controlled tourniquets are used in only clinical practices to maintain occlusion pressure without manual control (e.g., during surgical operations on limbs). What is missing from present tourniquet technology is a device that can be worn in a soldier's uniform on each limb that will be available for immediately tourniqueting a wounded limb by using one hand. In addition, there is not a designed combat tourniquet that automatically controls occlusion pressure that would be of critical importance to an unconscious soldier who cannot

adjust tourniquet pressure around a limb. There is a need for a one-hand operated and an automatically operated tourniquet that can be permanently worn within the soldier's combat uniform. In the event of a wound, a soldier often cannot seek medical assistance and has to administer self-care to avoid death due to blood loss that has happened too often in past military conflicts. Also, such a tourniquet carried in an individual emergency kit or care-giver's medical pack would be invaluable to the injured hunter or hiker in isolated mountains.

The following sections discuss the requirements for a battlefield tourniquet based on the operational scenario of the modern battlefield and on the medical issues involving tourniquet use, before describing the development of the one-handed, automatic tourniquet.

5.2 Fundamentals of Tourniquet Technology

An important feature missing from present combat tourniquets is a proper design that minimizes damage to the tissue and nerves; conventional tourniquets have often damaged limbs to the point that they were amputated after removing the tourniquet. Typically, a combat tourniquet is drawn around the limb so tightly (far above the systolic pressure) that the tissue and nerves are permanently damaged because the systolic pressure is not monitored. A tourniquet that can be adjusted just near or slightly above the systolic pressure to occlude major arteries without substantive damage is needed. Tourniquets used safely in clinical surgical facilities have been studied to provide a combat tourniquet device that minimizes damage to limbs.

Field tourniquets must be capable of reliably applying enough pressure to occlude arterial bleeding. If a tourniquet applies enough pressure to stop only the venous flow, then the rate of blood loss from an arterial wound will most likely increase. It should be noted that some small blood seepage may continue in the case of traumatic amputation due to medullar blood flow even with a properly tightened tourniquet.

Tourniquet tension must increase dramatically with the diameter of the limb. This is primarily due to the linear geometrical increase in contained pressure area with limb diameter. The required increase in tourniquet belt tension for a leg 25 in. in circumference to that of an arm with a 12.5 in. circumference is a factor of 2. This would be approximately 30 pounds tension for a one and one-half inch belt width applying 230 mmHg pressure on the 25-in. leg and 15 pounds tension for the 12.5-in. arm. In addition to the geometric considerations, there is a pressure spreading effect. This spreading results in the pressure inside the limb at a distance below the surface of the tourniquet being lower than that at the limb surface immediately below the tourniquet. This effect is primarily associated with the edges of the tourniquet and is due to the pseudoplastic nature of blood and the viscoelastic nature of blood vessels and surrounding tissue. Tourniquet edge-effects, illustrated in Fig. 5.2, mean that for a tourniquet with a width substantially greater than the limb

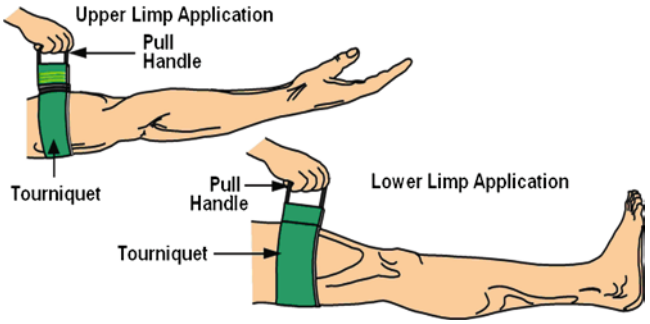

Fig. 5.2 Application of one-hand tourniquet to upper and lower limbs

radius, the pressure along the center of the limb, centered below the tourniquet will approach the pressure at the limb surface just under the tourniquet. There is, however, in addition to the impracticality of an exceedingly wide tourniquet a tradeoff in belt tension. As the belt width increases, the required pressure drops but the required tension for a given pressure increases linearly with the belt width. This can be seen in the equation for surface pressure, P_S (mmHg):

$$T_B = 0.0097 P_S d_L w_B = 0.0031 P_S C_L w_B$$

where T_B is the belt tension in pounds, d_L is the limb diameter (inches), C_L is the limb circumference (inches), and w_B is the belt width in inches. Figure 5.2 shows the pressure edge effects for a wide and narrow tourniquet width.

Graham et al. (1993) measured the occlusion pressure of the proximal leg as a function of cuff width and found the following relationship for the occlusion pressure, P_{occ} (mmHg), systolic pressure, P_{sys}, (mmHg), and the diastolic pressure, P_{dia}, (mmHg):

$$P_{occ} = P_{dia} + \left(P_{sys} - P_{dia}\right) C_L / \left(3 w_B\right)$$

The occlusion pressure in the following equation is the surface pressure applied by the tourniquet required to occlude the arterial blood flow. Belt tension required for occlusion, T_{Bocc} (pounds), is found by combining the two equations above to be:

$$T_{Bocc} = 0.0031 \left[P_{dia} + \left(P_{sys} - P_{dia}\right) C_L / \left(3 w_B\right) \right] C_L w_B$$

The primary cause of nerve and tissue damage in the proximity of the tourniquet is not the absolute pressure but the shear stresses associated with the edges of the tourniquet. Since the wider tourniquet belts reduce the required pressure, they also reduce the shear stresses at the edge of the tourniquet, thus reducing the potential for nerve damage. The primary means to reduce the shear is to use the minimal

occlusion pressure. This implies that some form of fine adjustment and intelligent tightening is required to achieve the proper tourniquet tension. Another method to reduce the edge shear stresses would be to smoothly reduce the surface pressure from the center toward the edges of the tourniquet belt. Pneumatic bladder style tourniquets have this quality due to bladder geometry.

The typical application of a one-hand operated tourniquet is demonstrated in Fig. 5.3. The relationship between occlusion pressure and width/circumference is shown in Fig. 5.3. Minimum occlusion pressure is important for minimizing the damage to tissue because the limb must be treatable after removing the tourniquet. The obvious conclusion from this plot is that the occlusion pressure decreases as the width/circumference ration increases. Graham et al. (1993) proposed the equation shown as the inset in this figure for the purpose of calculating the minimum occlusion pressure for a limb tourniquet. Shaw and Murray (1982) showed the relationship between tourniquet pressure and underlying soft tissue in the thigh and measured (as shown in Fig. 5.4) and reported (in Fig. 5.5). The damage to nerve tissue by over pressuring soft tissue with a tourniquet is illustrated in Fig. 5.6, the distortion of the nodes of Ranvier (Hodgson 1994), and which is reason for proper design of the cuff edges is important as diagramed in Fig. 5.6 (i.e., θ should be as low as possible).

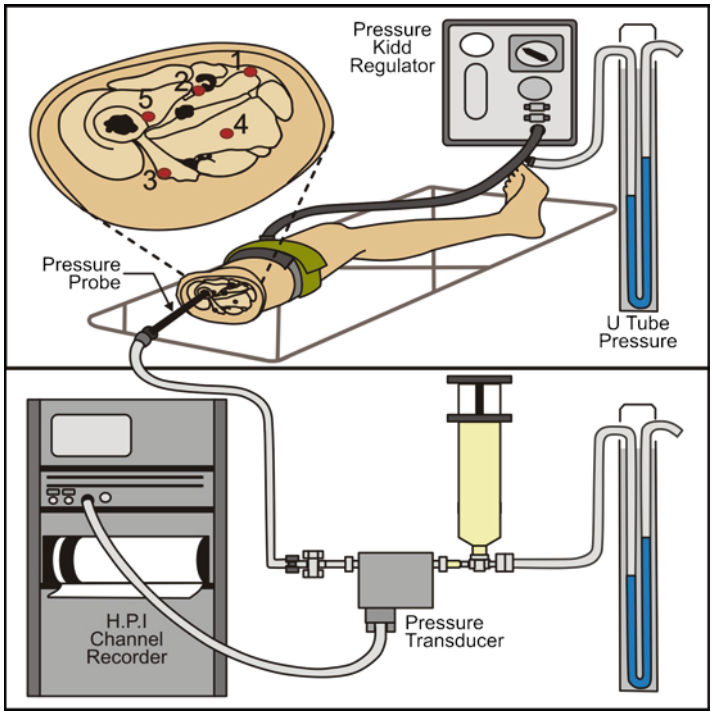

Fig. 5.3 Measurement of tourniquet pressure, soft-tissue pressure (Shawand Murray 1982). Reprinted with permission of the Journal of Bone & Joint Surgery, Inc

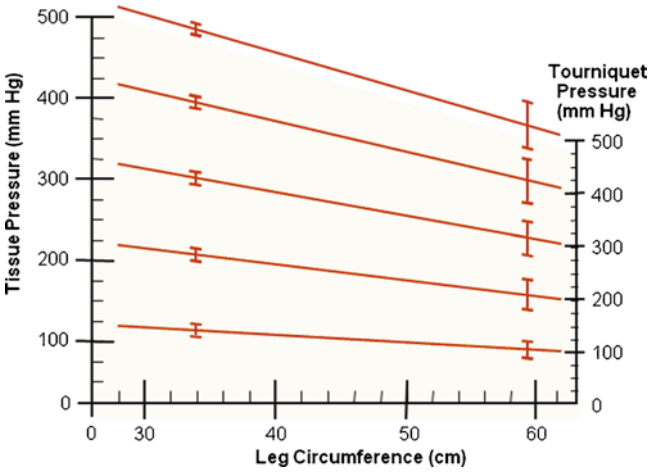

Fig. 5.4 Tissue pressures versus leg circumference (Shaw and Murray 1982). Reprinted with permission of the Journal of Bone & Joint Surgery, Inc

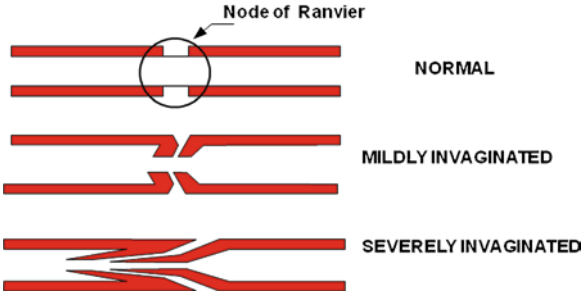

Fig. 5.5 Damage to nerves by tourniquet pressure, invagination, nodes of Ranvier (Hodgson 1994). Reprinted with permission of ASME International, Three Park Avenue, New York, NY 10016

The relationship of occlusion pressure to the ratio of width/circumference for tourniquets is plotted in Fig. 5.7.

In addition to the edge-effect shear described above, belt tightening of the tourniquet can cause asymmetric surface shear due to the friction of the tourniquet belt and underlying material with the skin. The most notable effect with windlass and ratchet-tightening tourniquets is pinching observed in the region of the buckle or twist negated by the sloping and smooth edge of the cuff in Fig. 5.8. Asymmetric forces usually range from purely radial compression in the region directly opposite the buckle to circumferential compression directly under the buckle or twist region of the tourniquet. The side regions range between these two extremes and are mostly under circumferential tension. Less apparent is the fact that this shear can add to the edge-effect shear to increase the potential for nerve and other tissue damage in the proximity of the tourniquet, and the extent of this effect is not known at

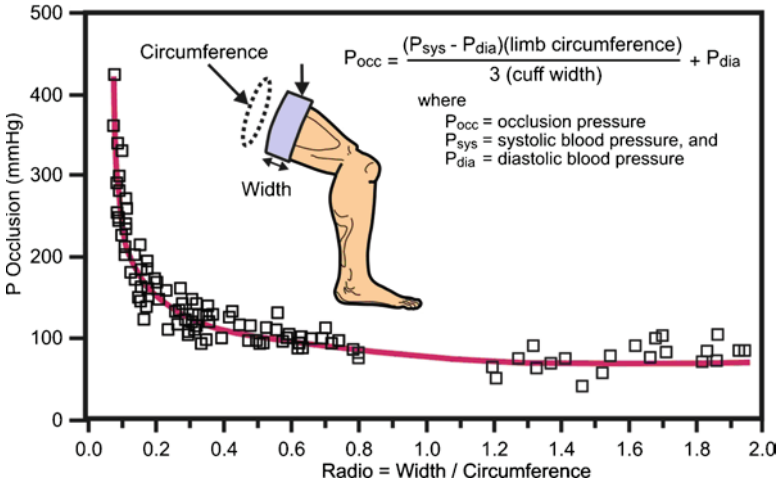

Fig. 5.6 Occlusions pressure versus ration (width/circumference for tourniquets)

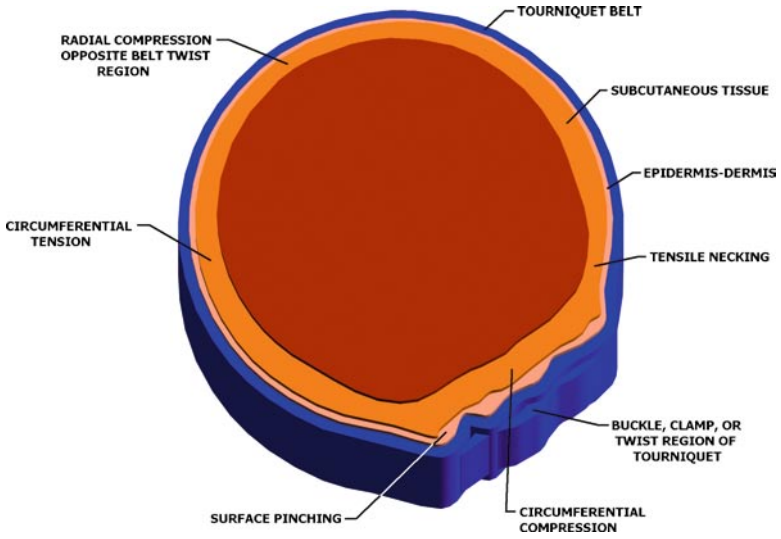

Fig. 5.7 Circumferential compression and surface pinching in twist region

this time. However, Fig. 5.9 shows an example of how the stress distribution caused limb distortion due to this asymmetric shear effect. It should be noted that this surface shear is not present in pneumatic tourniquets which apply almost exclusively radial compression.

The fundamentals outlined above clearly demonstrate that the width and occlusion pressure are related, and that low ratios of width/circumference necessitate

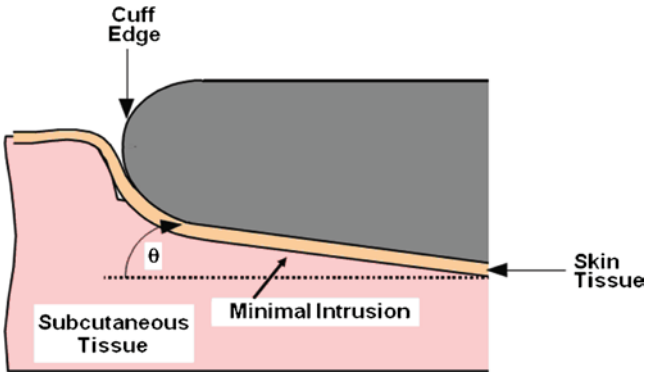

Fig. 5.8 Optimal cuff design

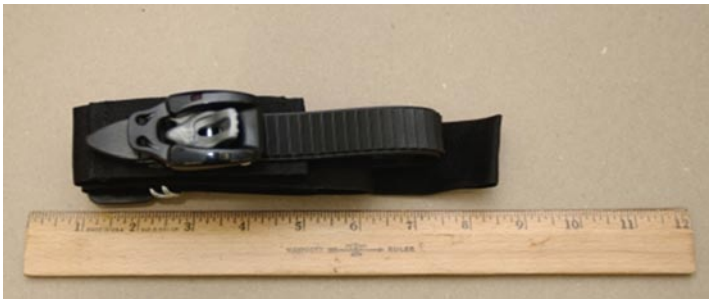

Fig. 5.9 Burke device (Biomedical Innovations, Southern Pines, NC, reprinted with permission)

large occlusion pressures. The damage to tissue and nerves increases with any occlusion pressure increase. This relationship dictates that there is a lower limit to the width of a tourniquet that is effective without serious tissue and nerve damage. At the same time, the larger circumference limb by its nature requires greater occlusion pressure; therefore, larger limbs would have a greater lower limit on tourniquet width that can successfully occlude the arterial blood flow without significant nerve and tissue damage. So, the greatest challenge for the combat tourniquet is the effective occlusion of blood flow of a large upper and lower limb using occlusion pressure that does not permanently damage tissue and nerves. At any time, it is of critical importance that the limb can be saved after removing the tourniquet in a hospital.

These relationships imply that a wider tourniquet not only requires lower pressure applied to the limb to occlude blood flow, but lower pressure translates to less damage to nerves and tissue. A good design for a battlefield tourniquet should be flexible to allow different widths to accommodate the needs of soldiers with respect to the range of limb sizes. On the other hand it is recognized that the solider must carry a personal tourniquet of very limited size and weight, possibly worn within the uniform, so a narrow tourniquet may be necessary, and a wide tourniquet (e.g., 3–4 in.) may not be practical.

5.3 Critical Properties of a Combat Tourniquet

In August of 2003, the current U.S. Army tourniquet requirements were discussed by the Advanced Technology Applications for Combat Casualty Care Conference (ATACCC) Tourniquet panel. This panel not only emphasized the need for battlefield tourniquets but also established an initial outline of item and test requirements for the current battlefield tourniquets. In October, a follow-up committee drafted requirements for the testing of candidate commercial tourniquets for military issue. The committee stated, as expected, that the primary function of a battlefield trauma tourniquet was to completely occlude the arterial blood flow in order to stop hemorrhaging. They emphasized that partial occlusion, especially when only venous flow was occluded, was often worse than no occlusion at all. As mentioned above they pointed out that tourniquet tension increases rapidly with limb size. In addition, the committee felt that with narrow belts of 1 in. or less it was virtually impossible to occlude the arterial flow in a leg without the aid of mechanical advantage. They concluded that simple strap and buckle tourniquets were not capable of reliably occluding the flow in large limbs. The benefit of wide belts was further acknowledged as a factor in lowering the necessary occlusion pressure. The use of pneumatic tourniquets relying on air pressure as opposed to belt tension was deemed effective and more easily and precisely controlled. The committee also understood that the contoured edge of pneumatic tourniquets reduced the edge-effect shear and therefore the amount of shear damage to nerves and other tissue. These factors have made them the standard for surgical applications, but the committee observed that weight and reliability concerns (the potential for punctures and leaks) have prevented their acceptance on the battlefield.

The committee established four major mechanical considerations:

1. The tourniquet must reliably occlude blood flow in large limbs up to 27 in. in circumference.
2. The belt must be greater than 1 in. in width.
3. The belt must have some form of mechanical advantage for tightening.
4. The tourniquet must not have mechanical limitations that prohibit its functioning reliably on small limbs of 11 in. in circumference.

On the basis of these requirements, a study was initiated by the United States Army Institute of Surgical Research (USAISR) to evaluate nine commercially available tourniquets for applicability to combat use. Of these nine only the seven that were within the mechanical specifications of size and weight were tested. In a report covering the study issued in 2005, Walters et al. concluded that three of the candidate tourniquets met the requirements, the Combat Applications Tourniquet (CAT), the Special Operations Forces Tactical Tourniquet (SOFTT), both are windlass type tourniquets, and the Emergency Military Tourniquet (EMT), which is a hand-operated bulb pump pneumatic type tourniquet. These three were rated as 100% effective in blood flow occlusion. Of these three, the EMT was judged as the least painful, but it was recommended only for medical personnel and not for the field

soldier due to perceived reliability problems of possible bladder punctures that would render the device useless. Owing to this factor, the windlass type tourniquets were deemed the most reliable and were perceived to have the best overall performance under battlefield conditions. The CAT was recommended for issue to combat soldiers over the SOFTT because of the significantly higher pinch pain of the SOFTT. It should be noted that a fourth tourniquet that could reliably occlude blow flow failed in nearly one quarter of the trials due to intolerable pain caused by pinching. In a later analysis of field data from the Iraq conflict on 428 applied tourniquets, Kragh et al., found the Emergency Medical Tourniquet to be 92% effective and the Combat Application Tourniquet to be 79% effective. The implication is that the pneumatic type tourniquet is more effective when used properly than purely mechanical type tourniquet.

This study was not meant to be comprehensive in that no attempt to simulate battlefield conditions or to measure the effects of environmental stresses on the candidate tourniquet types was made. Additional information for a full evaluation and specification of battlefield tourniquets was slated for future evaluation.

In 2005 and 2007 (Phase 2), a more recent study for the U. S. Marine Corps, by Hill et al., evaluated 13 self-applied tourniquets for their applicability in combat applications. This study attempted to measure the functionality of the candidate tourniquets in battlefield conditions by immersing them in a simulated blood/sand mixture prior to testing. In contrast to the earlier Army study by Walters et al., the conclusion drawn from this study was the recommendation that one of the ratcheting or stretch-retention type tourniquet systems be adopted for combat deployment. These types had the best user subjective ratings as well as the lowest application times especially on the upper extremities where one-handed application was required. The recommended group had application times 30–50% lower on the upper extremities than the windlass types recommended by the Army study. Velcro® was observed to lose its effectiveness as a clamp when it became fouled with wet sand or mud and, therefore, should be avoided. It should be noted that none of the tourniquet types used in the Marine Corps study were pneumatic.

A total of 19 different types of tourniquets were used in these studies (see Figs. 5.5–5.23). The tourniquet types ranged from the simple elastic type tourniquets to a pneumatic operated tourniquet. The tourniquet types included: elastic, windlass, mechanical advantage cam, block and tackle, ratchet, and pneumatic. The tourniquets tested in these studies included:

Burke Device (Biomedical Innovations, Southern Pines, NC).
Combat Application Tourniquet, C-A-T (North American Rescue Products, Greenville, SC).
Emergency & Military Tourniquet (Delfi Medical Innovations, Inc., Vancouver, Canada).
Flow Stopper Tourniquet (Creative & Effective Technologies Inc, Raeford, NC).
Hemodyne (Hemodyne Inc., Richmond, VA).
Last Resort Tourniquet (Hammerhead, LLC).

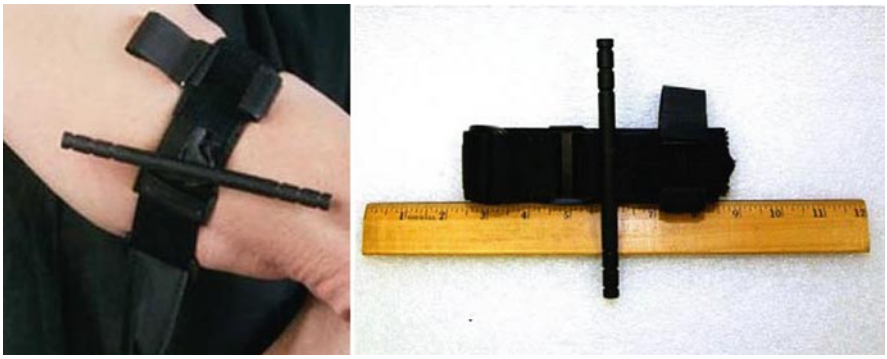

Fig. 5.10 Com application tourniquet (North American Rescue Products, Greenville, SC, reprinted with permission)

Fig. 5.11 Emergency and military tourniquet (Delfi Medical Innovations, Inc., Vancouver, Canada, reprinted with permission)

Fig. 5.12 Flow stopper tourniquet (Creative and Effective Technologies Inc, Raeford, NC, reprinted with permission)

Fig. 5.13 Hemodyne tourniquet (Hemodyne Inc., Richmond, VA, reprinted with permission)

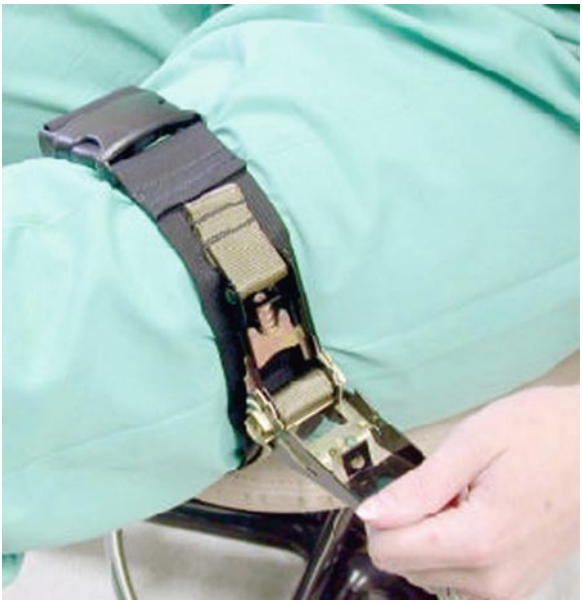

Fig. 5.14 Last resort tourniquet (Hammerhead, LLC, reprinted with permission)

McMillan Tourniquet (CSM Tactical Gear, Temecula, CA).
Mechanical Advantage Tourniquet (Bio Cybernetics International, LaVerne, CA).
Military Emergency Tourniquet (ATSCC, Virginia Beach, VA).

Fig. 5.15 McMillan tourniquet (CSM Tactical Gear, Temecula, CA, reprinted with permission)

Fig. 5.16 Mechanical advantage tourniquet (Bio Cybernetics International, LaVerne, CA, reprinted with the permission)

Fig. 5.17 Military emergency tourniquet (ATSCC, Virginia Beach, VA, reprinted with permission)

NATO Tourniquet (Deployment Medical Resources, Partlow, VA).
One-Hand Tourniquet-1 in. width (Canvass Specialties, Inc.; San Antonio, TX).
One-Hand Tourniquet-2 in. width (Canvass Specialties, Inc.; San Antonio, TX).
Q-Tourniquet (Blade Tech Industries, Lakewood, WA).
Quickette (Ivy Off, Laxahatchee, FL).

Fig. 5.18 NATO tourniquet (Deployment Medical Resources, Partlow, VA, reprinted with permission)

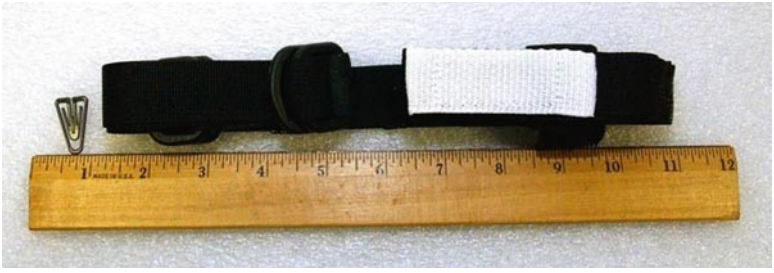

Fig. 5.19 One-hand tourniquet-1 in. width (Canvass Specialties, Inc.; San Antonio, TX, reprinted with permission of Canvass Specialities, Inc)

Fig. 5.20 One-hand tourniquet-2 in. width (Canvass Specialties, Inc., San Antonio, TX, reprinted with permission)

Special Applications Tourniquet System (Marketing Tactics, LLC, Lake Worth, FL).
Special Operations Forces Tactical Tourniquet (Tactical Medical Solutions, Anderson, SC).

Fig. 5.21 Q-Tourniquet (Blade Tech Industries, Lakewood, WA reprinted with permission of Blade Tech Industries)

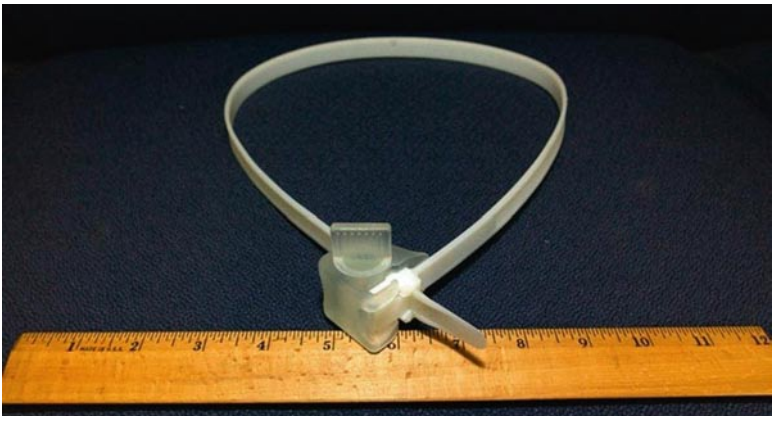

Fig. 5.22 Quickette (Ivy Off, Laxahatchee, FL, reprinted with permission)

TIAX Tourniquet (TIAX, LLC, Cambridge, MA).
Tourni-Kwik-3 (H & H Associates, Bena, VA).
Tourni-Kwik-4 (H & H Associates, Bena, VA).

The tourniquet pictures above were reproduced from the above mentioned studies by Hill et al. (2007), Ruterbusch et al. (2005), and Walters et al. (2005)

The following requirements for a combat tourniquet are the result of data, discussions, and conclusions drawn from the above conferences and studies. The requirements listed below, which are different than those for a clinical tourniquet, emphasize factors including self-application and operation, mobility, and light-weight construction.

- Sufficient belt width to occlude blood flow without shearing tissue, greater than 1 in.
- Narrow enough belt width to allow placement close to groin or arm pit, less than 4 in.
- Belt mesh design to allow blood and fluids to pass through the belt without trapping under the belt
- Appropriate width/circumference ratio to minimize occlusion pressure in order to minimize damage to tissue and nerves

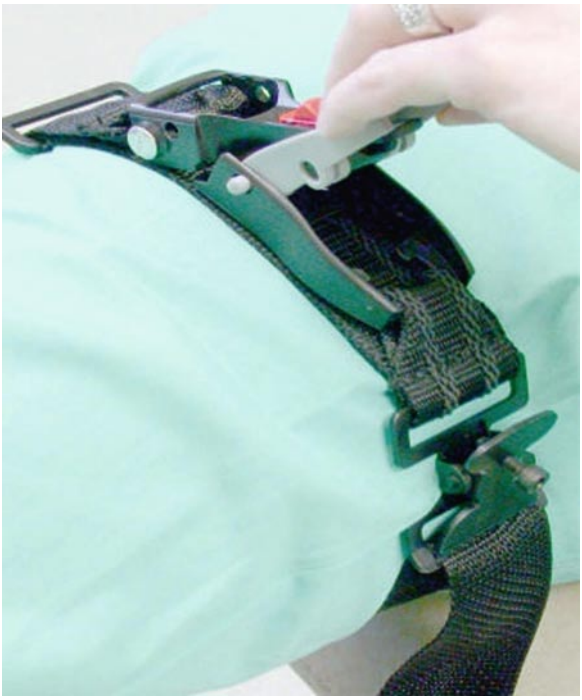

Fig. 5.23 Special applications tourniquet system (Marketing Tactics, LLC, Lake Worth, FL, reprinted with permission of Marketing Tactics, LLC)

- Low in weight and cube, less than 400 grams, preferably 200 grams or less.
- One-hand operation
- Mechanical advantage device for belt tension adjustment
- Manual microadjustment of occlusion pressure to avoid excessive pressure that may damage tissue and nerves
- Foul resistant belt fastening mechanism, no Velcro®-like materials
- Time to occlude blood flow not exceed 60 s
- Resistance to heat (exposure to air currents near fire, tropical climates, nonair conditioned storage, etc.)
- Capable of operating manually after damage by bullets, shrapnel, etc.
- Operational in mud, bloody field, and rain
- Capable of manual removal using buckle release
- Capable of being removed by patient or clinician by severing the belt with a knife or scissors
- Reusable buckle and disposable belt
- Long shelf storage without damage to any part of the tourniquet
- Low gloss and dark appearance
- Optional automatic occlusion pressure control, battery operated over 24 h

5.4 One-Hand Operated and Automatic Tourniquets for the Battlefield

The development of a tourniquet for battlefield application (Gooch et al. 2003) occurred in two stages. The first stage was the development of a one-handed ratcheting buckle strap tourniquet that would work reliably by itself and could be easily optionally augmented with an automatically controlled pneumatic pressure bladder. The second stage was the development of an automatically controlled pneumatic tourniquet that could occlude the blood flow within 15 s of application and would still function as a manually operated one-handed tourniquet in the event of a failure of the automatic system due to damage on the battlefield.

The one-hand and automatic tourniquets described in this section represent an innovation over the traditional battlefield devices presently employed. A one-hand operated combat tourniquet (see Figs. 5.24–5.36) has been designed and fabricated

Fig. 5.24 Special operations forces tactical tourniquet (Tactical Medical Solutions, Anderson, SC, reprinted with permission)

Fig. 5.25 TIAX tourniquet (TIAX, LLC, Cambridge, MA, reprinted with permission)

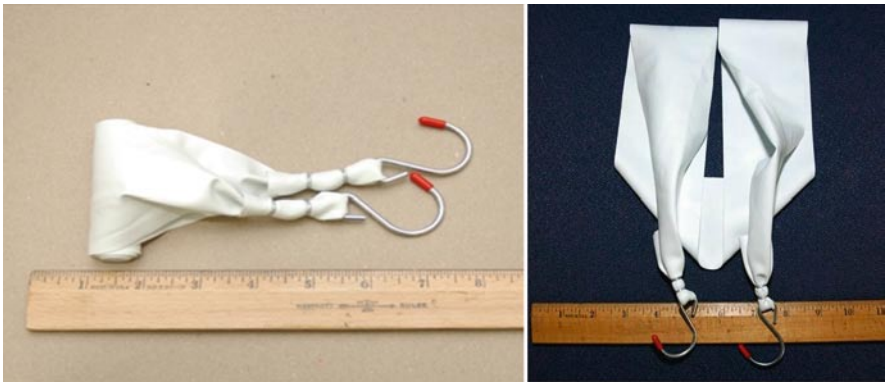

Fig. 5.26 Tourni-Kwik-3 (H & H Associates, Bena, VA, reprinted with permission)

Fig. 5.27 Tourni-Kwik-4 (H & H Associates, Bena, VA, reprinted with permission)

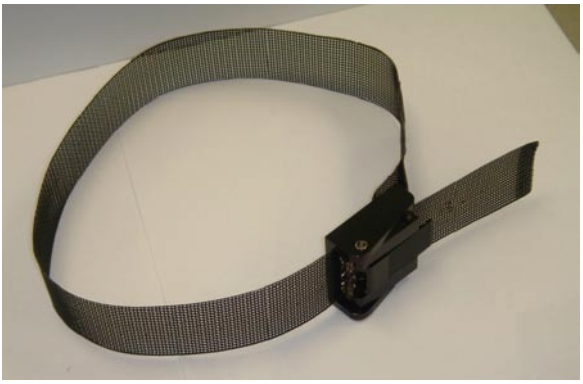

Fig. 5.28 Full view of one-hand cinch buckle tourniquet

to fulfill the requirements listed above. The one-hand operated cinch-buckle was built in two sizes: a version for a 1.5-in. strap that was intended to be used by itself as a manual tourniquet and a version for a 3.0-in. strap that was intended to be used with the autoinflation pneumatic tourniquet.

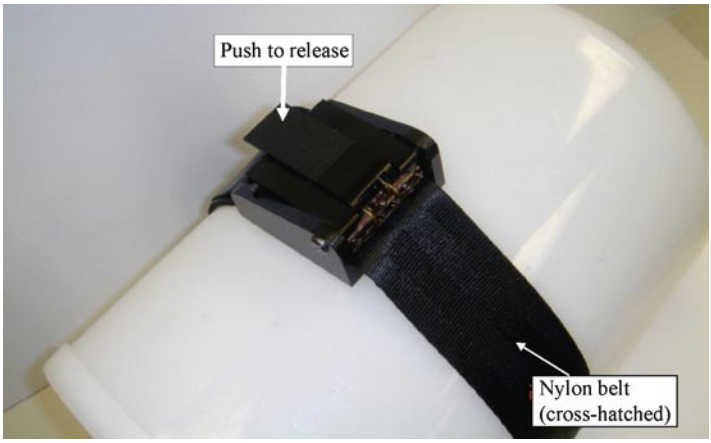

Fig. 5.29 View of cinch buckle deployed around limb-facsimile

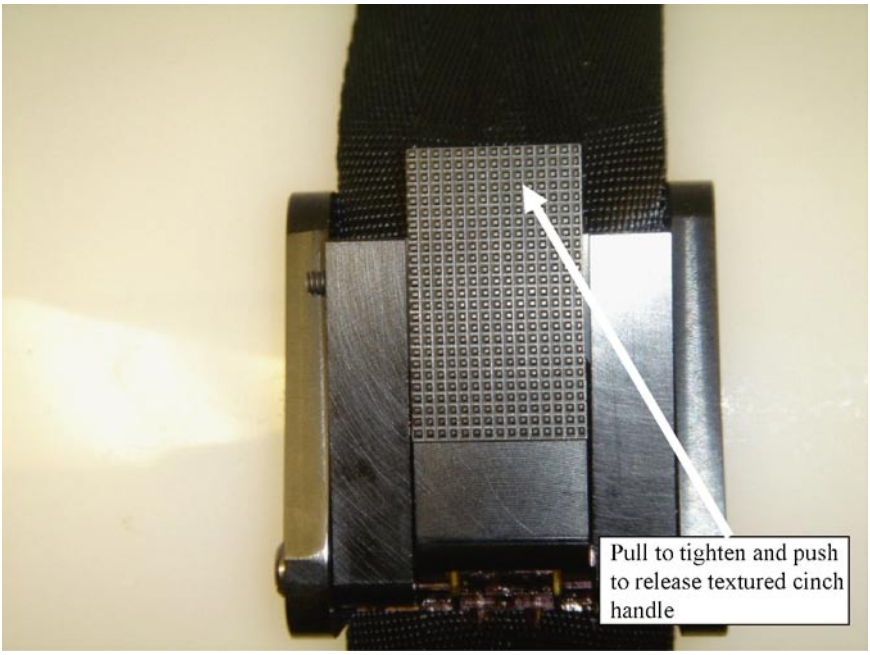

Fig. 5.30 View of textured cinch, cross-hatched, to assist a soldier in darkness where only tactile sensing is necessary to operate the tourniquet

A full view of the black-anodized buckle and belt are shown in Fig. 5.28. The buckle is colored to insure that this tourniquet has low visibility. The dark anodizing not only provides a dark color with low gloss (~5% at 60°) but also acts as a protective coating to inhibit corrosion and abrasive ware. Since a soldier often does not have the

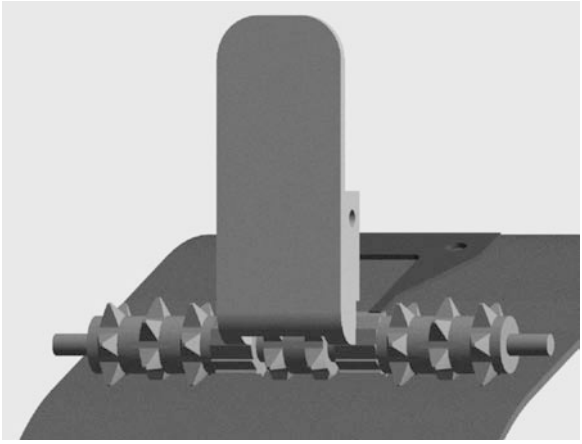

Fig. 5.31 Cinch buckle ratchet assembly

Fig. 5.32 One-hand cinch buckle open to adjust strap

luxury of proper lighting, the buckle is provided with a textured cinch-lever. This lever (featured in Fig. 5.30) provides the soldier with tactile-sensing as to the location of the cinch and point to apply force, so that the buckle can be operated blind.

The ratcheting cinch-buckle is capable of micro adjustment in 2 mm (0.08 in.) steps. The buckle's ratcheting assembly (depicted in Figs. 5.31–5.33) shows the ratchet gear, cinch-lever, and retention spring. The ratchet gear has 48 teeth organized into eight columns and six staggered rows. The teeth are long enough to penetrate the strap in order to prevent slippage under tension. The staggered layout of the teeth insures a minimum of four full and eight almost full strap penetrations at any position in the rotation of the gear. This insures that strap slippage will not occur until the tension is high enough to tear the strap by the teeth penetrations. As can be

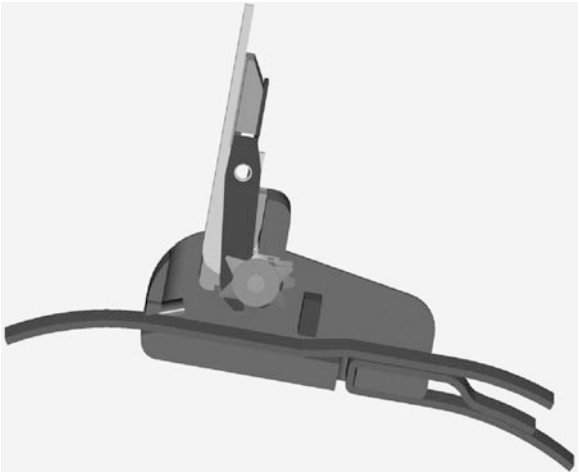

Fig. 5.33 Cinch buckle open to adjust strap, *cut-away view*

seen in the figure, there are two redundant columns of ratcheting teeth integrated between columns of the strap penetrating teeth. They are simultaneously engaged by the ratchet cinch-lever and retention spring. Each column alone is capable of maintaining the proper function of the assembly in the event that the other fails. The position of the retention spring's contact with the ratchet gear is offset with respect to that of the ratchet cinch-lever's contact. This offset reduces the wear on the gear steps which helps maintain reliable operation of the ratcheting mechanism. In addition, the offset allows for more efficient self-clearing of debris from the ratchet.

There are two quick-release mechanisms. The first is activated by pulling up the entire cinch mechanism handle as shown in Figs. 5.32 and 5.33 so that the strap can be adjusted and pulled tight prior to ratcheting to the proper tension for occlusion.

The second is a release-bar (the dark rectangle seen in Fig. 5.36 in the middle of the buckle housing) which is used to disengage the ratchet retention spring. This is accomplished by pressing down hard on the buckle handle.

The operation of the cinch-buckle tourniquet is simple and easily executed with a minimum of physical force as depicted in Figs. 5.34–5.37 in three simple steps. The first is to lift the full handle assembly. In this position, the ratchet assembly is lifted well above the strap and the teeth fully disengage from the strap. In this position, the free end of the strap can be completely removed from the cinch-buckle. This allows the strap to be placed around a trapped limb. In the case of a free limb, the strap which should be stored with the free end already threaded through the buckle can be slid over the limb to a position above the wound. The strap should then be pulled snug to remove the slack from the strap. Removing the slack at this time is highly recommended; however, if the user forgets to do so, the slack, no matter how great, can be removed by cinching in step 3. At this point, the handle assembly is then closed (step 2) as depicted in Fig. 5.35. In this position, the ratchet assembly teeth are fully engaged and penetrate the strap preventing it from moving.

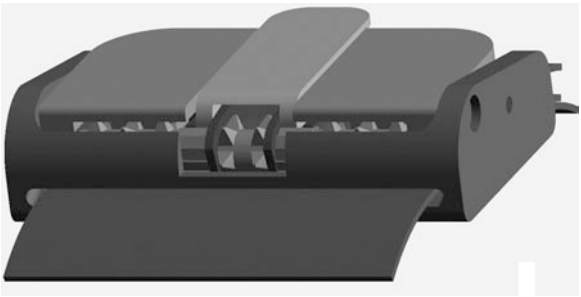

Fig. 5.34 One-hand cinch buckle closed to secure strap

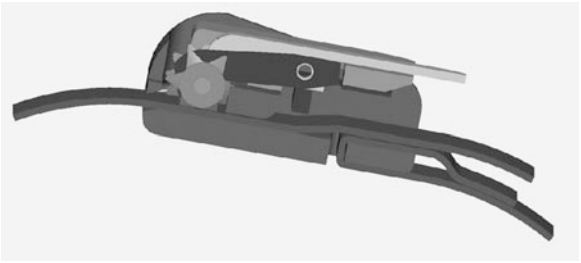

Fig. 5.35 Cinch buckle closed to retain strap, *cut-away view*

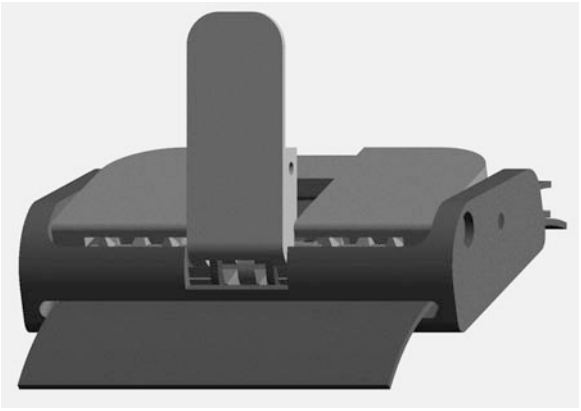

Fig. 5.36 One-hand cinch buckle ratcheting to tighten strap

In the third and final step, the cinch-lever is lifted, as depicted in Figs. 5.36 and 5.37, to adjust the tension on the strap to that needed for occlusion of blood flow in the limb, distal to the tourniquet. The cinch-lever can be repeatedly lifted or partially lifted to achieve proper strap tension.

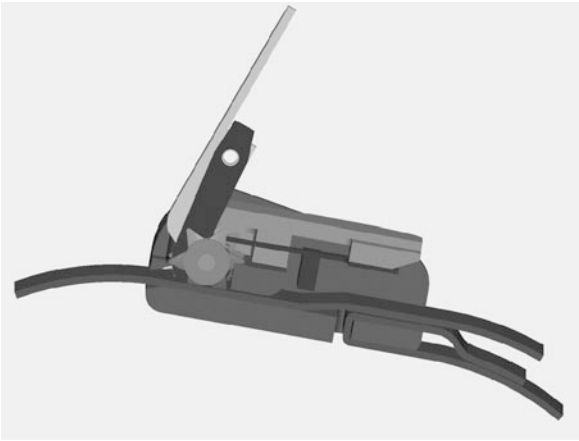

Fig. 5.37 Cinch buckle ratcheting to tighten strap, cut-away view

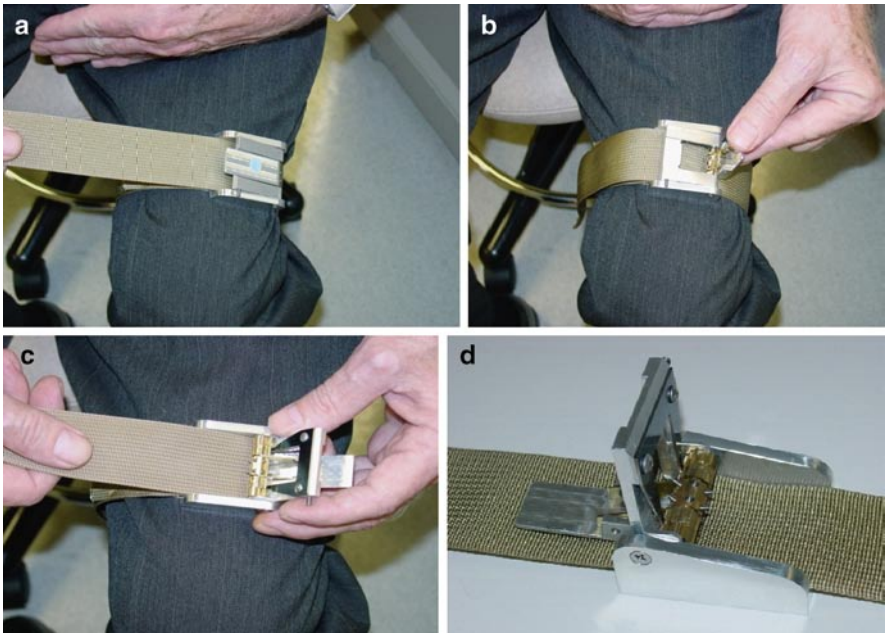

Fig. 5.38 Operation of one-handed cinch-buckle tourniquet: (**a**, **b**) lift the buckle handle assembly and thread the strap through the buckle and (**b**) pull to remove slack in strap, (**c**) close the buckle handle assembly to secure the strap. (**d**) Lift the cinch-lever to tighten the tourniquet to the proper occlusion pressure

The operation of the one-handed cinch-buckle is shown as demonstrated on a human subject in Fig. 5.38. The tourniquet buckle is not anodized and the strap is light in color for high visibility in these images. In views (a) and (b), the buckle

Fig. 5.39 View of extended tourniquet showing buckle in soldier's right hand and end of belt in left hand

handle assembly is in the open position and the strap is first threaded through the buckle and pulled taut with one hand to remove slack from the tourniquet. The handle assembly is then closed (c) to secure the tourniquet strap. The thumb-finger cinch (d) is pushed forward to tighten the belt and occlude blood flow. A unique and tissue-saving feature of the tourniquet is the micropressure that can be applied to the tourniquet by adjusting the cinch in the small 0.08 in. steps which will limit overpressure and damage to tissue. The tourniquet is released by raising the cinch handle assembly to the original position as shown in (a) in order to release the strap tension and remove the pressure from the limb, thus allowing the strap to move freely. The strap can then be removed. In the event of damage to the buckle, the sewed strap can be cut free with a knife or scissors from the buckle.

The tourniquet is low in weight (97 grams without autocontrol) and low in cube as shown in Fig. 5.39, and it is easily rolled-up and stored/carried in a pocket as illustrated in Fig. 5.40.

5.5 Automatic Occlusion Pressure Controller

To augment the one-hand operated cinch-buckle tourniquet in order to create a pneumatically operated battlefield tourniquet, an automatic occlusion pressure controller (Gooch et al. 2004) that could be used with the manually operated tourniquet was designed and

Fig. 5.40 Illustration of convenient storage in breast-pocket of rolled-up tourniquet

developed. The requirements for the battlefield tourniquet outlined in Sect. 3 translate to the following requirements for the automatic occlusion pressure controller:

- Battery power source with 24-h operation time
- Minipneumatic pump to inflate bladder capable of generating 500 mmHg pressure
- Elastomeric polymer bladder, with minimum 6 in. length × ¾ of tourniquet width
- Moisture resistant housing to protect components
- Exterior controls: ON/OFF power, adjustable occlusion pressure, and deflation of bladder
- Controller weight of less than 10.7 oz (300 grams)
- An electronic control circuit with pressure sensor that will automatically control pneumatic pressure to the bladder by sensing and maintaining pressure within ±10 mmHg.

The weight limit of 300 grams for the controller when added to the 97 grams for the manually operated section meets the maximum weight restriction for battlefield tourniquets of 400 grams. The controller as designed has a package weight of approximately 200 grams, which makes the tourniquet weight less than 300 grams.

In addition to the light weight in the above requirements, the pressure controller was designed to have a low profile for easy stowage and to facilitate its incorporation into future combat soldier uniforms. The packaging was also designed so that tourniquet straps from 1.5 to 3 in. in width could be used. This consideration would allow for wider tourniquets to be used on larger limbs and proxel limbs, and narrower

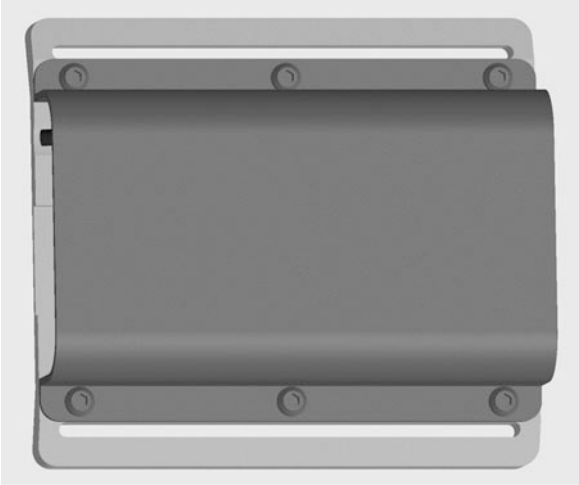

Fig. 5.41 Automatic occlusion pressure controller, *top view*

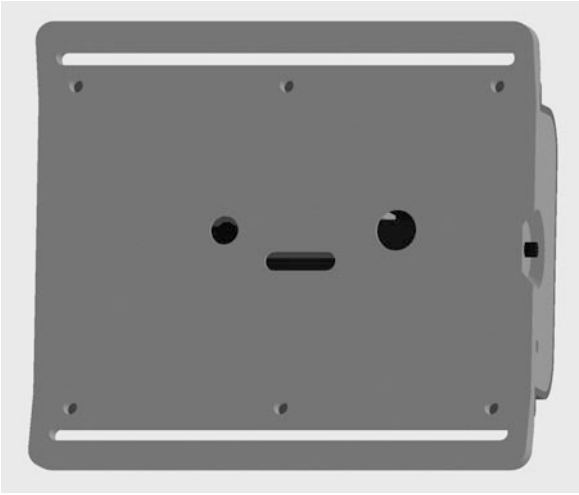

Fig. 5.42 Automatic occlusion pressure controller, *bottom view*

tourniquets on smaller limbs and distal limbs. Figs. 5.41–5.44 depict the automatic occlusion pressure package. The controller package has external dimensions of 3.25 in. long by 1.875 in. wide by 0.75 in. thick (8.2 cm by 4.8 cm by 1.9 cm), with 0.5 in. wide slotted flanges to retain the tourniquet strap. The external controls can be seen in Figs. 5.43 and 5.44. The ON/OFF power switch and the pressure adjustment control are located on the front panel (Fig. 5.43). These controls are recessed

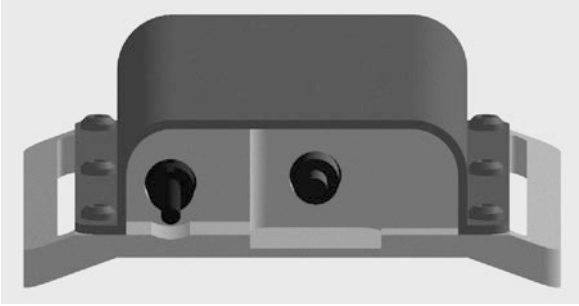

Fig. 5.43 Automatic occlusion pressure controller, *front view*

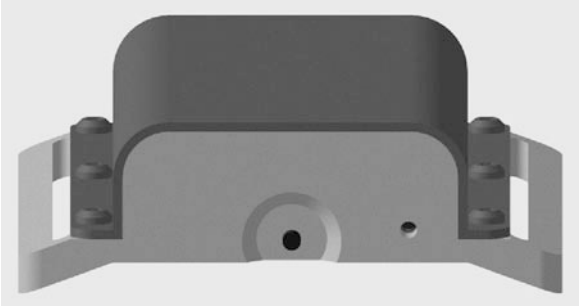

Fig. 5.44 Automatic occlusion pressure controller, *rear view*

under the case cover to prevent accidental activation of the controller. The pressure release button is located in a recess on the rear of the controller (Fig. 5.44). As can be seen from the front and rear views, the controller case is contoured to better fit the limb shape. The upper corners of the case are rounded so as to minimize the cases catching on clothing and other objects.

The automatic occlusion pressure controller is built around a microrolling air pump (Oken Seiko Co.) which can produce minimum pressures in excess of 520 mmHg at 6 VDC, with a typical flow of 90 $in.^3$/min (1,500 cm^3/min). The pump is mounted directly on the controller's printed circuit board (Fig. 5.45).

The pump and controller electronics are powered by two type CR2 3 VDC lithium photo batteries which are designed for high current applications. This makes them perfectly suited for driving the pump which will draw several hundred milliamps under full load. The actual full charge voltage of the batteries is 3.3 V which puts the total drive for the pump at 6.6 VDC. This adds a typical value of 100 mmHg above the nominal pressure for the pump. These batteries have a shelf life greater than 2 years. This means a long lifetime for the controller.

The controller electronics are located on a single multilayer printed circuit board. The circuit board is shown in Fig. 5.46.

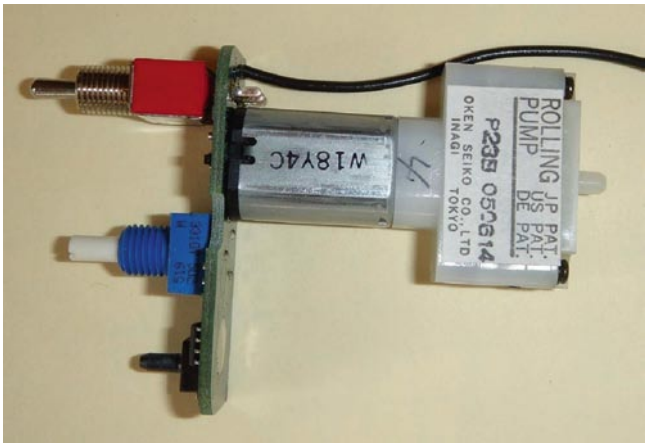

Fig. 5.45 Microrolling pump mounted on PC board

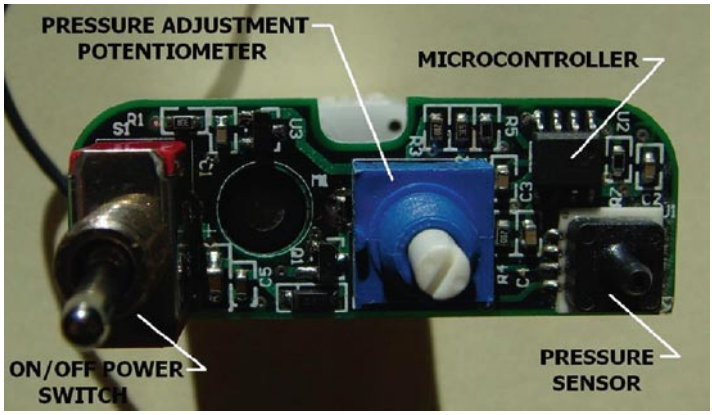

Fig. 5.46 Automatic occlusion pressure controller electronics PC board

An 8-bit microcontroller located on the upper right corner of the printed circuit board controls the system. When the power is activated, the microcontroller calculates the desired pressure from the readings it makes of the pressure adjustment potentiometer. It also measures the voltage output from the pressure sensor and calculates the pressure in the tourniquet's bladder. The microcontroller then compares the bladder pressure to that of the desired pressure. If the bladder pressure is lower than the desired pressure by an amount that is greater than the allowable pressure error, the microcontroller activates the pump to increase the pressure until the bladder pressure is just above the desired pressure. The microcontroller continuously monitors and adjusts the pressure in this manner as long as the power is on. The error range, and therefore the pressure variations, is set by the program and can be changed to suit any particular design. The typical error range is set to ±10 mmHg. The microcontroller is capable of recording the applied pressure versus time for the

duration of the tourniquet's use. These data could be made available to attending medical personnel for use in treatment decisions or injury statistics.

The components are arranged efficiently within the controller's case as depicted in Fig. 5.46. The components of the pneumatic system, pump, pressure bladder, pressure sensor, and pressure release switch, are connected by 0.125-in. outer diameter by 0.063-in. inner diameter silicone rubber tubing and two plastic tee fittings. The pressure release valve, located at the rear of the case, uses a spring arm lever to seal a knife edge style port with a silicone rubber sheet. The spring arm can be pushed open to release the bladder pressure (using the button shown in Fig. 5.47). This release valve also acts as a pressure relief valve to prohibit excessive overpressures in the tourniquet's bladder. The relief pressure can be adjusted by changing the thickness of the spring arm or by shimming it at its screw mount.

The PVC inflatable pressure bladder shown in Fig. 5.48 is nominally 2 in. wide by 7 in. long and has a maximum inflated volume of 15 $in.^3$. The typical inflation volume when secured under the tourniquet strap is less than 10 $in.^3$. The Oken Seiko pump will inflate the bladder in less than 10 s in typical tourniquet use. The pressure bladder is fastened to the bottom of the controller housing with waterproof adhesive and positioned by the two location nibs inserted into the hole and slot in the bottom of the housing. The bladder fill port extends into the controller housing through the third hole and is connected to the pneumatic system as described above with silicone rubber tubing. The adhesive serves not only to attach the pressure bladder but also to seal the penetrations in the bottom of the housing. Figure 5.49 shows the pressure bladder as attached to the controller housing.

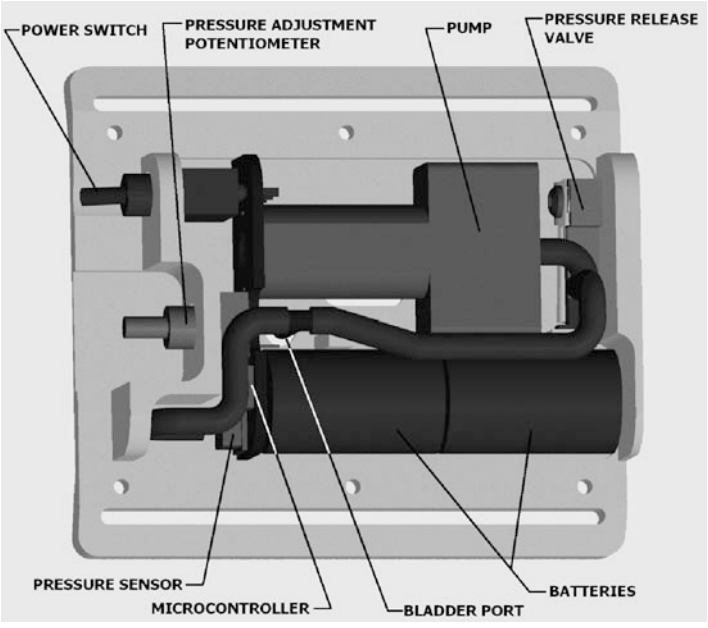

Fig. 5.47 Automatic occlusion pressure controller, *with lid removed*

Fig. 5.48 Pressure bladder for automatic occlusion pressure controller

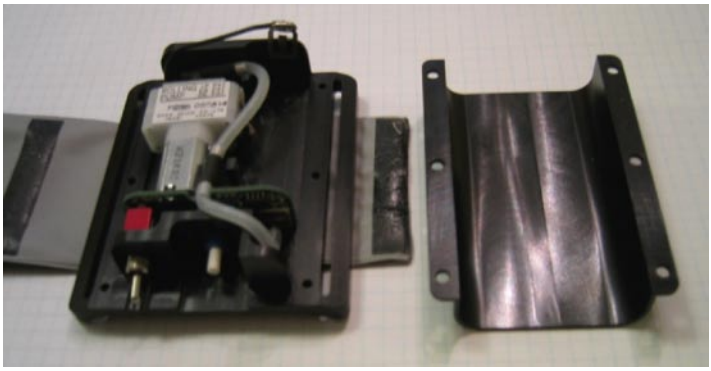

Fig. 5.49 Automatic occlusion pressure controller, with pressure bladder attached, *inside view*

The complete automatic occlusion pressure controller assembly (Fig. 5.50) is easily added to the basic cinch-buckle tourniquet by sliding the free end of the tourniquet strap over the pressure bladder, under the controller housing flange, and up through the slot in the housing flange. The strap is then passed over the top of the controller housing and down through the flange slot on the other side. In this manner, both the pressure bladder and the controller housing are positioned under the tourniquet strap and positioned by the controller housing flange slots. Figure 5.50 depicts the controller in position on the tourniquet strap with the bladder partially inflated and (Fig. 5.51) is a side view of the automatic occlusion pressure controller mounted on a tourniquet strap.

The combined one-hand operated automatic tourniquet has the following specifications:

- Tourniquet strap width: 1.5–2.9 in. (3.8–7.4 cm)
- Tourniquet strap length: 27 in. (68.6 cm)
- Open web strap to allow passage of blood and fluids, to prevent under strap trapping
- Power source: 6 VDC lithium battery pack
- Battery pack operation time greater than 24 h of typical tourniquet activation
- Battery pack shelf life greater than 2 years
- Micropneumatic pump to inflate bladder capable of generating 500 mmHg pressure

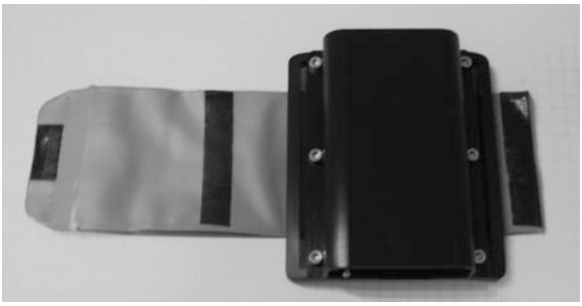

Fig. 5.50 Automatic occlusion pressure controller full assembly

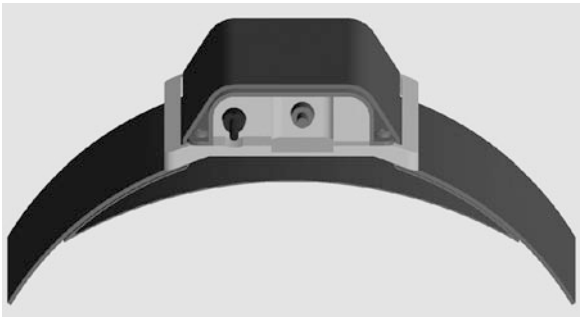

Fig. 5.51 Automatic occlusion pressure controller mounted on tourniquet strap

- Bladder inflation time typically less than 10 s
- Elastomeric polymer bladder, 7 in. length × 2 in. width
- Moisture resistant housing to protect components
- Exterior controls: ON/OFF power, adjustable occlusion pressure, and deflation of bladder
- Total weight of combined tourniquet unit: less than 10.7 oz. (300 grams)
- Microcontroller based electronics capable of automatically controlling pneumatic pressure to the bladder by sensing and maintaining pressure within ±10 mmHg
- A cinch-type buckle with ratcheting mechanical advantage, which will tighten the flexible tourniquet belt around the limb before engaging the pneumatic pump
- Capability to function manually using the mechanical advantage of the cinch-buckle in the event of pressure controller or bladder failure.

5.6 Summary

The needs and usefulness of tourniquets are obvious. An effective tourniquet is one-hand operated and applied and operated with the least amount of force and attention. Also, manually adjusted variable pressure tourniquets are safer than just

total exclusion of blood flow designs. An automatic tourniquet that will control pressure without the attention of the patient is the optimal device because the patient may lose consciousness, and attention to the tourniquet under combat conditions may not be possible. Better still, the perfect tourniquet is a device that can be worn on the limbs and will already be instantly in position when needed and automatic in operation.

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Appendices

Appendix A: Statistical Tests

Introduction

The use of statistical tests to analyze and quantify the significance of sample data is widespread in the study of biological systems where precise physical models are not readily available. Statistical tests are used in conjunction with measured data as an aid to understanding the significance of a result. Their aid in data analysis fills a need to answer the question of whether or not the inferences drawn from the data set are probable and statistically relevant. The statistical tests go further than a mere qualitative description of relevance. They are designed to provide a quantitative number for the probability that the stated hypothesis about the data is either true or false. In addition, they allow for the assessment of whether there are enough data to make a reasonable assumption about the system.

The primary statistical tests used in the studies described in this text are based on the chi-square tests which are in turn derived from the chi-square distribution which is based on the chi distribution. These tests include the chi-square test for goodness of fit, the chi-square test of independence, and Fisher's Exact Test. There are also corrections to some of the tests that account for small number deviations, Yates' Correction for Continuity, and for multiple studies attempting to verify the same procedures or processes, Bonferroni's correction.

Chi (χ) and Chi-Square (χ^2) Probability Distributions

For a set of random variables, such as set of x, y, and z coordinates of a distribution of random particles in space, common metrics such as average position and higher moments can be used to describe the distribution of these variables. For three-dimensional space, the most often used metric is the average position, (x_0, y_0, z_0), which is found by summing the individual types of coordinates and dividing by the number of positions. For example, x_0 would equal the sum of all of

the x-coordinates in the set divided by the number of particles. This is equivalent to finding the mean of the $x_i s$. If one were interested in how tightly the particles were clustered, the root-mean-square (rms) distance would be an appropriate measure. The rms distance from the mean position is calculated by taking the square root of the sum of the square distances from the mean position and dividing by the number of particles. In many cases, the variables are scaled or scattered differently due to the particular nature of the sets being described. Since the standard deviation of each variable gives a measure of its spread, an appropriate scaling factor for each variable would be the standard deviation of that variable. This scenario can be generalized to any set of N independent variables, $\boldsymbol{X}_i$, in which case the normalized distance, d, of any N-dimensional point from the mean point is given by:

$$d = \left[\sum_{i=1}^{N} \left(\frac{X_i - \mu_i}{\sigma_i} \right)^2 \right]^{1/2} \tag{1}$$

where: μ_i and σ_i are the mean and standard deviation of the ith independent random variable.

This formulation for the N-dimensional distance is directly related to the chi distribution. The chi distribution or χ distribution is a probability distribution that describes the variation from the mean value of the normalized distance of a set of continuous independent random variables that each has a normal distribution. More formally if $\boldsymbol{X}_1$, $\boldsymbol{X}_2$, …, $\boldsymbol{X}_N$ are a set of N continuous independent random variables, where each $\boldsymbol{X}_i$ has a normal distribution, then the random variable, $\boldsymbol{Y}$, given by:

$$Y = \left[\sum_{i=1}^{N} \left(\frac{X_i - \mu_i}{\sigma_i} \right)^2 \right]^{1/2} \tag{2}$$

where: μ_i and σ_i are the mean and standard deviation of the ith independent random variable.

The random variable, $\boldsymbol{Y}$, as defined above has the chi distribution, which is described by the following continuous probability density function, $f(x, N)$:

$$f(x,N) = \frac{x^{N-1} e^{-x^2/2}}{2^{N/2-1}\Gamma(N/2)} \tag{3}$$

for x greater than or equal to 0. $f(x, N)$ is equal to 0 when x is less than 0.

In the above formula, $\Gamma(\ )$ is the Gamma function. When N is even, $N/2$ is an integer and $\Gamma(N/2)$ is equal to $[(N-2)/2]!$ that is $\Gamma(1)=0!$, $\Gamma(2)=1!$, $\Gamma(3)=2!$, … When N is odd, $N/2$ contains a half integer and $\Gamma(N/2)$ is equal to $\pi^{1/2}[(N-2)/2]$ $[(N-4)/2]$ … $[3/2][1/2]$ that is $\Gamma(1/2)=\pi^{1/2}$, $\Gamma(3/2)=[1/2]\pi^{1/2}$, $\Gamma(5/2)=[3/4]\pi^{1/2}$, …

N is referred to as the degrees of freedom of the chi distribution. The chi distribution for an N of one is the half-normal distribution centered on zero with a standard deviation of $(\pi/2)^{1/2}$. For larger N, the distribution has a mean, μ, given by:

$$\mu = 2^{1/2}\Gamma[(N+1/2]/\Gamma(N/2) \tag{4}$$

and a standard deviation, σ, given by:

$$\sigma = \left(N - \mu^2\right)^{1/2} \tag{5}$$

The chi distribution is often confused with and used to describe the chi-square distribution or χ^2 distribution which is the distribution of the continuous random variable that represents the sum of the normalized squares of the $\boldsymbol{X}_i$ random variables. This is equal to the probability distribution that describes the square of the chi distribution, $\boldsymbol{Y}^2$, which is given by:

$$Y^2 = \sum_{i=1}^{N}\left(\frac{X_i - \mu_i}{\sigma_i}\right)^2 \tag{6}$$

The chi-square distribution has the probability density function, $f(x, N)$:

$$f(x,N) = \frac{x^{N/2-1}e^{-x/2}}{2^{N/2}\Gamma(N/2)} \tag{7}$$

for x greater than or equal to 0. $f(x, N)$ is equal to 0 when x is less than 0. The chi-square distribution has a mean that is equal to the degrees of freedom of the distribution, N, and a standard deviation of $(2N)^{1/2}$, making these quantities easy to visualize. As N increases, the chi-square distribution approaches the normal distribution with the same mean and standard deviation.

Chi-Square Tests

Chi-square distribution is extremely useful in determining whether a hypothesis is valid for large populations. For example, if a measurement is made on a group of randomly selected individuals from a large population and the measurements are divided into k ranges, the sum of the squares of the number of measurements falling into each range minus the expected number in that range normalized by the expected number in each range will exhibit a discrete probability distribution that can be very closely approximated in most instances by the continuous chi-square probability distribution, which as stated above has well known and well understood properties and readily available values. The value of this sum, $\boldsymbol{Y}^2$, for a single measurement is given by:

$$Y^2 = \sum_{i=1}^{k} \frac{(x_i - n_i)^2}{n_i} \tag{8}$$

where: x_i is the measured number in range i and n_i is the expected number in range i. Note that the $x_i s$ and $n_i s$ are actual measured quantities or numbers and not percentages.

The chi-square distribution for the above example will have $N = k - 1$ degrees of freedom and not k. This is due to the fact that the total number of measurements is a constant and thus only $k - 1$ of the measured numbers, x_i, are independent; since the last measured x_i is the difference of the total number of measurements minus the sum of the rest of the $x_i s$. The probability for having $\boldsymbol{Y}^2 s$ greater than or equal to the $\boldsymbol{Y}^2$ calculated from the actual measured data can be calculated from the chi-square distribution with $N = k - 1$. This value is often referred to as the P-value or probability value. This chi-square probability calculation is usually performed using interpolation from precalculated tables or by using calculators or computer analysis programs designed for this purpose. If the probability calculated is less than that expected from chance alone then the measurement is significant in that the hypothesis that it occurred by chance is improbable.

If an event occurs and the calculated probability of the event's occurring is so small that it is unlikely that the event occurred due to chance alone, the hypothesis, from which the probability that indicated the event would not occur was calculated, should be considered invalid. This qualitative decision based on "unlikely" is insufficient for comparison; therefore, the decision is based on a specific value that should always be specified along with the hypothesis. The limiting value chosen for this decision is termed the significance level and is usually represented by α. Significance levels of 0.01 (1%) and 0.05 (5%) are common. Some researchers use α values as low as 0.001. One would generally believe that events whose probabilities of occurrence are less than 1% or 5% did not occur through chance alone. The significance level commonly used in medical and biological research is $\alpha = 0.05$, which means a calculated probability, P-value < 0.05. At this level, there is only a one in 20 chance that the outcome was due to a random fluctuation in the variables. On the other end of the probability distribution range, if the probability is too high, greater than 95% or 99%, that the hypothesis is true, one might expect that things are too good to be true; since, it is statistically improbable that random-measured data would agree so well even with a distribution that correctly describes it. This often implies that the measurements were not statistically independent or that there was some error in the measurement. The steps in using this test are summarized as follows:

1. Determine the hypothesis to be evaluated and the method to calculate the expected results.
2. Gather the data by conducting the proposed experiment.
3. Determine the expected numbers for each observational class.
4. Calculate $\boldsymbol{Y}^2$ using the formula above.
5. Determine degrees of freedom, N, from the number of ranges, k, and the number of parameters estimated from the data, m.

6. Determine a significance level, α, to serve as the basis for accepting or rejecting the hypothesis, 0.05, 0.01, etc.
7. Use the chi-square distribution table, program, etc., to determine $\boldsymbol{Y}_{\alpha}^{2}$ value associated with the chosen significance value and degrees of freedom.
8. Accept (if $\boldsymbol{Y}^2 < \boldsymbol{Y}_{\alpha}^{2}$) or reject (if $\boldsymbol{Y}^2 \geq \boldsymbol{Y}_{\alpha}^{2}$) your hypothesis.
9. Repeat steps 6–8, choosing a significance level for the lower end, α, 0.95, 0.99, etc.

The above analysis is referred to as the chi-square test for goodness of fit. It is also known as Pearson's chi-square test. The freedom in choosing the method of generating the expected value, n_i, for the measurement ranges allows for a wide range of uses of this test. For example, this test can be used to determine whether or not a measured variable can be described by a particular probability distribution by using that distribution to calculate the expected values, n_i. This can be accomplished even if the particulars of the distribution are not known. The unknown probability parameters such as mean and standard deviation can be calculated using the measured data. There is, however, an adjustment that needs to be made for this case. The degrees of freedom, N, of the chi-square distribution needs to be reduced by the number of independent parameters that are estimated from the measurements; that is $N = k - 1 - m$, where: m is the number of independent parameters estimated from the measurements. It should be noted that the standard deviation and the variance are not independent since the variance is equal to the square of the standard deviation and would only decrease the degrees of freedom, N, by one. In addition to the freedom of choosing the expected values, the ease of calculation of $\boldsymbol{Y}^2$ and the broad availability of tables and programs for calculating the chi-square probabilities have contributed to the widespread use of the chi-square tests.

Chi-Square Test of Independence

The chi-square test for goodness of fit as described above was concerned with the distribution of ranges of a single variable. In many cases, especially in biological measurements, there is a desire to determine whether one type of procedure has an effect on the outcome of the measurement. This can be accomplished by using the chi-square test of independence. In this situation, there are usually two variables, the type of procedure and the outcome. An example would be a study to determine whether a treatment changes the survival rate of rats with any specific affliction. The experiment is designed with two groups, the treated group and the control group. Each group would then have two outcomes, survived and did not survive. At the end of the experiment, the data are organized into a contingency table. A contingency table is a row column matrix type table whose rows show the segregation of the population into types, e.g., treated and not treated. The contingency table columns show the range groups of the outcome, e.g., survived, and not survived. There is usually an extra row showing the outcome range group totals and an extra

column showing the population type totals. These are referred to as the marginal totals. A possible contingency table for the example study described above would be as follows:

Study groups	Survived	Did not survive	Group totals
Treated group	25	35	60
Control group	8	37	45
Outcome totals	33	72	105

The table above shows that there were 105 total individuals of which 60 were treated and 45 were not treated. Of the total group, 33 individuals survived, 25 in the treated group and 8 in the untreated group. In the example, the null hypothesis to be verified or rejected is that the treatment had no effect on the number of surviving individuals. The verification is made using the chi-square test of independence. The contingency table above is referred to as a 2 by 2 contingency table. This concept can be expanded to any number of rows and columns, i.e., an N by M contingency table and to any number of dimensions, i.e., an N_1 by N_2 by … by N_k contingency table. The general form of an N by M contingency table would be:

Study groups	Range 1	Range 2	…	Range M	Group totals
Study groups 1	x_{11}	x_{12}	…	x_{1M}	Group 1 total
Study groups 2	x_{21}	x_{22}	…	x_{2M}	Group 2 total
Study groups N	x_{N1}	x_{N2}	…	x_{NM}	Group N total
Range totals	Range 1 total	Range 2 total	…	Range M total	Study total

where x_{ij} is the number of measurements that fall in both group i and range j. Equation 10.3.1 can be generalized for the two-dimensional case to become:

$$Y^2 = \sum_{i=1}^{N} \sum_{j=1}^{M} \frac{(x_{ij} - n_{ij})^2}{n_{ij}} \tag{9}$$

where: n_{ij} is the expected number in group i and range j. For the k-dimensional case, $\mathbf{Y}_2$ is given by:

$$Y^2 = \sum_{i=1}^{N_1} \sum_{j=1}^{N_2} \cdots \sum_{s=1}^{N_k} \frac{(x_{ij} - n_{ij\ldots s})^2}{n_{ij\ldots s}} \tag{10}$$

These forms for $\mathbf{Y}^2$ as with the case for (10.3.1) are also very closely approximated by the chi-square distribution when the numbers are large; therefore, the $\mathbf{Y}_{\alpha}^{\ 2}s$ that are associated with the probabilities of the chi-square distribution having the same number of degrees of freedom can be compared with the $\mathbf{Y}^2$ calculated from (10.4.1) or (10.4.2) to determine whether or not to reject the null hypothesis of no effect. If the probability P-value associated with the $\mathbf{Y}^2$ is less than or equal to the significance level, α, chosen for the study, i.e., $\mathbf{Y}^2 \leq \mathbf{Y}_{\alpha}^{\ 2}$, then the hypothesis is rejected and the variables are not independent. This rejection would imply that the difference in results would be due to the difference in the variable, i.e., for a biological study, due to the treatment or procedure.

The number of degrees of freedom used with the chi-square distribution associated with the 2-dimensional distribution would be $(N-1)(\mathrm{M}-1)-m$, where: m is the number of independent parameters estimated from the measurements. For the k-dimensional case, the degrees of freedom used with the chi-square distribution would be $(N_1-1)(N_2-1)\ldots(N_k-1)-m$, where: m is the number of independent parameters estimated from the measurements. The steps used in the implementation of the chi-square test of independence are essentially the same as those listed for the chi-square test for goodness of fit. The only difference is that the expected values must be calculated for all $N \times M$ cases in the two-dimensional distribution and for all $N_1 \cdot N_2 \ldots N_k$ cases in the k-dimensional distribution. The expected values for the cells are often arranged in a table that resembles the contingency table or are sometimes included, inside parentheses, within the same cell of the contingency table as the measurement.

Due to the fact that the chi-square distribution is only an approximation to the $\boldsymbol{Y}^2$ distribution, a correction factor is sometimes applied. Some of the disagreement with the chi-square distribution is due to the fact that the Y^2 distribution is a discrete distribution while the chi-square distribution is a continuous distribution. The difference is greatest when the degree of freedom is only 1 and the values in some of the cells in the contingency table are small. The approximation commonly made when the degree of freedom is one of the Yates' Correction for Continuity (also known as Yates' correction, Yates' adjustment, or chi-squared correction) where the squared term in the $\boldsymbol{Y}^2$ equations is replaced by $(|x-n|-0.5)$; therefore, the equation for $\boldsymbol{Y}^2$ becomes:

$$Y^2 = \sum_{i=1}^{N}\sum_{j=1}^{M} \frac{(\left|x_{ij} - n_{ij}\right| - 0.5)^2}{n_{ij}} \tag{11}$$

This correction is generally applied when one or more cells of the contingency table have small values of 5 or less. Note: some sources state that this correction should be applied for values of 10, 20, or less.

Fisher's Exact Test

The chi-square tests described above use the chi-square distribution as a continuous approximation to the actual discrete probability distribution. As stated, the approximation is accurate for large numbers but becomes more inaccurate as the numbers decrease. The accuracy becomes so suspect for small numbers that corrections such as the Yates' correction are applied. When the numbers are small, it is possible to calculate the probabilities and the significances exactly. The methods for accomplishing this are referred to as exact tests. One such test is Fisher's Exact Test which uses the hypergeometric distribution to calculate the probability of having the set of values in any given contingency table. The method is to calculate the probability of obtaining a contingency table pattern that has the same marginal totals and that

is equally or more extreme than the observed table pattern. This is accomplished by calculating the probabilities for all table patterns with the same marginal totals and summing the ones that are equally or more extreme than the observed pattern. The sum should contain only those patterns that are extreme in the same way as the observed data.

The probability, P, for a given 2 by 2 contingency table pattern is given by:

$$P = \frac{(e_{11} + e_{12})!(e_{21} + e_{22})!(e_{11} + e_{21})!(e_{12} + e_{22})!}{(e_{11} + e_{12} + e_{21} + e_{22})!e_{11}!e_{12}!e_{21}!e_{22}!} \tag{12}$$

where: e_{ij} is the number of occurrences in the cell in the ith row and jth column. Since the 2 by 2 contingency table has only 1 degree of freedom, only the probabilities for tables whose cells give the same marginal totals as the measured contingency table need to be calculated. This means that the number of probabilities that need to be collected is equal to the smallest marginal total plus one. This is quite practical for small numbers but gets increasingly difficult as the numbers increase.

Once the set of trial probabilities has been calculated, the probabilities that are less than or equal to the probability of the measured contingency table are summed. They can be summed in two ways. The first and easiest is to find the sum of all the Ps in the set. This sum gives the probabilities at both extremes, those that are more extreme in the direction of the measured table, and those that are more extreme in the other direction. This will give the two-tail P-value and the test is termed a two-tailed test. The two-tail consideration describes the probability that a measured contingency table as far away from the expected contingency table as was the measured consistency table would occur. If this probability is less than or equal to the two-tail significance level, α, chosen for the study, then the null hypothesis of "no effect" is rejected; otherwise, the null hypothesis is accepted.

The second type of probability sum calculated from the set of Ps contains only those trial tables that are more extreme in the same direction as the measured contingency table. As with the chi-square test, if this probability is less than or equal to the significance level, α, chosen for the study, then the null hypothesis of "no effect" is rejected; otherwise, the null hypothesis is accepted. This P is referred to as a one-tail or one-sided P-value and its associated test, a one-tailed test. The difficulty in this type of test is to correctly identify the trial tables from the set that are more extreme in the same direction as the measured contingency table.

The hypergeometric distribution can be generalized to a multivariable form, the multivariate hypergeometric distribution, which can be used to extend Fisher's Exact Test to contingency tables larger than 2 by 2 and to multidimensional contingency tables. There is statistical software available to perform these calculations; however, due to the complexity of the calculations and the large number of trial tables whose probability of occurrence must be calculated, this extension has received limited use.

Multiple Studies: Bonferroni's Correction

When there are multiple studies to evaluate one procedure or method, the choice of the significance level becomes nontrivial. For one test, a significance level, α, of 0.05 indicates a 1 in 20 chance that the result was due to a large random fluctuation in the measurement and a 19 in 20 chance that it was not. If more than one study were made to evaluate the same procedure, there would be a be a $[1-(1.0-\alpha)^M]$, where M is the number of studies undertaken, probability that there is at least one result that was less than α due to a random fluctuation. On the other hand, there would be a probability of only α^M that all studies would have resulted in probabilities less than α due to random fluctuations in the measurements. For an α of 0.05 and an M of 2, there would be a 1 in 10 chance that at least one of the studies would result in a probability less than α due to a random fluctuation of the data, but there would be only a one in 400 chance both results would have probabilities less than α due to random fluctuations. The results become rapidly more extreme as M increases beyond 2.

If there are no results above the significance level, α, then the null hypothesis would be accepted. If all results are above the significance level then the null hypothesis would be rejected and the procedure would be deemed to have an effect. The middle range, where some studies have results with probabilities above and some have probabilities below the significance level, becomes ambiguous. Bonferroni's correction is useful in assigning significance to this middle range. Bonferroni's correction is applied by assigning each of the M studies a significance level of α/M. If the significance level is assigned in this manner the probability that at least one study will result in a probability less than or equal to α/M is α or the same as the original significance level. For example, a set of four studies each with a significance level of 0.05/4 or 0.0125 would have a probability of 0.05 that at least one study will have a probability of 0.0125 or less, in which case the null hypothesis should be rejected.

When using the Bonferroni's correction, care should be taken to ensure that the studies are not related or connected in a manner as to make the correction inappropriate. An example would be studies whose outcomes are correlated.

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Appendix B: Phenol Coefficient

Joseph Lister introduced phenol (carbolic acid) as a disinfectant in 1967. It has been the standard disinfectant to which other disinfectants are compared under the same conditions. The result of this comparison is the phenol coefficient. *Salmonella typhi*, a pathogen of the digestive system, and *Staphlococcus aureus*, a common wound pathogen, are typically used to determine phenol coefficients. A disinfectant with a phenol coefficient of 1.0 has the same effectiveness as phenol (Dorland's Illustrated Medical Dictionar). A coefficient less than 1.0 means that the disinfectant is less effective than phenol and greater than 1.0 is more effective than phenol. Phenol coefficients are reported separately for the different test organisms.

The procedure for determining the phenol coefficient is pervasion throughout the published literature, including a summary description by Black (2005), and examples of phenol coefficients are listed in the following table (Table A.1).

Table A.1 Phenol coefficients of common agents (Black 2002)

Chemical agent	*Staphylococcus aureus*	*Salmonella typhi*
Phenol	1.0	1.0
Chlorlamine	133.0	100.0
Cresols	2.3	2.3
Ethyl alcohol	6.3	6.3
Formalin	0.3	0.7
Hydrogen peroxide	–	0.01
Lysol™	5.0	3.2
Mercury chloride	100.0	143.0
Tincture of iodine	6.3	5.8

The phenol coefficient provides an acceptable means of evaluating the effectiveness of chemical agents derived from phenol, but it is less acceptable for other agents. Another problem is that the materials in which organisms may affect the usefulness of a chemical agent by complexing with it or inactivating it. These effects are not reflected in the phenol coefficient number.

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Appendix C: Antimicrobial Activity and Resistance

Typical chemical antimicrobial agents, their actions and uses are listed in Table A.2, restructured from Black (2002) and others.

The follow information (*italics*) is from Russell, A. D., Chapter 3. Principles of Antimicrobial Activity and Resistance, Disinfection, Sterilization, and Preservation, Block, S. S., editor., 5th ed., Lippincott, Williams and Wilkins, New York, 2001:

Antimicrobial agents may be of several different types, either physical or chemical or, sometimes, a combination of the two. Table [A.3] depicts the various processes that are available. Several physical processes such as moist heat, dry heat, and ionizing radiations usually can be relied on to kill all types of microorganisms, including bacterial spores, and thus will achieve sterilization. Temperatures well below 100°C may be of value in pasteurization or in the preparation of certain bacteria vaccines when the aim is to inactivate all the cells without affecting their antigenic identity. Ultraviolet (UV) radiation can kill spores but is considered a surface sterilizer only, and therefore effects disinfection rather than sterilization. Hydrostatic pressure is a method that uses the high pressures exerted by liquids on bacterial spores and other microorganisms. Some infectious agents, the prions, are highly resistant to physical and chemical processes (Taylor 1999).

Data from Russell et al. (1979 and 1984), Russell (1981 and 1998b), Dey and Engley (1983), Russell and Hugo (1994), and Liau et al. (1997). A "Universal" neutralizing solution is described by Dey and Engley (1983).

Mechanisms of Antibacterial Action

An immense amount of time and effort has been spent studying the effects of antibiotics on microorganisms, yet the precise mechanism of action of many biocidal compounds remains unclear [Table A.4]. Matters are undoubtedly improving, however, because the importance of understanding the mechanism whereby these agents exert their inhibitory and lethal effects on bacteria (and other types of microorganisms) is now better appreciated. Studies on mechanisms of microbial inactivation can lead to the design of improved molecules or to combinations of molecules, the overall aim always being to enhance activity while ensuring low toxicity to the user and the environment (Russell and Chopra 1996; Russell et al. 1997).

Table A.2 Chemical antimicrobial agents and properties (Black 2002)

Agent	Actions	Uses
Soaps/detergents	Lower surface tension, make microbes accessible to other agents	Hand washing, laundering, sanitizing kitchen equipment
Surfactants, emulsifiers	Dissolve lipids, disrupt membranes, denature proteins, and inactivate enzymes in high concentrations, and act as wetting agents	Cationic detergents are used to sanitize utensils, anionic detergents are used to launder clothes and clean household objects, and quaternary ammonium compounds are sometimes used as an antiseptic on skin
Acids	Lower pH and denature proteins	Food preservatives
Alkalis	Raise pH and denature proteins	Utilized in hand washing and other soaps
Heavy metals	Denature proteins	Silver nitrate is used to prevent gonococcal infection, mercury compounds are used to disinfect skin and objects, copper inhibits algal growth, and selenium inhibits fungal growth
Halogens	Oxidize cell components in absence of organic matter	Chlorine is used to kill pathogens in water and to disinfect utensils, and iodine/iodine compounds are as antiseptics
Alcohols	Denature proteins when mixed with water	Isopropyl alcohol is used to disinfect skin, and ethylene and propylene glycol are used in aerosols
Phenols	Disrupt cell membranes, denature proteins, and inactivate enzymes; phenol is not impaired by organic matter	Phenol is used to disinfect surfaces and destroy cultures; amylphenol destroys vegetative organisms and inactivates viruses on skin and objects; chlorhexidine gluconate is effective as surgical scrub
Oxidizing agents	Disrupt sulfide bonds	Hydrogen peroxide is used to clean puncture wounds; potassium permanganate is used to disinfect instruments
Alkylating agents	Disrupt structures of proteins and nucleic acid	Formaldehyde is used to inactivate viruses without destroying antigenic properties, glutaraldehyde is used to sterilize equipment, betapropiolactone is used to destroy hepatitis viruses, and ethylene oxide to sterilize objects that would be harmed by elevated temperatures

Table A.3 Types of antimicrobial processes

Type of process	Agent	Application	Comments
Physical	Dry heat (≥160°C)	Sterilization	Less effective than moist heat
	Moist heat (≥121°C)	Sterilization	Use of autoclave
	Moist heat (<100°C)	Disinfection	Inactivation of bacterial cells (56°C) in vaccine production
	Cold/freezing	Preservation	See text
	Ionizing radiation	Sterilization	
	Ultraviolet radiation	Disinfection	Considered a surface sterilizer only
	Hydrostatic pressure	Disinfection (?)	Activity dependent on concentration and temperature
Chemical (vapor phase)	Ethylene oxide	Disinfection	Ethylene oxide also used as sterilizing agent
	Propylene oxide		
	Formaldehyde		
	β – Propiolactone		Possible carcinogenic activity of β-propiolactone
Chemical (liquid phase)	Acids and esters, alcohols, aldehydes and aldehyde-releasing agents, halogens (including chlorine-releasing agents), metals, phenols and cresols, quaternary ammonium compounds, biguanides	Disinfection or preservation	Glutaraldehyde (pentanedial) has been considered a "chemosterilizer" Quaternary ammonium compounds: also used as antiseptics Chlorhexidine salts are important antiseptics, disinfectants, and preservatives
	Dyes (acridines, triphenylmethane)	Antisepsis	Now little used
	Metal chelate complexes	Antisepsis	
	Organic mercury compounds	Preservation or antisepsis	Important pharmaceutical preservations
	Silver compounds	Application to wounds	Effective against *Pseudomonas aeruginosa*

(continued)

Table A.3 (continued)

Type of process	Agent	Application	Comments
Other	Bacterial vaccines and toxoids Viral vaccines	Prophylaxis	Subunit vaccines sometimes available
	Rickettsial vaccines	Therapy	
	Antisera	Chemotherapy	
	Antiviral protein (interferon)		
Combined processes	Heat + chemical	Sterilization	Method at one time used in United Kingdom for sterilization of certain injections and eye drops
	Irradiation + chemical	Sterilization	Use of lowered radiation dose in combination with substerilizing temperature
	Thermoradiation	Sterilization	
	Heat + hydrostatic pressure	Sterilization (?)	
	Chemical + ultrasonics	Sterilization (?)	

Table A.4 Cellular targets of antimicrobial action[a]

Target	Agents	Effect
Cell Wall	Lysozyme	Attacks peptidoglycan (β, 1–4 links)
	Aldehydes	Interaction with $-NH_2$ groups
	Lysostaphin	Peptidase liberates *N*-terminal glycine and alanine
	Anionic surfactants	High concentrations: lysis
Outer membrane	EDTA (and similar chelating agents)	Chelates cations, induces release of up to 50% of lipopolysaccharide of outer membrane
	Lactoferrin, transferrin	Iron-binding proteins with effects apparently similar to ETDA
	Polycations (e.g., polylysine)	Displace cations
Cytoplasmic membrane	Moist heat, phenols, quaternary ammonium compounds, biguanides, parabens, hexachlorophene	Leakage of low-molecular-weight material; proton flux (for more specific information, see Table 3.4)
	Acridines, dyes, alkylating agents peroxygens, hypochlorites, ionizing and ultraviolet radiations	Possible binding of chemical agents to nucleic acids extensively studies (see text and Table 3.5)
Enzymes or proteins	Metal ions	–SH groups of enzymes, which may be membrane associated
	Alkylating agents, oxidizing agents	May also combine with DNA and RNA

EDTA ethylenediamine-tetraacetic acid

Appendix D: Halogens and Solutions for Disinfection

An antiseptic solution containing sodium hypochlorite (e.g., 0.05% by weight) has been developed to treat infected wounds. First used during World War I, Dakin's solution was the product of a long search by an English chemist, Henry Drysdale Dakin, and a French surgeon, Alexis Carrel, for an ideal wound antiseptic (Encyclopedia Britannica 2009). Stronger germicidal solutions, such as those containing carbolic acid (phenol) or iodine, either damage living cells or lose their potency in the presence of blood serum. Dakin's solution has neither disadvantage, and its solvent action on dead cells hastens the separation of dead from living tissue. Dakin's solution is prepared by passing chlorine into a solution of sodium hydroxide or sodium carbonate. The solution is unstable and cannot be stored more than a few days. The Carrel–Dakin treatment consists of the periodic flooding of an entire wound surface with the solution.

The following information (in italics) was taken directly from: Sidgwick, N. V., The Chemical Elements and Their Compounds, Vol. II, Clarendon Press, Oxford, V. K., 1950. This is the "earliest" comprehensive literature on chlorine solutions. The K_a values have been more accurately measured since 1950, but the equilibrium mechanisms remain unchanged. Many different forms of disinfectant solution have come from halogen solutions. This information is valuable because halogens and their corresponding salt solutions were some of the earliest disinfecting agents and continue to be widely used although the chemistry of halogen solutions is not well understood.

Elementary Chlorine

Chlorine is the most abundant of the halogens, especially in sea water, a ton of which contains in grams chlorine 15,000, bromine 97, iodine 0–17 (ratio 106:6,000:1). Its preparation depends on the discharge of its ion, either directly (i.e., electrolytically) or by oxidation. The older methods of oxidation (by manganese dioxide or by air in presence of certain catalysts) have now been replaced for technical purposes by the electrolysis of sodium chloride, which is primarily for the production of caustic soda, the chlorine being a by-product; the chloroparaffins which are now so much used as solvents were developed to utilize this chlorine.

Chlorine has two isotopes of masses 34.979 and 36.978 in the proportions 75.4, 24.6. They have been separated almost completely by thermal diffusion in a 20-m tube; their electrolytic separation factor is 1.006 on platinum electrodes, or 1.007 on graphite. The boiling and melting points data are given by Giauque. For the high purification of chlorine by distillation and freezing, see reference[294]. The dipole moment of chlorine is zero.

Chemically, chlorine is extremely reactive, though less so than fluorine. Water absorbs about twice its volume of the gas at 25°. It is present in the solution partly

as such, and partly as what may be called the hydrolytic products HCl and HOCl. In an N/40 solution of chlorine in water about one-half is present as the two acids. On standing, and more rapidly in sunlight, this "chlorine water" evolves oxygen through the conversion of HOCl into HCl.

Atomic Chlorine

This highly active monatomic form of chlorine, analogous to those of hydrogen, oxygen, and nitrogen, was discovered simultaneously by Rodebush and by Schwab. It is formed by the action of the electric discharge on chlorine gas at low temperatures. The metal of the electrode is liable to catalyze the recombination of the atoms. Hence Rodebush used external electrodes, while Schwab found that internal water-cooled electrodes of iron were more effective. Below 1 mm., the gas can be atomized up to some 20%. The atoms recombine rapidly; the rate – or rather the amount of uncombined atoms left – may be measuredby the heat evolved on the thermo-junction inserted in the gas is passing at a known rate. At about 0.1 mm. pressure the mean life in a glass tube was found to be 6×10^{-3} s. The combination occurs only on the walls of the tube. It is promoted by magnesium andcopper, and still more by silver, but platinum has no more effect than the glass. Methane "kills" the catalytic power of the glass, presumably by occupying the surface. Carbon monoxide promotes it.

Atomic chlorine is highly reactive, combining slowly with sulphur and red phosphorus, rapidly with copper and chromium sesquioxide. It also can be shown to start the long chains in a mixture of hydrogen and chlorine.

Chemical Properties

Hydrogen chloride gas, like the bromide and iodide, has a curious power of forming solid compounds of definite composition with certain anhydrous salts of oxy-acids, especially the sulphates, phosphates, and phosphites of di- and trivalent (mainly transitional and B) metals.[443] Many of these are formed at the ordinary temperature, and do not decompose below 200°, where the HCl compounds lose their HCl, but those of HBr and HI usually have the anion of the oxy-acid reduced, with liberation of the halogen. These compounds have as many molecules of halogens hydride to one metal atom as the latter has valencies; thus salts $M'''PO_4$ *have three molecules, but* $M''SO_4$, $M''HPO_4$, *and* $M''(HPO_2)_2$ *only two molecules of the halide to 1 M.*

Hydrogen chloride, which, like the bromide and iodide, is covalent in the pure state, remains covalent and unsolvated in nonionizing solvents such as the hydrocarbons and accordingly, has the minute solubility to be expected of a gas of so low a boiling-point. For example, the solubilities in grams per liter at 25° are hexane

6.20, benzene 13.7, o-nitro-toluene 180, chloroform at 0° 10. In such solutions, Henry's law is followed.[446] From the vapor pressures, the heat of the solution of the acid can be calculated[447–8]; it is 4.3 in benzene, 3.0 in chloroform, and 3.2 kcal/mole in ethylene dibromide.

On the other hand, the solubility in water is enormously greater (7.70 g per liter of solution at 20° and 1 atm.), while the vapor pressure rises much more rapidly than the concentration. Roscoe and Dittmar's results (1859) for water and HCl at 20° give these values of p, the pressure of the gas in mm, and c, the concentration of liquid in grams/liter, with those of c/p, which would be constant if Henry's law held:

p	60	500	1,300 mmHg
c	613	782	895 g/l
c/p	10.3	1.56	0.68

In the same way, Wrewsky[449] shows that to raise the solubility from 8 to 16 mol% at 25°, the pressure must be increased more than 100 times. See further Wynne-Jones.[450]

Ether, in which the solubility is 220 g/l at 20°, is intermediate between the other two classes of solvents, owing to the formation of the oxonium compound.

In water, at ordinary concentrations, the hydrogen chloride is practically all present as the hydrated ions. The infrared absorption bands characteristic of HCl, and shown by the liquid hydride and its solutions in nonionizing solvents do not appear in the aqueous solutions.[451] In dilute solutions, the conductivities agree with the Debye-Huckel-Onsager formula.

But the large vapor of the very concentrated solutions show that they contain a considerable amount of undissociated hydrogen chloride, and that in the general equation for ionization

$$M(A\square)M[A]\square\quad[[M]]^{-}++[[A]]^{-}-$$

(where according to Debye, the middle term M[A] represents only a limiting case) with hydrogen chloride that covalent H–Cl thoughnegligible at low concentrations becomes quite considerable at high concentrations. This behavior must be contrasted, on the one side, with that of weak acids like acetic, where the unionized form always predominates except at extreme dilutions, and, on the other, with salts like the alkaline halides, where this first (unionized or covalent) term must be entirely absent, since the salt can be shown to be ionized in all states, solid, liquid, and gaseous.

On the other hand, the halogen acids HCl, HBr, and HI, though they appear in dilute aqueous solution to be among the strongest acids, and to be highly dissociated as any, go over to the covalent form more easily than such acids as perchloric if the conditions become less favorable to ionization, either through the increase of concentration or when the water is replaced by a less powerfully ionizing solvent. Of the familiar strong acids, the first to "shut up" in this way is nitric, the next the halogen hydrides (other than HF, which is quite peculiar), and the last perchloric acid. These

facts were first pointed out by Hantzsch[452] and have been entirely confirmed by later work. Thus Hartley finds[453] that E.M.F. measurements of solutions of HCl even in methyl alcohol show the ionization to be less complete than in water.

Hydrogen chloride is often used in nonaqueous solvents as a condensing agent. It can be used in some reactions in the place of hydrogen fluoride. Simon[454] has carried out a series of alkylations of aromatic hydrocarbons using initial pressures of hydrogen chloride of 7–27 atm., and working at 75–235°. As with hydrogen fluoride, the products are always paracompounds. Thus tert. Butyl chloride reacts with toluene giving with either hydrogen chloride or fluoride only the para-di-derivative, while with aluminum chloride only 30–35% of para is formed, along with 65–70% of meta.

Hypohalous Acids, (H–O–X)

These acids, which in the undissociated form obviously have the structure H–O–X, occur with all three halogens, and are formed by the action of water on the elementary halogen, which leads (at any rate primarily) to a reversible hydrolysis of the type.

The three acids show a marked gradation in properties, which is especially clear with the most important of these, the completeness of the hydrolytic reaction by which they are formed, their strength as acids, and the tendency, which is common in varying degree to all of them, to change over into the halide and the halite. The approximate values of the constants of these three reactions are given below, the temperature being 25°C unless otherwise stated (Table A.5).

Table A.5 Dissociation constant of halogens in water

	Chlorine	Bromine	Iodine
$K_1 = \frac{[H^+]\cdot[H^-]\cdot[HOX]}{[X_2]}$	3.4×10^{-4}	5.8×10^{-9}	3×10^{-13}
K_2, Classical dissociation constant	3.2×10^{-8}	2×10^{-9}	3×10^{-11}
K_3 for reaction $3HOX\rightarrow 2HX+HXO_3$ (relative)	1	100	30,000

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Encyclopedia Britannica, Inc., Chicago, Illinois, 2009.

Appendix E: Tourniquet Testing Protocol

On the battlefield, a properly applied tourniquet can be an extremely effective means of controlling severe extremity wound hemorrhage and could prevent seven out of ten deaths (Bellamy 1984; Mabry 2000). However, a great deal of confusion exists among soldiers, medics, and military medical officers on a number of tourniquet-related issues. What is an appropriate combat tourniquet? When is it appropriate to use a tourniquet? When and by whom should a tourniquet be removed? Under what conditions a tourniquet should not be released or removed? What are the most effective ways to increase limb salvage while using a tourniquet?

Tourniquet use remains controversial and is the source of a good deal of confusion (Navein 2003). In civilian emergency medicine, the fear of tourniquet-related complications has all but eliminated their use. Yet, the Israeli Defense Force (IDF) advocates the liberal use of tourniquets (Lakstein 2003), as do members of the Special Operations Forces community (Butler 1996). These divergent views have led soldiers, combat life savers (CLS), medics, and other military medical personnel to considerable confusion.

In addition to the references mentioned above, other useful references for the readers' information are listed at the end of each chapter and at the end of the appendix.

Appendix F: One Hand Operated Cinch-Buckle Assembly and Details

The assembly and details of subcomponents of the cinch-buckle (Gooch 2003) are shown below and augment by engineering drawing and details contained in Tourniquet Engineering Drawings. The finished surface of the buckle is "anodized low-gloss black", but the parts are imaged in original aluminum and brass to show detail because anodized parts do not reflect light significant and do not image well. The tourniquet belt is a light-tan in color for imaging purposes only, and the actual tourniquet belt is low-gloss black as well. The over-all appearance of the tourniquet is low-gloss black (Figs. A.1–A.6).

Reference

Gooch, J. W., One-Hand Operated Tourniquet Design and Prototype, National Research Council Final Report, 2004.

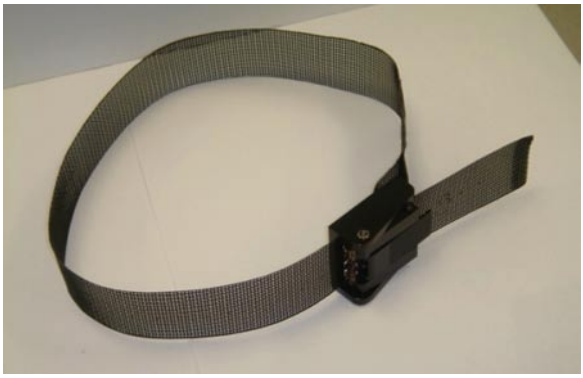


Fig. A.1 One-hand operated cinch-buckle tourniquet

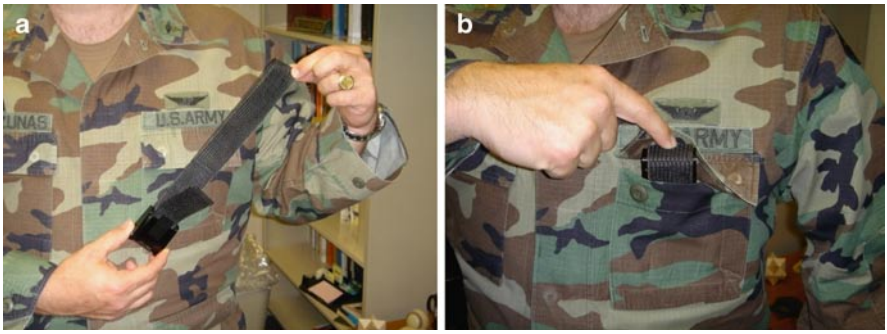


Fig. A.2 Soldier demonstrating (**a**) low cube and weight of tourniquet, and (**b**) rolled-up shape for convenient carrying shape in issue blouse pocket

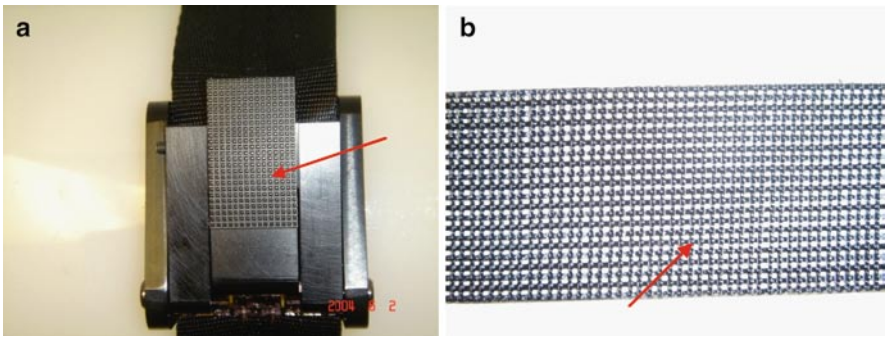


Fig. A.3 View of the (**a**) black anodized and textured thumb-finger operated cinch, convenient in darkness when the cinch can be located by touch rather than by sight, and (**c**) the *black*, 840 × 1680 denier nylon mesh belt with tractor holes for the spikes

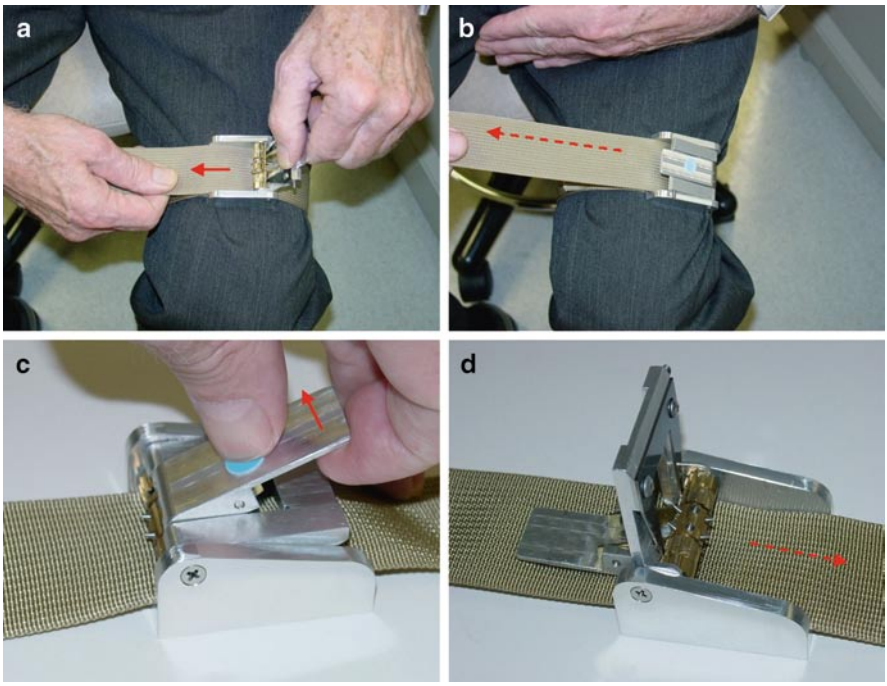


Fig. A.4 Demonstration of the application of the cinch-buckle tourniquet on lower left limb: (**a**) opening the buckle cinch and removing slack in belt, (**b**) pulling the belt taunt, (**c**) cinching the buckle to tighten belt (*blue dot* placement of thumb and index finger is used to apply pressure), and (**d**) removing the tourniquet by raising the cinch assembly releasing the tension and allowing the belt to travel backwards as indicated with the *red arrow*

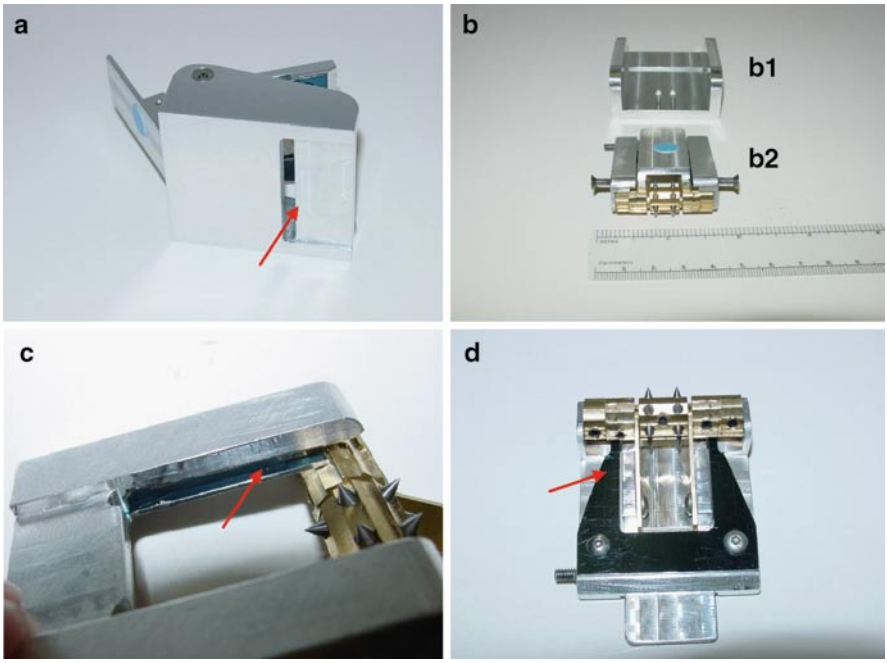


Fig. A.5 The base-plate is shown in (**a**) with the tourniquet belt slot indicated with the *red arrow*, the base-plate (**b1**) and cinch-assembly (**b2**) are shown together in (**b**) where b2 fit into b1 and secured with the shoulder mounted screw-fasteners; and the spring-steel stop-plate (*red arrow*) for controlling the travel of the cog-wheel containing the grasping spikes; the reverse side of the cinch-assembly riveted to the top section and detailed in (**d**) that shows the contact between the stop and cog-wheel

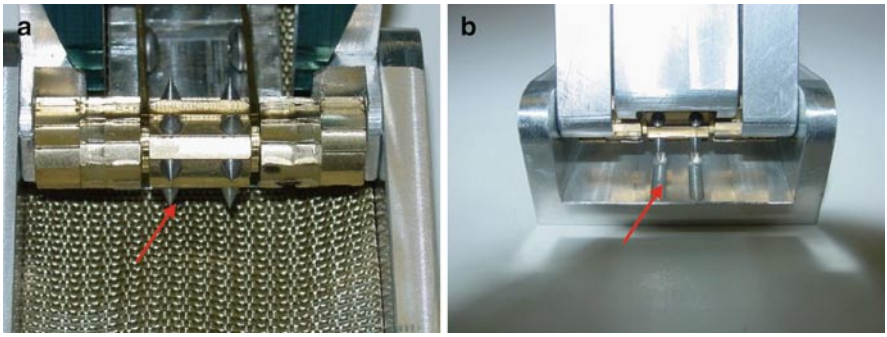
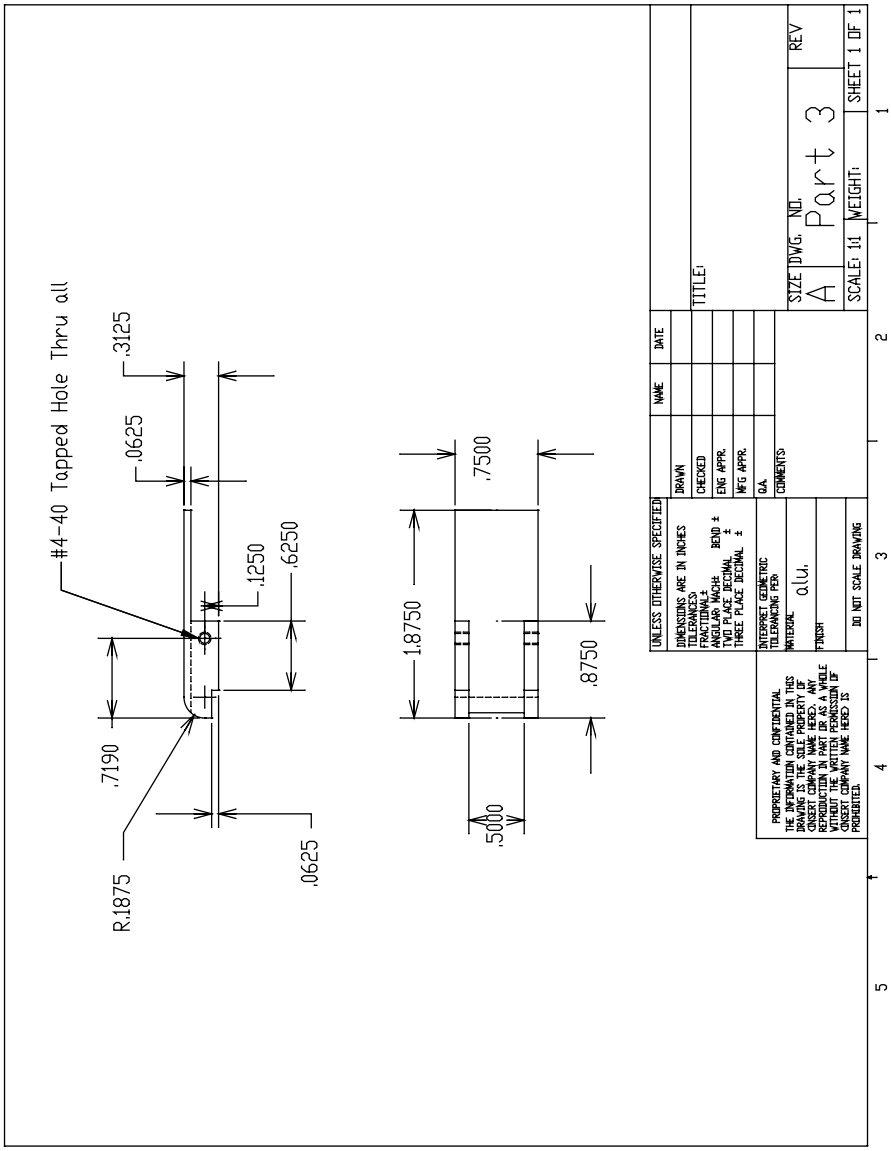
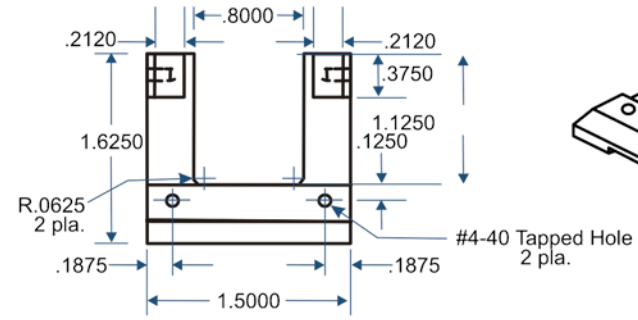


Fig. A.6 View of front section of buckle and (**a**) spikes for grasping mesh belt (*red arrow* points to spikes) with detail of cinch lever engaged on cog wheel, and (**b**) view of buckle minus belt detailing spikes and grooves (*red arrow*) for full penetration of belt

Appendix G: Tourniquet Engineering Drawings

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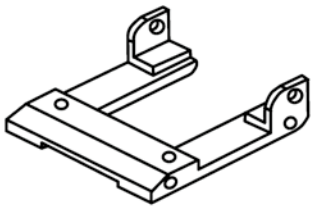
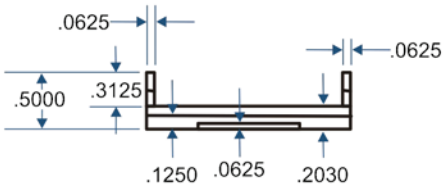
1.5000

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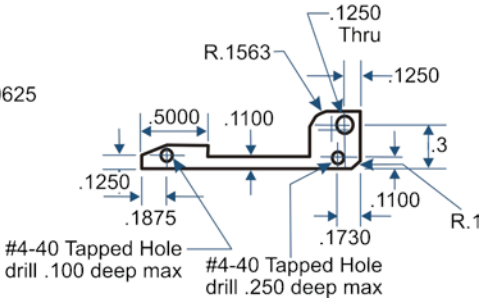
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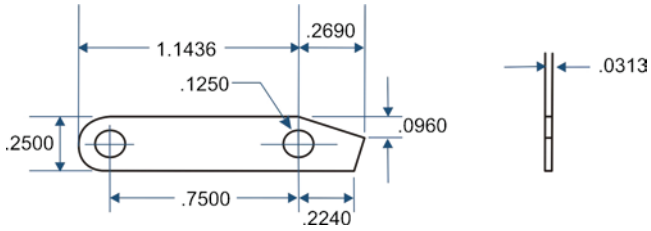
#4-40 Tapped Hole
drill .250 deep max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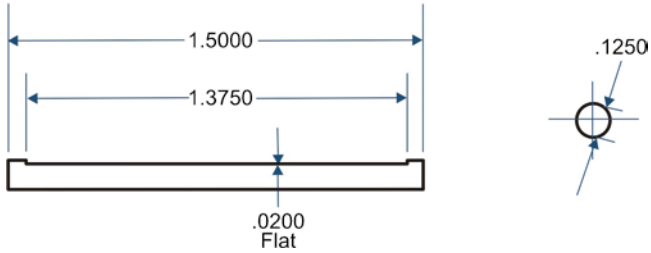
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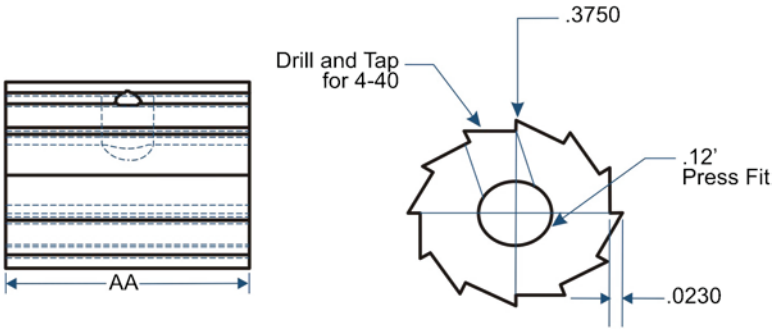

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