CHAPTER II

LITERATURE REVIEW

2.1 **Polymer Membrane** (Ulbricht, 2006)

A membrane is an interphase between two adjacent phases acting as a selective barrier, regulating the transport of substances between the two compartments. The main advantages of membrane technology as compared with other unit operations in (bio) chemical engineering are related to this unique separation principle, i.e. the transport selectivity of the membrane. Separations with membranes do not require additives, and they can be performed isothermally at low temperatures and—compared to other thermal separation processes—at low energy consumption. Also, up-scaling and down-scaling of membrane processes as well as their integration into other separation or reaction processes are easy.

The membrane process conditions must be engineered very carefully, but the performance limits are clearly determined by the membrane itself. This will be briefly explained by giving an overview on the main membrane processes and separation mechanisms. Even when ceramic, metal and liquid membranes are gaining more importance, the majority of membranes is and will be made from solid polymers. In general, this is due to the wide variability of barrier structures and properties, which can be designed by polymer materials. Current (1^{st} generation) membrane polymers are biopolymers (mainly cellulose derivatives) or (less than 20 major) synthetic engineering polymers, which had originally been developed for different purposes. The typical membrane structures and manufacturing technologies will be briefly summarized.

The development of synthetic membranes had always been inspired by the fact that the selective transport through biological membranes is enabled by highly specialized macromolecular and supramolecular assemblies based on and involved in molecular recognition. The focus of this feature article will be onto improved or novel functional polymer membranes (the 'next generation' of membrane materials), and important trends in this field include:

- the synthesis of novel polymers with well-defined structure as 'tailored' membrane materials
- advanced surface functionalizations, yielding novel barrier structures or enabling the combination of existing barrier structure with 'tailored' modes of interactions (from 'affin' to 'inert')
- the use of templates for creating tailored barrier or surface structures for membranes
- preparation of mixed matrix or composite membranes for the synergistic combination of different functions by different (polymeric) materials
- improved or novel processing of polymers for membranes, especially thin-layer technologies or the miniaturization of membrane manufacturing.

The barrier structure of membranes can be classified according to their porous character (Table 2.1).

Table 2.1 Classification of membranes and membrane processes for separations via passive transport

Membrane barrier structure	Trans-membrane gradient		
	Concentration	Pressure	Electrical field
Non-porous	Pervaporation (PV)	Gas separation (GS) Electrodialysis (ED)	Reverse Osmosis (RO)
Microporous pore diameter $d_p \leq 2$ nm	Dialysis (D)	Nanofiltration (NF)	
Mesoporous pore diameter $d_p = 2–50$ nm	Dialysis (D)	Ultrafiltration (UF)	Electrodialysis
Macroporous pore diameter $d_p = 50–500$ nm		Microfiltration (MF)	

An overview of the state-of-the-art polymeric materials, used for the manufacturing of commercial membranes, is given in Table 2.2. A closer inspection reveals that most of the membranes currently on the market are based on relatively few polymers which had originally been developed for other engineering applications.

An example is polyacrylonitrile fiber (PAN), a common and cheap commercial product. Polyacrylonitrile is used for very few products an average consumer would be familiar with, except to make another polymer, carbon fiber. Homopolymers of polyacrylonitrile have been uses as fibers in hot gas filtration systems, outdoor awnings, sails for yachts, and even fiber reinforced concrete. An interesting application is a membrane for filtration. Therefore, PAN is produced for Ultrafiltration (UF) such as a chelating fiber for adsorbing trace (metal ions) (Gong, 2001; Chang, 2001; Chang, 2002; Deng, 2003).

In this work, the membrane application for antibacterial activity should be interested in medical application such as surgical mask or industrial application such as air filtration. Therefore, the incorporation of antibacterial agent (likes silver) in PAN was prepared and investigated for further application.

Table 2.2 Polymers as materials for industrially established separation membranes

Polymer	Morphology			
	Barrier type	**Cross-section**	**Barrier thickness (mm)**	**Membrane process**
Cellulose acetates	Nonporous Mesoporous Macroporous	Anisotropic Anisotropic Isotropic	~0.1 ~0.1 50–300	GS, RO UF MF
Cellulose nitrate	Macroporous	Isotropic	100–500	MF
Cellulose, regenerated	Mesoporous	Anisotropic	~0.1	UF,D
Perfluorosulfonic acid polymer	Nonporous	Isotropic	50–500	ED, fuel cell
Polyacrylonitrile	Mesoporous	Anisotropic	~0.1	UF
Polyetherimides	Mesoporous	Anisotropic	~0.1	UF
Polyethersulfones	Mesoporous Macroporous	Anisotropic Isotropic	~0.150–300	UF MF
Polyethylene terephthalate	Macroporous	Isotropic track-etched	6–35	MF
Polyphenylene oxide	Nonporous	Anisotropic	~0.1	GS
Poly(styrene-co-divinylbenzene), sulfonated or aminated	Nonporous	Isotropic	100–500	ED
Polytetrafluoroethylene	Macroporous Nonporous	Isotropic	50–500 ~0.1	MF GS
Polyamide, aliphatic	Macroporous	Isotropic	100–500	MF
Polyamide, aromatic	Mesoporous	Anisotropic	~0.1	UF
Polyamide, aromatic, in situ synthesized	Nonporous	Anisotropic/composite	~0.05	RO, NF
Polycarbonates, aromatic	Nonporous Macroporous	Anisotropic Isotropic track-etched	~0.1 6–35	GS MF
Polyether, aliphatic crosslinked, in situ synthesized	Nonporous	Anisotropic/composite	~0.05	RO, NF

Table 2.2 Polymers as materials for industrially established separation membranes (continued)

Polymer	Morphology			
	Barrier type	**Cross-section**	**Barrier thickness (mm)**	**Membrane process**
Polyethylene	Macroporous	Isotropic	50–500	MF
Polyimides	Nonporous	Anisotropic	~0.1	GS, NF
Polypropylene	Macroporous	Isotropic	50–500	MF
Polysiloxanes	Nonporous	Anisotropic/composite	w0.1<1–10	GS PV, NF (organophilic)
Polysulfones	Nonporous Mesoporous	Anisotropic Anisotropic	~0.1 ~0.1	GS UF
Polyvinyl alcohol, crosslinked	Nonporous	Anisotropic/composite	<1–10	PV (hydrophilic)
Polyvinylidenefluoride	Mesoporous Macroporous	Anisotropic Isotropic	~0.1 50–300	UF MF

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2.2 Burns

A burn is an injury caused by *heat, cold, electricity, chemicals, light, radiation,* or *friction.* Burns can be highly variable in terms of the tissue affected, the severity, and resultant complications. Muscle, bone, blood vessel, and epidermal tissue can all be damaged with subsequent pain due to profound injury to nerve endings. Depending on the location affected and the degree of severity, a burn victim may experience a wide number of potentially fatal complications including shock, infection, electrolyte imbalance and respiratory distress. Beyond physical complications, burns can also result in severe psychological and emotional distress due to scarring and deformity.

2.2.1 Classification by Degree

The most common system of classifying burns categorizes them as first-, second-, or third-degree. Sometimes this is extended to include a fourth or even up to a sixth degree, but most burns are first- to third-degree, with the higher-degree burns typically being used to classify burns post-mortem. The following are brief descriptions of these classes (Burn Degrees, 2008).

2.2.1.1 First-degree Burns are usually limited to redness (erythema), a white plaque and minor pain at the site of injury. These burns only involve the epidermis.

2.2.1.2 Second-degree Burns manifest as erythema with superficial blistering of the skin, and can involve more or less pain depending on the level of nerve involvement. Second-degree burns involve the superficial (papillary) dermis and may also involve the deep (reticular) dermis layer.

2.2.1.3 Third-degree Burns occur when most of the epidermis is lost with damage to underlying ligaments, tendons and muscle. Burn victims will exhibit charring of the skin, and sometimes hard eschars will be present. An eschar is a scab that has separated from the unaffected part of the body. These types of burns are often considered painless, because nerve endings have been destroyed in the burned area. Hair follicles and sweat glands may also be lost due to complete destruction of the dermis. Third degree burns result in scarring and may be fatal if the affected area

is significantly large. If extensive enough, it can increase the risk of infection, including bacterial, and can result in death.

2.2.1.4 Fourth-degree Burns damage bone tissue and may result in a condition called compartment syndrome, which threatens both the life of the limb and the patient. These are burns in which most of the hypodermis is lost, charring and exposing the muscle underneath. Fourth-degree burns are frequently fatal.

Other classifications

A newer classification of "Superficial Thickness", "Partial Thickness" (which is divided into superficial and deep categories) and "Full Thickness" relates more precisely to the epidermis, dermis and subcutaneous layers of skin and is used to guide treatment and predict outcome.

Table 2.3 A description of the traditional and current classifications of burns

Nomenclature	Traditional nomenclature	Depth	Clinical findings
Superficial thickness	First-degree	Epidermis involvement	Erythema, minor pain, lack of blisters
Partial thickness — superficial	Second-degree	Superficial (papillary) dermis	Blisters, clear fluid, and pain
Partial thickness — deep	Second-degree	Deep (reticular) dermis	Whiter appearance, with decreased pain. Difficult to distinguish from full thickness
Full thickness	Third- or fourth-degree	Dermis and underlying tissue and possibly fascia, bone, or muscle	Hard, leather-like eschar, purple fluid, no sensation (insensate)

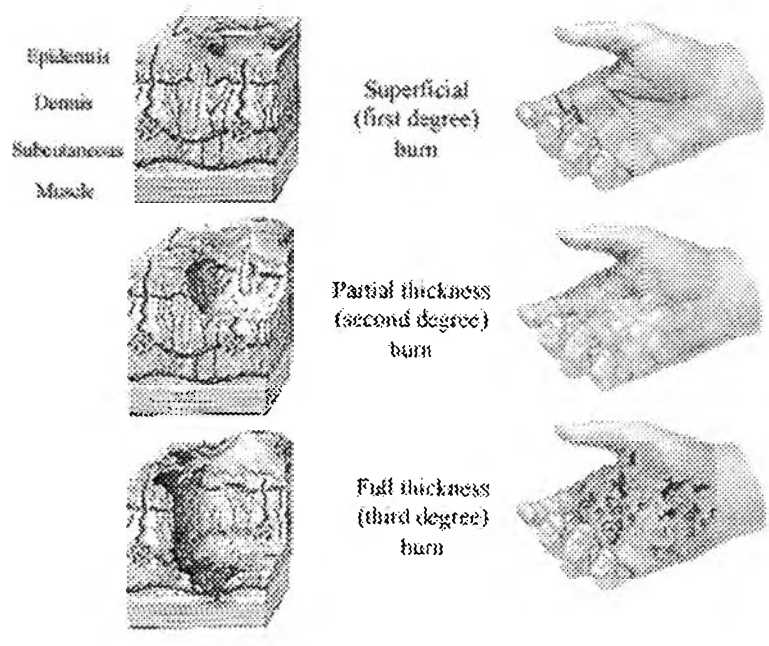


Figure 2.1 Classification of burn injuries (www.burn-recovery.org/injuries.htm).

2.2.2 Burn Wound Infection (Church, 2006)

Burn wound infection is a serious problem because it causes a delay in epidermal maturation and leads to additional scar tissue formation (Edwards, 2004; Singer, 2002). Invasion of microorganisms into the tissue layers below the dermis may also result in bacteremia, sepsis, and multiple-organ dysfunction syndrome (Baker, 1979; Mason, 1986).

Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers (Weber, 1997; Wurtz, 1995). Immediately following injury, gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound (Wysocki, 2002). Endogenous gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury (Manson, 1992). Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora (Rennie, 2000).

Staphylococcus aureus became the principal etiological agent of burn wound infections (Lilly, 1979; Phillips, 1989). Although *Staphylococcus aureus* remains a common cause of early burn wound infection,

Pseudomonas aeruginosa from the patient's endogenous gastrointestinal flora and/or an environmental source is the most common cause of burn wound infections in many centers (Altoparlak, 2004).

MRSA, methicillin-resistant coagulase-negative staphylococci, vancomycin-resistant enterococci, and multiply resistant gram-negative bacteria that possess several types of beta-lactamases, including extendedspectrum beta-lactamases, ampC beta-lactamases, and metallobeta-lactamases, have been emerging as serious pathogens in hospitalized patients (Clark, 2003; Dalamaga, 2003).

Fungal pathogens, particularly Candida spp., have increasingly become important opportunistic pathogens due to the use of broad-spectrum topical and systemic agents when infection occurs in the burned patient and have demonstrated increasing degrees of antifungal drug resistance (Appelgren, 2002; Baddley, 2004).

2.2.2 Types of Burn Wound Infection

2.2.2.1 Burn Wound Impetigo

Impetigo involves the loss of epithelium from a previously reepithelialized surface, such as grafted burns, partial-thickness burns allowed to close by secondary intention, or healed donor sites.

2.2.2.2 Burn-Related Surgical Wound Infection

Surgical wound infections in burn patients include both excised burn and donor sites that have not yet epithelialized. Surgical wound infections in open areas of the burn show loss of synthetic or biological covering of the wound, changes in wound appearance (such as hyperemia), and erythema in the uninjured skin surrounding the wound.

2.2.2.3 Burn Wound Cellulitis

Burn wound cellulitis results from an extension of infection into the healthy, uninjured skin and soft tissues surrounding the burn wound or donor

site. This condition is recognized by extension of erythema in the uninjured skin surrounding the burn beyond what is expected from the injury itself.

2.2.2.4 Invasive Infection in Unexcised Burn Wounds

Patients with areas of unexcised deep partial-thickness or full-thickness burn wound have an increased risk of developing an invasive infection. This complication may be heralded by a rapid associated change in burn wound appearance or character such as separation of the eschar or dark brown, black, or violaceous discoloration of the eschar.

Table 2.4 Lists the most common microorganisms colonizing and infecting burn wounds

Microorganisms causing invasive burn wound infection[a]	
Group	**Species**
Gram-positive organisms	*Staphylococcus aureus* Methicillin-resistant *S. aureus* Coagulase-negative staphylococci *Enterococcus* spp. Vancomycin-resistant enterococci
Gram-negative organisms	*Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella pneumoniae* *Serratia marcescens* *Enterobacter spp.* *Proteus spp.* *Acinetobacter spp.* *Bacteroides spp.*
Fungi	*Candida spp.* *Aspergillus spp.* *Fusarium spp.* *Alternaria spp.* *Rhizopus spp.* *Mucor spp.*
Viruses	Herpes simplex virus Cytomegalovirus Varicella-zoster virus

[a] Data are from references Agnihotri (2004), Bang (1999), Becker (1991), Burdge (1988), Gallagher (1970), Sheridan (2000)

2.3 Silver

Silver has a long history as an antimicrobial agent (Klasen, 2000; Klasen, 2001), especially in the treatment of burns. Silver (as well as copper) is used as a disinfectant in hospital and hotel water sanitization systems. It is used in the food industry in chicken farming and oyster cleaning to inhibit bacterial and fungal growth and in the space program to sterilize recycled water aboard the MIR space station and the NASA space shuttle (Searle, 1919). Although not employed in allopathic mainstream medicine, colloidal silver taken orally has been used as a complementary health aid, the overuse of which in some cases has lead to argyria (also referred to as "blue skin disease," where the reduced silver is deposited in dermal cells. This explains how the term "blue blood" originated). Silver also is used as a preservative in cosmetics and toiletries and has been incorporated into plastics of various forms to protect against microbial contamination.

Different silver delivery systems exist, including those that deliver silver from ionic compounds, such as silver calcium phosphate and silver chloride, and those that deliver silver from metallic compounds, such as nanocrystalline silver (Warriner, 2005; Kirsner, 2001). However, the difficulties with many current topical silver antimicrobials lie in their low silver release levels, the limited number of silver species released, the lack of penetration, the rapid consumption of silver ions, and the presence of nitrate or cream bases that are pro-inflammatory negatively affecting wound healing. Other issues include staining, electrolyte imbalance, and patient discomfort. Over the past few years, there has been a rapid increase in the number of silver dressings made available to physicians to address these issues (Warriner, 2005; Kirsner, 2001, Wright, 1999).

2.3.1 Types of Silver Products

Various available silver products may be summarized as follows (Atiyeh, 2007):

2.3.1.1 Colloidal Silver Solutions—Electrically Charged

This is the most common delivery system prior to 1960. Charged pure silver particles (3–5 ppm) are held in suspension by small electric

currents. Positive ions repel each other thus remain in solution even when applied topically to a wound.

2.3.1.2 Silver Proteins

Consist of silver complexed to small proteins in order to improve stability in solution. These however proved to possess much less antibacterial action than pure ionic silver and were rapidly replaced by silver salts in the 1960s.

2.3.1.3 Silver Salts

Delivery system becomes more stable when positively chargedsilver ion is complexed to negatively charged ions (AgCl, $AgNO_3$, $AgSO_4$). 0.5% Silver nitrate is the standard and most popular silver salt solution used for topical burn wound therapy. Concentrations exceeding 1% silver nitrate are toxic to the tissues. Ionic silver solutions are highly bactericidal, with no reported resistance and have a beneficial effect in decreasing wound surface inflammation. The solutions, however, are unstable and when exposed to light produce typical black stains therefore extremely unpractical. On the other hand, nitrate is toxic to wounds and to cells and appears to decrease healing offsetting to some degree the beneficial antibacterial effect of silver. Moreover, the reduction of nitrate to nitrite causes oxidant induced cell damage. This effect is most likely the reason for the impaired re-epithelialization reported with its use in partial thickness burns or donor sites. Bacterial resistance to $AgNO_3$ has been described.

2.3.1.4 Silver Compounds—Silver Sulfadiazine

Silver sulfadiazine (Flammazine®, Silvadene®) was introduced by Fox (Fox, 1967) in 1970s as an antibacterial agent for topical treatment of burns and wounds. Silver is complexed to propyleneglycol, stearyl alcohol, and isopropyl myrislate and mixed with the antibiotic Sulfadiazine producing a combined formulation made from silver nitrate and sodium sulphadiazine by substituting a silver atom for a hydrogen atom in the sulphadiazinemolecule and combining the inhibitory action of the silver with the antibacterial effect of sulphadiazine (Klasen, 2000, Stanford, 1969). This silver complex acts on the bacterial wall in contradistinction to the silver ions which act on the bacterial energy system. All kinds of combinations of sulpha drugs with silver were tested in vitro, but silver

sulphadiazine appeared to be the most effective (Stanford, 1969). A possible explanation of this effectiveness could be the relatively strong bonding of silver sulphadiazine to DNA (Klasen, 2000) which differs from that of silver nitrate or other silver salts (Klasen, 2000, Fox, 1971). Bacterial resistance to these products does develop. Impaired re-epithelialization has been described. Observed bone marrow toxicity with silver sulfadiazineis primarily due to the propylene glycol component.

2.3.1.5 Sustained Silver Releasing Systems—Nanocrystalline Silver

Various silver-based dressings have been introduced in the past few years and have become the latest and greatest ''innovation'' in wound care products. The ''innovation'' involved in these new wound care products is the simple fact that silver itself is incorporated within the dressing rather than being applied as a separate salt, compound, or solution. The basic issues in choosing a silver-containing dressing can be broadly conceptualized in terms of: (1) the characteristics of the ''carrier'' dressing and (2) the delivery of silver by the dressing to the wound. Keeping these basic issues in mind can help make sense of some of the media marketing blitz accompanying these products (Mooney, 2006). The following list of available silver dressings is not intended to be exhaustive, as the list is growing rapidly. Rather, it should be seen as illustrating various carrier dressing materials used in conjunction with various silver delivery ''reservoirs'' (Mooney, 2006).

- Acticoat-7 (Smith & Nephew, Hull, United Kingdom) dressing consists of three layers of polyethylene mesh coated with nanocrystalline (<20 nm diameter) silver and two layers of rayon polyester. The nanocrystalline silver provides an initial large bolus of silver to the wound followed by a sustained release.
- Actisorb Silver 220 (Johnson & Johnson, New Brunswick, N.J.) is an activated charcoal dressing to which silver is bound. Actisorb works by adsorbing bacteria onto the charcoal component, where they are killed by silver. The ''odoreating'' nature of the charcoal is used as a marketing focus.

- Aquacel-Ag hydrofiber (Convatec, Skillman, N.J.; 70:30 sodium: silver carboxymethylcellulose hydrofiber) is an absorptive dressing. Silver ion is displaced from the carboxymethylcellulose carrier as it is hydrated, thereby achieving a gradual, sustained slow release.
- Arglaes (Medline, Mundelein, Ill.) is silver-impregnated polymer film. The silver reservoir is Ag/CaPo4, formed as glasses co-extruded in a polymer matrix.
- Contreet-H (Coloplast, Marietta, Ga.) is a dense hydrocolloid dressing that has silver bound to the hydrocolloid.
- SilvaSorb (Medline) is a polyacrylate matrix with a silver halide reservoir.
- Silverlon (Argentum LLC, Willowbrook, Ill.) is a polymeric fabric coated with metallic silver by autocatalytic electroless chemical plating. A marketing focus is the three-dimensional fabric, which has a large surface area and is flexible.

2.3.1.6 Silver Nanoparticles

Metal nanoparticles and nanostructured materials are novel classes of materials, which have attracted great attention in catalysis (Daniel, 2004; Lewis, 1993), optics (Hayward, 2000; Ispasoiu, 2000), electronics (Kiesow, 2003; Poizot, 2000) and biomedicine (Daniel, 2004; Geckeler, 2006) as well as quantum-size domain applications (Wang, 2001) due to their unusual physicochemical properties that are quite different from those of the bulk solids. The synthesis of metal nanoparticles is a major research area in nanoscience and technology. Chemical reduction (Lisiecki, 1993), co precipitation (Chen, 2002), carbon nanotubes (Kim, 2005) and polymer protection (Yanagihara, 2001; Gao, 2004) has been extensively used as the best way to obtain metal nanoparticles with a narrow size distribution.

Nanotechnology has provided a way of producing pure silver nanoparticles. This system also markedly increases the rate of silver ion release (Fan, 1999). Silver nanoparticle is one of the most effective antimicrobial agents because of the high specific surface or volume fraction so that a large proportion of metal

atoms are directly contact with the environment. Silver nanoparticle is an effective antimicrobial agent, is non-toxic to human tissue and can kill a wide range of bacteria. Moreover, it can help in wound healing process.

Table 2.5 outlines the most widely used topical antimicrobial agents and new silver nanocrystalline dressings that are based on the bactericidal properties of the silver ion (Falcone, 1986; Heggers, 2002; Monafo, 1980). Nowadays, silver-based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers. The examples of silver-containing wound dressings that are commercially available such as Aquacle, Acticoat, Contreet Foam, Urgotul, PolyMem, Actisorb, Arglaes and Silverlon as shown in Table 2.6 and the pictures of these products are shown in Figure 2.2.

Table 2.5 Profile of commonly used topical antimicrobial agents [a]

Topical agent	Preparation	Eschar Penetration	Antibacterial activity[b]	Major toxicity
Silver nitrate ($AgNO_3$)	0.5% solution	None	Bacteriostatic against aerobic gram-negative bacilli, *P. aeruginosa*, limited antifungal	Electrolyte imbalance
Silver sulfadiazine (Silvodene, Flamazine, Thermazine, Burnazine)	1% water-solution cream (oil-in-water emulsion	None	Bactericidal against aerobic gram-negative bacilli, *P. aeruginosa*, some *C. albicans*	Leukopenia
Nanocrystalline silver dressings (Acticoat A.B. dressing, Silverlon etc.)	Dressing consisting of two sheets of high-density polyethylene mesh coated with nanocrystalline silver	Moderate	Protent activity against aerobic gram-negative bacilli, *P. aeruginosa*, aerobic gram-positive bacilli, MRSA,VRE, mutidrug-resistant *Enterobacteriaceae*	Limited toxicity

[a] Data are from references Falcone, 1986; Heggers, 2002; Monafo, 1980.

[b] VRE, vancomycin-resistant enterococci.

Table 2.6 Commercially Topical Dressings

Dressing Name	Manufacturer	Basic dressing	Silver composition	Silver release
AQUACEL® Ag	ConvaTec (Deeside, UK)	Hydrocolloid fiber (sodium carboxymethyl cellulose)	1.2% w/w ionic silver (silver nitrate)	Ag^+
Acticoat™	Smith & Nephew (Hull, UK)	Absorbent polyester inner core sandwiched between two outer layers of silver-coated polyethylene net (Vapor-deposition)	Metallic nanocrystallinesilver	Ag^+
Contreet ® Foam	Coloplast (Humlebaek, Denmark)	Polyurethane foam	Ionicsilver (silver sodium hydrogen zirconium phosphate)	Ag^+
Urgotul ® S.S.D	Laboratory URGO (Chenove, France)	Polyester gauze dressing impregnated with hydrocolloid particles dispersed in a Vaseline paste	Silver sulfadiazine	Ag^+
PolyMem ® Silver	Ferris Mfg. Corp. (Burr Ridge, IL)	Polyurethane foam containing a safe nontoxic cleanser (F-68 surfactant), a moisturizer (glycerol) and an absorbing agent (superabsorbent starch copolymer)	Elemental nanocrystalline silver (124 ug/cm^2)	Ag^+
Actisorb Silver 220	Johnson & Johnson, New Brunswick, N.J.	Antisorb works by adsorbing bacteria onto the charcoal component, when they are killed by silver	Silver is bound with an activated charcoal dressing.	Ag^+
Arglaes	Medline, Mundelein, ill	Silver is impregnated polymer film	The silver reservoir is $Ag/CaPo_4$, formed as glasses co-extruded in a polymer matrix	Ag^+
Silverlon	Argentum LLC, Willowbrook, ill	Polymeric fabric is coated with metallic silver by autocatalytic/electroless chemical plating	Metallic Silver	Ag^+

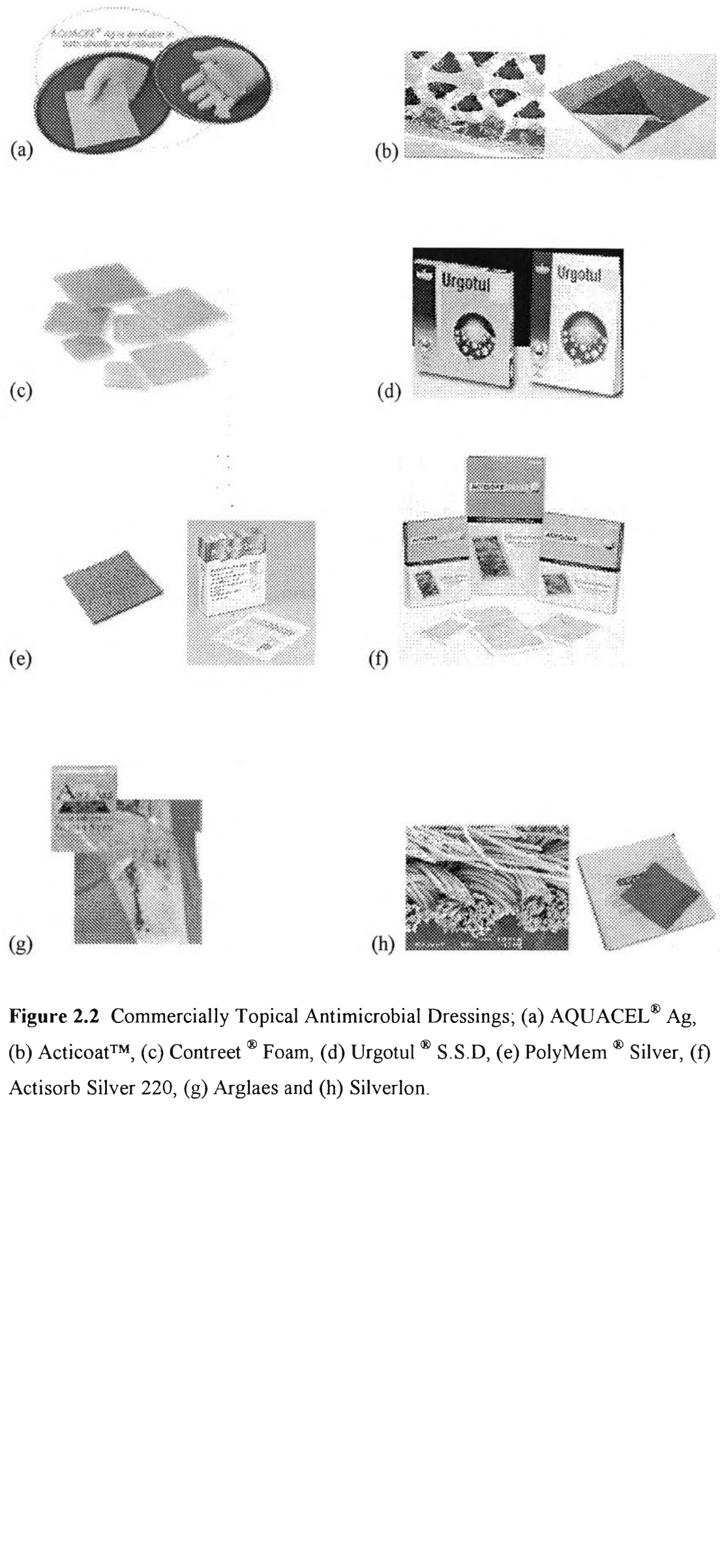


Figure 2.2 Commercially Topical Antimicrobial Dressings; (a) AQUACEL® Ag, (b) Acticoat™, (c) Contreet ® Foam, (d) Urgotul ® S.S.D, (e) PolyMem ® Silver, (f) Actisorb Silver 220, (g) Arglaes and (h) Silverlon.

2.3.2 Mechanism of Silver Ions Against Bacteria

The increasing number of commercially available silver-based dressings, there is a distinct lack of comparative data on their clinical effectiveness. What is known is that silver can be effective against a wide range of microorganisms, including aerobic, anaerobic, Gram-negative and Gram-positive bacteria, yeast, fungi, and viruses. Elemental silver (Ag^{o}) appears to have no antibacterial, whereas its cation (Ag^{+}) is highly reactive (Brett, 2006; Maillard, 2006; Lansdown (silver I and II), 2002), particularly at a concentration between 5 and 40 mg/l (Burrell, 2003), and its low concentration component means it retains efficacy even when dilute. The antimicrobial effect of silver can be explained by various mechanisms:

2.3.2.1 The inhibitory action of silver is due to its strong interaction with thiol groups present in the respiratory enzymes in the bacterial cell (Lansdown (silver II), 2002; Lansdown, 2005).

Unlike antibiotics, silver is toxic to multiple components of bacterial cell metabolism. These include damage to the bacterial cell wall, and membrane permeability leads to gross cellular structural changes, blockage of transport and enzyme systems such as the respiratory cytochromes, alteration of proteins and binding of microbial deoxyribonucleic acid and ribonucleic acid to prevent transcription and division.

2.3.2.2 Silver has also been shown to interact with structural proteins and preferentially bind with DNA nucleic acid bases to inhibit replication (Lansdown (silver I), 2002; Lansdown (silver II), 2002).

Like other antiseptics, silver is soon inactivated by protein binding, but this inactivation can also be caused by tissues and anions such as chloride, phosphate and sulphide. For this reason, silver has recently been shown to be highly toxic to keratinocytes and fibroblasts and may delay burn wound healing if applied indiscriminately to debrided healing tissue areas (Cooper, 1990; Lansdown (silver I), 2002).

Dressings that can sustain release of silver do not need to be changed so often, thereby representing a nursing management time benefit. A reduced number of

dressing changes could affect positively a patient's quality of life, particularly in burn management.

Organisms do vary in their susceptibility to silver, but there is good evidence that silver has activity against the common pathogens, *S. aureus* and *Pseudomonas spp.*, which are commonly encountered in chronic wound care. The newer dressings present silver ions differently from silver nitrate and SSD. These include forming unique Ag^{+}/Ag^{o} complexes by the use of nanocrystalline technology, or a high silver availability (Ag^{+}) through other means, to give a large and effective sustained bolus delivery (Wright, 1998; Thomas, 2004). Clinical evidence of bacterial resistance to silver ions, involving organisms cultured from chronic wounds, is awaited, but it would be inappropriate to discount that the possibility could occur. Local staining by silver dressings does not appear to be a major complication and is usually temporary. This probably relates to sustained release and high bioavailability, which is furnished by many of the new dressings. Although the level of staining relates to the silver concentration presented by dressings at the wound–skin interface, penetration into the tissues is small. This is more likely with the use of silver nitrate (Walker, 2005). Systemic toxicity, argyria, is unlikely as absorption from dressings is so small and probably depends on wound size (Lansdown, 2005). This systemic risk is probably overstated, just as the risk of thyroid disorder is after the use of povidone–iodine in chronic wounds (Teot, 2004). Nevertheless, argyria may theoretically result when there is a very large open wound and dressings that release large amounts of silver ions are used. There have been no consistent reports of silver allergy, unlike the use of topical antibiotics, such as neomycin, and some other antiseptics.

2.4 Advances in Wound Dressing

2.4.1 Wound Healing Process

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. It is a complex biological process involving haemostasis and inflammation, migration, proliferation, and maturation (Debra, 1998).

2.4.1.1 Haemostasis and Inflammation

Bleeding usually occurs when the skin is injured and serves to flush out bacteria and/or antigens from the wound. In addition, bleeding activates haemostasis which is initiated by exudate compounds such as clotting factors. Fibrinogen in the exudates elicits the clotting mechanism resulting in coagulation of the exudates (blood without cells and platelets) and, together with the formation of fibrin network, produces a clot in the wound causing bleeding to stop. The clot dries to form a scab and provides strength and support to the injured tissue. Haemostasis therefore, plays a protective role as well as contributing to successful wound healing.

The inflammatory phase occurs almost simultaneously with haemostasis, sometimes from within a few minutes of injury to 24 h and lasts for about 3 days. It involves both cellular and vascular responses. The release of protein-rich exudates into the wound causes vasodilation through release of histamine and serotonin, allows phagocytes to enter the wound and engulf dead cells (necrotic tissue). Necrotic tissue which is hard is liquefied by enzymatic action to produce a yellowish coloured mass described as sloughy. Platelets liberated from damaged blood vessels become activated as they come into contact with mature collagen and form aggregates as part of the clotting mechamism.

2.4.1.2 Migration

The migration phase involves the movement of epithelial cells and fibroblasts to the injured area to replace damaged and lost tissue, These cells regenerate from the margins, rapidly growing over the wound under the dried scab (clot) accompanied by epithelial thickening.

2.4.1.3 Proliferation

The proliferative phase occurs almost simultaneously or just after the migration phase (Day 3 onwards) and basal cell proliferation, which lasts for between 2 and 3 days. Granulation tissue is formed by the in-growth of capillaries and lymphatic vessels into the wound and collagen is synthesized by fibroblasts giving the skin strength and form. By the fifth day, maximum formation of blood vessels and granulation tissue has occurred. Further epithelial thickening takes place until collagen bridges the wound. The fibroblast proliferation and

collagen synthesis continues for up to 2 weeks by which time blood vessels decrease and oedema recedes.

2.4.1.4 Maturation

This phase (also called the "remodeling phase") involves the formation of cellular connective tissue and strengthening of the new epithelium which determines the nature of the final scar. Cellular granular tissue is changed to an acellular mass from several months up to about 2 years.

2.4.2 Choosing a Wound Dressing (Cuzzell, 1997)

In choosing appropriate wound dressings for elderly patients, the geriatric nurse must consider such factors as the type of wound, method of healing, and condition of the skin.

In the geriatric population, age-related skin changes and alterations in the healing response may limit dressing selection. For example, thinning of the dermis with flattening of the epidermodermal junction alters skin integrity. As a result, overly adhesive dressings can predispose elderly patients to skin tears, especially if dressings require frequent changing. Wrinkling and Sagging of the skin requires a wound covering that is flexible enough to conform to pendulous skin folds while maintaining an adequate seal. Alterations in the barrier function of the skin with aging increase the potential for irritant reactions to certain products. Finally, altered immune response and diminished dermal vascularity increase the potential for infection, requiring a dressing that maintains an effective barrier to outside contaminants without increasing the bacterial load on the wound surface (Table 2.7) (Wysocki, 1992; Jones, 1990). In figure 2.3 shows the anatomy of normal skin.

Table 2.7 Impact of age-related skin changes on dressing selection

Impact of age-related skin changes on dressing selection	
Age-related skin change	**Result**
Dermal thinning and flattening of epidermodermal junction	Separation of dermis and epidermis when friction or shearing forces are applied (i.e., skin tears)
Skin wrinkling and sagging	Pendulous skin folds that interfere with adequate maintenance of dressing seal
Alterations in barrier function of skin	Increased irritant reactions to dressing products
Altered immune response and diminished dermal vascularity	Increased potential for wound infection especially under occlusive dressing products

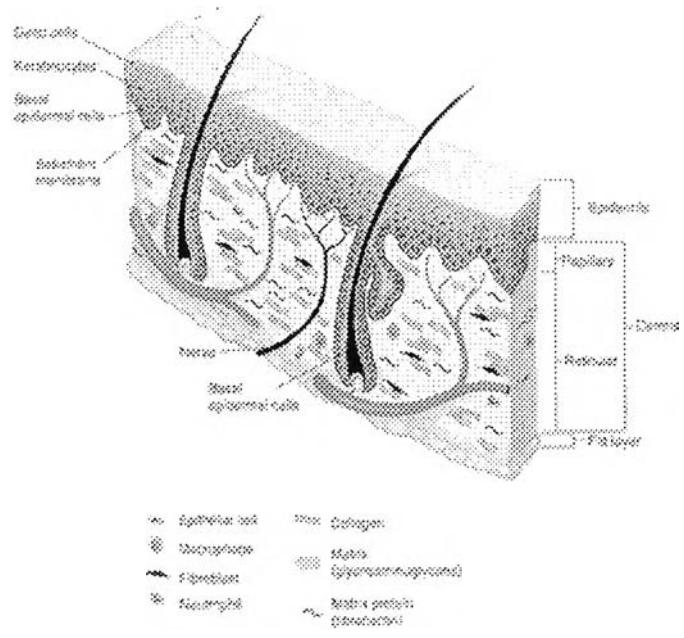


Figure 2.3 The anatomy of normal skin.

In the clinical setting, dressing selection can often be facilitated by using systematic approach to wound assessment and intervention. When customizing a

dressing protocol to a specific patient situation, begin by considering the wound type and method of healing.

2.4.2.1 Dressing Primarily Closed (Surgical) Wounds

Healing by primary intention occurs in surgical incisions and clean lacerations when tissue planes are brought together and held in approximation by sutures or staples. A dressing placed over a suture line serves to protect the wound from injury when the patient is moved or turned, provides a barrier to exogenous contamination, and helps immobilize the surgical site, minimizing pain (Cooper, 1992).

2.4.2.2 Dressing Open Wounds

Before selecting a wound dressing, correlate the properties of the dressing category with: (1) wound color, (2) wound infection, (3) amount of exudate, (4) depth, and (5) condition of the peri-wound skin (Table 2).

1. Wound Color

The color of an open wound represents the balance between necrotic tissue and new scar tissue. A wound that is satisfactorily progressing along the healing continuum appears red or pink, with minimal black eschar or yellow slough.

Black Wounds. A black eschar represents full-thickness tissue destruction, and is a common finding in patients with Stage 3 or 4 pressure ulcers In large black wounds and those associated with bacteria, surgical or sharp debridement of the eschar is warranted to control infection and promote timely healing. Moisture-retentive dressings, such as saline-moistened gauze or synthetic dressing materials, are usually contraindicated in these wounds because moisture increases bacterial proliferation. This particularly applies to patients with altered resistance to infection, such as those who are immune compromised or have poor circulation.

Red Wounds. As yellow slough is removed from the wound surface, an increasing amount of red or pink granulation tissue should become visible, converting the wound to a "red" wound. The goal of red wound management is to select a dressing procedure that maintains a clean and slightly moist wound environment and minimizes damage to healing tissue (Bolton, 1991; Field, 1994).

2. *Wound Infection*

Prevention of infection requires that a balance be maintained between local tissue resistance to bacterial invasion and the number and virulence of microorganisms. Local management of clinically infected wounds includes aggressive removal of necrotic tissue, thorough and frequent wound cleansing, draining of deep tissue abscesses, and use of systemic and topical antimicrobial preparations to control bacterial proliferation (Hutchinson, 1991; Kerstein, 1996).

3. *Wound Exudates*

In the presence of drainage, a nonabsorptive wound dressing can contribute to peri-wound maceration, promote the growth of bacteria and yeast, and impair healing. In general, expect wound drainage to increase with autolysis of necrotic tissue and gradually decrease as the wound fills with granulation tissue.

4. *Wound Depth*

While superficial wounds such as Stage 2 pressure ulcers and skin tears can be easily managed with sheet or wafer dressings, deep wounds require dressing "filler." Wound fillers maintain direct Contact with the wound surface, filling dead space (tissue pockets) where exudate can pool and contribute to the formation of soft-tissue abscesses.

5. *Condition of Peri-wound Skin*

Overly adhesive materials, such as some transparent film dressings, can easily strip or tear skin in an elderly patient. Avoid adhesive materials when possible, especially if the skin is already irritated or damaged. If adhesive dressings and taping are unavoidable, paint unbroken skin with protective skin sealants to facilitate nontraumatic removal.

2.4.3 Classification of Dressing (Ovington, 2007)

Materials used to cover wounds since Egyptian times have only slowly evolved from readily available materials in nature to materials specifically designed by man to provide particular benefits for wound healing.

2.4.3.1 Moist Wound Healing Dressings

Moist wound healing refers to the provision and maintenance of optimal hydration of the exposed tissues in the wound as opposed to allowing or encouraging those tissues to dehydrate and dry out. Since then, the use of dressings that keep wound tissues moist has been associated with increased healing rates, improved cosmesis (Nemeth, 1991), reduced pain (Nemeth, 1991), reduced infection (Hutchinson, 1993), and reduced overall health care costs (Ovington, 2001; Jones, 2006).

To achieve or maintain moist tissues does not mean that the wound should be covered in fluid. Wound tissues should be physiologically moist, not dry but not wet. In this case, the dressing must be able to manage the exudates by absorption to establish optimal tissue moisture levels. Moist wound healing dressings usually fall into 1 of these 3 performance categories with regard to their effects on tissue moisture levels—dressings that absorb excessive wound exudates, dressings that maintain existing levels of tissue moisture, and those that add moisture to the tissues (see Table 2.8).

2.4.3.2 Advanced Dressings that Absorb Exudates

In any case where the wound is generating moderate to high levels of exudate, an absorbent dressing is needed. Absorbent dressings are those types that have a high capacity for capturing and holding fluid. Foams and calcium alginate dressings are both excellent wound dressing choices when absorbency is needed.

2.4.3.3 Advanced Wound Dressings that Maintain Hydration

As wounds progress in healing and begin to granulate or fill in with new connective tissue, their exudates production lessens and dressing absorbency becomes less important. Indeed, when the exudates levels decrease, continued use of an absorbent dressing may actually dehydrate the wound tissues. At this phase of healing, what is needed is a dressing that can maintain the natural moisture level of the newly forming tissues without active absorption. Two types of dressings that can provide this function are hydrocolloid dressings and transparent film dressings.

2.4.3.4 Advanced Dressings that Donate Moisture

When wounds are already dehydrated and therefore covered by dry, dead tissues, these tissues need to be removed to allow the wound heal optimally. When the amount of dead tissue is not significant or when the patient is not a candidate for surgical debridement, the health care professional may opt for autolytic debridement. Autolytic debridement is the slow digestion of the dead cells by endogenous phagocytes and enzymes, and maintaining a moist local wound environment facilitates this process. This is a scenario in which the goal is to actively add moisture to the wound, not just prevent the loss of moisture. It may also be of interest to add moisture to wounds that are just beginning to dry out before desiccation has already set in. To effectively add moisture, a wound dressing must contain water, and the dressings that fulfill the function of hydration are the amorphous hydrogels and sheet or wafer hydrogels. These 2 types of hydrogels are similar in composition—containing significant portions of water and smaller amounts of polymers and thickening agents.

2.4.3.5 Moving Beyond Tissue Moisture Management

- Addressing the local biochemical environment

Recently, dressings have been developed that do more than simply manage moisture or exudate levels—a new function of advanced dressing is to interact with the biochemical environment of the wound. These dressings may be considered "active" in the sense that they quantitatively change something about the wound. A new approach to developing advanced dressings has therefore recently focused on managing more than just moisture levels in the wound environment, and we are beginning to see dressings enter the market that aim to address specific biochemical imbalances commonly found in chronic, nonhealing wounds.

- Addressing elevated protease levels in the local wound environment

Levels of proteolytic enzymes are found to be different in healing *vs* nonhealing wounds of many etiologies. A particular family of structurally related proteolytic enzymes called matrix metalloproteases (MMPs) has been found to be persistently elevated in chronic wounds that are not progressing toward closure (Ovington, 2002). An active dressing specifically targeted toward reducing local

levels of MMPs in nonhealing wounds is composed of a homogeneous mixture of 55% bovine collagen and 45% oxidized regenerated cellulose.

2.4.3.6 Biomaterials as Dressings

The biomaterials are typically extracellular matrix components and are designed to have an impact in the local wound environment beyond moisture management. Collagen dressings have been available on the market. The collagen in these dressings may be derived from a variety of animal sources including cowhide, cow or chicken tendon, and pig intestine. Because collagen is an evolutionarily conserved protein, the origin of the protein seems to make little difference in terms of its function. These collagen dressings are available in multiple physical formats such as gels or pastes, powders or granules, and sheets or sponges.

Advanced dressings composed of hyaluronic acid derivatives are also available. Hyaluronan is a component of the extracellular matrix and is thought to play a role in several aspects of the healing process. These hyaluronan-based dressings have shown promise in the management of chronic wounds such as venous leg ulcers (Taddeucci, 2004; Colletta, 2003).

Another recently introduced biomaterials based dressing is a viscous solution of extracellular matrix proteins (amelogenins) in propylene-glycol alginate. This dressing is thought to provide a temporary extracellular matrix for cell attachment. (http://www.dressings.org/Dressings/xelma.html.)

2.4.3.7 Antimicrobial Dressings

Recently, dressings that contain and release antimicrobial agents at the wound surface have entered the marketplace. These dressings usually provide a continuous or sustained release of the antiseptic agent at the wound surface to provide a long-lasting antimicrobial action in combination with maintenance of physiologically moist environment for healing.

- Iodine has been complexed with a polymeric cadexomer starch vehicle to form a topical gel or paste. The cadexomer moiety provides exudate absorption from the wound, which results in a concomitant slow release of low concentrations of free iodine from the vehicle. The wound healing effects of this particular iodophor has been studied in 9 randomized controlled trials.

- Silver has also been recently incorporated into a wide variety of semiocclusive dressing formats such as foams, hydrocolloids, alginates, and hydrofibers. All of these products release silver cations into the wound as they absorb or come in contact with wound exudate. The silver may be incorporated into the dressing by multiple methods such as a coating of metallic silver or the inclusion of a specific silver salt. Regardless of the method of incorporation, the active antimicrobial moiety being released in and from these dressing is the positively charged silver cation.
- Other antimicrobial agents that have been incorporated into wound dressings include polyhexamethyl biguanide and chlorhexidine gluconate. Table 2.9 lists examples of various antimicrobial dressings.

2.4.4 Electrospun Fibers Used in Wound Dressing

Wound dressing with electrospun nanofibrous membrane can meet the requirements such as higher gas permeation and protection of wound from infection and dehydration. The goal of wound dressing is the production of an ideal structure that gives higher porosity and a good barrier. To reach this goal, wound-dressing materials must be selected carefully, and the structure must be controlled to confirm that it had good barrier properties and oxygen permeability.

A collagen nanofibrous matrix produced by electrospinning process was introduced for application of wound dressing by Rho *et at.* (2006). The collagen nanofibrous matrix was chemically cross-linked by glutaraldehyde vapor with a saturated aqueous solution and then treated with aqueous 0.1 M glycine to block unreacted aldehyde groups. Effects on cytocompatibility, cell behavior, cell and collagen nanofiber interactions, and open wound healing in rats were examined. Relatively low cell adhesion was observed on uncoated collagen nanofibers, whereas collagen nanofibrous matrices treated with type I collagen or laminin were functionally active in responses in normal human keratinocytes. Collagen nanofibrous matrices were very effective as wound-healing accelerators in early-stage wound healing. The cross-linked collagen nanofibers coated with ECM protein,

particularly type I collagen, may be a good candidate for biomedical application such as wound dressing and scaffolds for tissue engineering.

Due to silver (Ag) ion and Ag conpounds have been widely used in various biomedical applications, such as wound dressing materials, body wall repairs, tissue scaffolds, antimicrobial filters, and so on. Thus, Hong *et al.* (2006) have prepared a novel wound dressing materials by electrospinning poly(vinyl alcohol) (PVA)/$AgNO_3$ aqueous solution into nonwoven mats and then treating the mats by heat or UV radiation. It was found that heat treatment as well as UV radiation reduces the Ag^+ ions in the electrospun PVA/$AgNO_3$ fiber mats into the Ag nanoparticles. Also the heat treatment improved the crystallinity of the electrospun PVA fiber mats and so it made the mats unsolved in moisture environment. Therefore, it was concluded that the only treated electrospun PVA/$AgNO_3$ fiber mat was a good material as wound dressing because it had structural stability in moisture environment as well as excellent antimicrobial ability and quick and continuous release of the effectiveness.

In 2007, Han *et al.* have investigated a biological wound dressing that improves early-stage wound healing and a technique that reduces the time between preparation and patient use. To achieve efficient wound dressing that contain proliferative cells, we cocultured dermal sheath (DS) and epithelial outer root sheath (ORS) cells on poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)-based nanofiber matrices of varying hydrophilicities. They found that cocultured hydrophobic PHBV had the most positive effect on wound closure and re-epithelization. In contranst, hydrophilic PHBV/collagen sets regenerated little of the epidermal layer, although they found faster cell attachment and better extracellular matrix (ECM) production.

The best biomaterials for wound dressing should be biocompatible and promote the growth of dermis and epidermis layers. Chen *et al.* (2008) have successfully produced a composite nanofibrous membrane composed of collagen and chitosan, which are known for their beneficial effects on wound healing. The membrane was found to promote wound healing and induce cell migration and proliferation. From animal studies, the nanofibrous membrane was better than gauze and commercial collagen sponge in wound healing.

Table 2.8 Examples of moist wound healing dressings

Dressing function	Dressing type	Dressing name	Manufacturer
Absorb exudate	Alginate	Sorbsan	Bertek Pharmaceuticals, Research Triangle Park, NC
	Alginate	Curasorb Calcium Alginate Dressing	Tyco Healthcare/Kendall, Mansfield, MA, USA
	Hydrofiber	Aquacel Hydrofiber Wound Dressing	ConvaTec, Skillman, NJ, USA
	Foam	Optifoam Nonadhesive Dressing	Medline Industries, Inc, Mundelein, IL, USA
	Foam	Tielle Hydropolymer Adhesive Dressing	Johnson & Johnson Wound Management, Somerville, NJ, USA
Maintains Moisture	Hydrocolloid	Duoderm CGF Sterile Dressing	Convatec, Skillman, NJ, USA
	Hydrocolloid	Replicare Thin Hydrocolloid Dressing	Smith & Nephew, Inc, Largo, FL, USA
	Transparent film	Tegaderm Transparent Dressing	3M Health Care, St. Paul, MN, USA
	Transparent film	Transeal	DeRoyal, Powell, TN, USA
Donate Moisture	Hydrogel—amorphous	Purilon Gel	Coloplast Corp, Marietta, GA, USA
	Hydrogel—amorphous	Skintegrity Hydrogel	Medline Industries, Inc, Mundelein, IL, USA
	Hydrogel—wafer	Nu Gel Wound Dressing	Johnson & Johnson Wound Management, Somerville, NJ, USA
	Hydrogel—wafer	FlexiGel Hydrogel Sheet Dressing	Smith & Nephew, Inc, Largo, FL, USA

Table 2.9 Examples of antimicrobial dressings

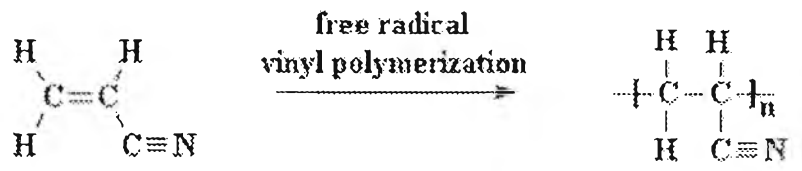
Dressing Name	Antimicrobial ingredient	Dressing Format	Manufacturer
Acticoat absorben	Ionic silver	Calcium alginate	Smith & Nephew, Inc, Largo, FL, USA
Actisorb Silver 220	Ionic silver and activated charcoal	Silver impregnatedactivated charcoal cloth	Johnson and Johnson Wound Management, Somerville, NJ, USA
Arglaes	Ionic silver	Transparent film or powder	Medline Industries, Inc, Mundelein, IL, USA
Aquacel AG	Ionic silver	Hydrofiber	Convatec, Skillman, NJ, USA
Contreet H	Ionic silver	Hydrocolloid	Convatec, Skillman, NJ, USA
Contreet F	Ionic silver	Foam	Coloplast Corp, Marietta, GA, USA
Iodosorb	Molecular iodine	Gel or paste	HealthPoint Ltd, Ft. Worth, TX, USA
Silvasorb	Ionic silver	Hydrogel sheet or amorphous gel	Medline Industries, Inc, Mundelein, IL, USA
Kerlix AMD Gauze	PHMB	Gauze	Tyco Healthcare/Kendall, Mansfield, MA, USA

PHMB indicates polyhexamethyl biguanide

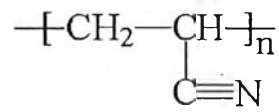
2.5 Polyacrylonitrile

2.5.1 Source

Polyacrylonitrile is a vinyl polymer, and a derivative of the acrylate family of polymers. It is made from the monomer acrylonitrile by free radical vinyl polymerization.



(a)



(b)

Figure 2.4 (a) Polymerization of Polyacrylonitrile and (b) Structural unit of Polyacrylonitrile.

2.5.2 Applications

Polyacrylonitrile is used for very few products an average consumer would be familiar with, except to make another polymer, carbon fiber. Homopolymers of polyacrylonitrile have been uses as fibers in hot gas filtration systems, outdoor awnings, sails for yachts, and even fiber reinforced concrete. But mostly copolymers containing polyacrylonitrile are used as fibers to make knitted clothing, like socks and sweaters, as well as outdoor products like tents and such. If the label of some piece of clothing says "acrylic", then it's made out of some copolymer of polyacrylonitrile. Usually they're copolymers of acrylonitrile and methyl acrylate, or acrylonitrile and methyl methacrylate.

Poly(acrylonitrile) in form of nanofibers can be fabricated by electrostatic spinning techniques, respectively (Song, 2008; Dong, 2007). It has been

used as a substrate for nanofiltration (NF) (Nam-Wun, 2001; Wang, 2006) and reverse osmosis (RO) (US Patent 4283359).

Table 2.10 Properties of poly(acrylonitrile)

Properties	
Glass transition temperature	85°C
Melting temperature	317°C
Amorphous density at 25°C	1.184 g/cm^3
Molecular weight of repeat unit	53.06 /mol

2.6 Gelatin

2.6.1 Source

Gelatin (also called gelatine) is a protein produced by partial hydrolysis of collagen extracted from the bones, connective tissues, organs, and some intestines of animals such as the domesticated cattle, and horses (Gelatin is prepared by the thermal denaturation of collagen, isolated from animal skin and bones, with very dilute acid. It can also be extracted from fish skins.). The natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Gelatin melts when heated and solidifies when cooled again. Together with water, it forms a semi-solid colloid gel. Gelatin forms a solution of high viscosity in water, which sets to a gel on cooling, and its chemical composition is, in many respects, closely similar to that of its parent collagen (Ward, 1977). Typically, gelatin can be dispersed in a relatively concentrated acid. Such dispersions are stable for 10-15 days with little or no chemical changes and are suitable for coating purposes or for extrusion into a precipitating bath. Gelatin is also soluble in most polar solvents. Gelatin gels exist over only a small temperature range, the upper limit being the melting point of the gel, which depends on gelatin grade and concentration and the lower limit, the ice point at which ice crystallizes. The mechanical properties are very sensitive to temperature variations, previous thermal history of the gel, and time. The viscosity of the gelatin/water mixture increases with concentration and when kept cool (≈40°F).

Although gelatin is 98-99% protein by dry weight, it has less nutritional value than many other protein sources. Gelatin is unusually high in the non-essential amino acids glycine and proline, (i.e., those produced by the human body), while lacking certain essential amino acids (i.e., those not produced by the human body) (Figure 2.4). It contains no tryptophan and is deficient in isoleucine, threonine, and methionine. The approximate amino acid composition of gelatin is: glycine 21%, proline 12%, hydroxyproline 12%, glutamic acid 10%, alanine 9%, arginine 8%, aspartic acid 6%, lysine 4%, serine 4%, leucine 3%, valine 2%, phenylalanine 2%, threonine 2%, isoleucine 1%,hydroxylysine 1%, methionine and histidine <1% and tyrosine <0.5%. These values vary, especially the minor constituents, depending on the source of the raw material and processing technique (Stevens, 1992).

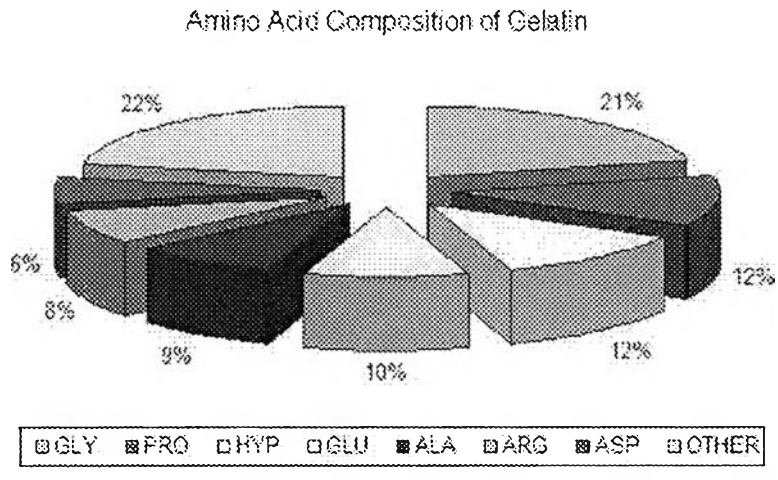


Figure 2.5 Amino acid composition of gelatin

Gelatin is commonly used for pharmaceutical and medical applications because of its biodegradability (Ikada, 1998; Kawai, 2000; Balakrishnan, 2005; Yamamoto, 2001) and biocompatibility in physiological environment (Kuijpers, 2000; Yao, 2004). These characteristics have contributed to gelatin's proven record of safety as a plasma expander, as an ingredient in drug formulations, and as a sealant for vascular prostheses (Kuijpers, 2000).

Two different types of gelatin can be produced depending on the method in which collagen is pretreated, prior to the extraction process (Ikada, 1998). The alkaline process, also known as "liming", targets the amide groups of asparagine and glutamine, and hydrolyses them into carboxyl groups, thus converting many of these residues to aspartate and glutamate. In contrast, acidic pre-treatment does little to affect the amide groups present. The result is that gelatin processed with an alkaline pre-treatment is electrically different in nature from acidic-processed gelatin. This is because the alkaline processed gelatin possesses a greater proportion of carboxyl groups, rendering it negatively charged and lowering its isoelectric point (IEP) compared to acidic-processed gelatin which possesses an IEP similar to collagen. By utilizing this technique, manufacturers now offer gelatin in a variety of IEP values (Figure 2.6).

The acidic treatment is suitable for less fully cross-linked collagens found in pig or fish skins, while the alkaline one is for the more complex collagens found in bovine hides. Gelatin obtained from acid-treated collagens is called type-A gelatin, while that obtained from alkali-treated ones is called type-B gelatin (Anonymous, 2007).

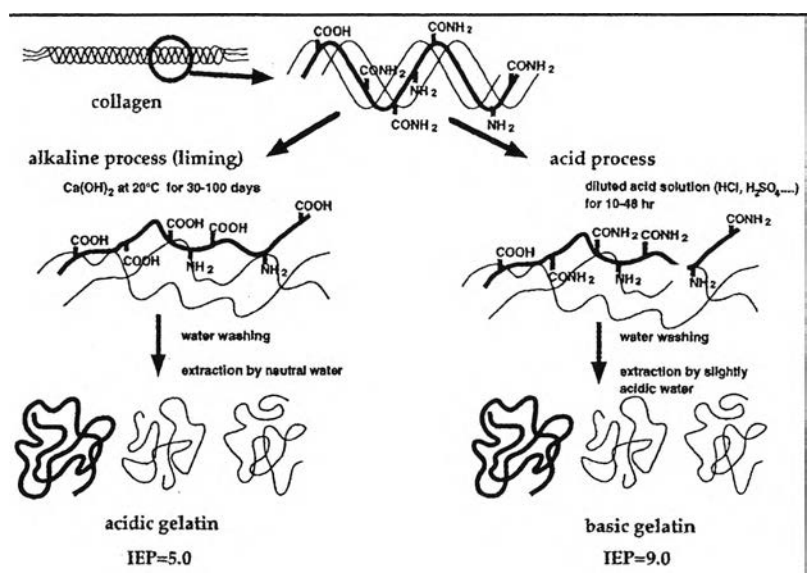


Figure 2.6 Preparative process for acidic and basic gelatins from collagen.

2.6.2 Structural Unit

Gelatin contains many glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-. (Figure 2.7)

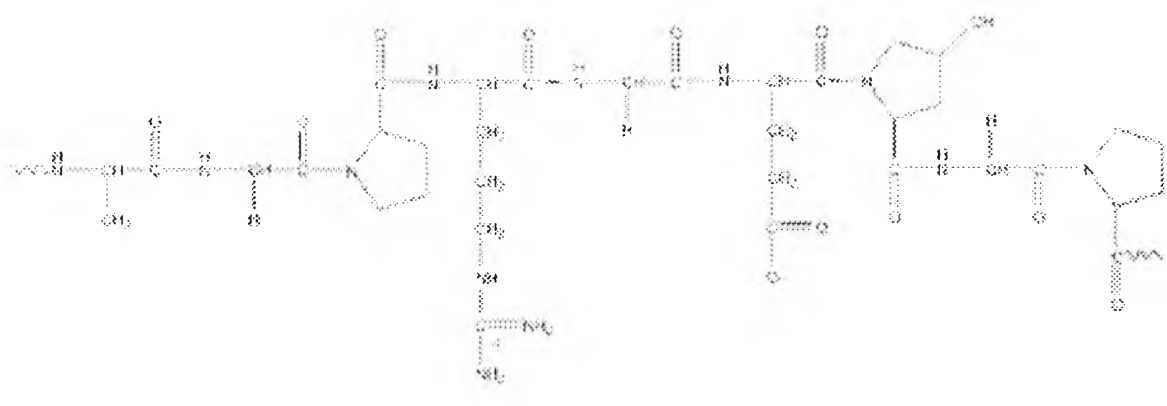


Figure 2.7 Structural unit of gelatin

2.7 The Electrospinning Process

2.7.1 Electrospinning

Electrospinning is a fiber spinning technique that produces polymer fibers of nanometer to micrometer range in diameters. In the electrospinning process, a polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field. Charge is induced on the liquid surface by an electric field. Mutual charge repulsion causes a force directly opposite to the surface tension. As the intensity of the electric field is increased, the hemispherical surface of the solution at the tip of the capillary tube elongates to form a conical shape known as the Taylor cone. When the electric field reaches a critical value at which the repulsive electric force overcomes the surface tension force, a charged jet of the solution is ejected from the tip of the Taylor cone. Since this jet is charged, its trajectory can be controlled by an electric field. As the jet travels in air, the solvent evaporates, leaving behind a charged polymer fiber which lays itself

randomly on a collecting metal screen. Thus, continuous fibers are laid to form a non-woven fabric (Doshi, 1995).

The formation of fibers from this spinning process can be divided into two parts:

2.7.1.1 The Initiation of The Jet

Before the electric field is applied to the polymer solutions, and when the capillary tube are in a vertical position and carries a drop at the tip of nozzle, the relation between the surface tension and the height of the column of liquid under equilibrium conditions is given by

$$2\gamma(1/R + 1/r) = \rho gh \qquad (1)$$

where γ is the surface tension of the liquid of density ρ, h is the height of the column of liquid above the lowest surface of the drop, R is the radius of curvature of the liquid at the upper liquid surface and r is the radius of curvature of the liquid at the lower surface of the liquid (Michelson, 1990).

Consider a droplet of polymer solutions that is applied to a high electric field. Charges that flow onto liquid surface repel each other. The repulsion forces are opposed to the forces from surface tension. The polymer droplet becomes unstable when the charge distributed on the surface overcomes the surface tension. The conditions that are necessary for a charged surface to become unstable are described by considering the equilibrium equation,

$$V_* = (4\pi r\gamma)^{1/2} \qquad (2)$$

where V_* is the critical potential, r is the droplet radius, and γ is the surface tension of the solutions (Koombhongse, 2001). For the droplets subjects to a higher potential, $V > V_*$, the droplet elongates into a cone-like shape that was described mathematically by Taylor and often referred to as a Taylor cone (Taylor, 1969).

As the potential is increased, which obtain the maximum instability of the liquid surface, a jet of liquid ejected from the tip of the cone. Taylor showed that the critical voltage V_c (expressed in kilovolts) at which the maximum instability develops is given by

$$V_c^2 = 4H^2/L^2 (\ln 2L/R - 1.5)(0.117\pi R\gamma) \quad (3)$$

where H is the distance between the electrodes, L and R are the length and radius of the capillary, respectively, and γ is the surface tension.

2.7.1.2 The Continuous Flow of The Jet

The mechanism of the appearance of a stable electrospinning jet is evidently established by the observation of the jet formation through the high speed electronic camera which recorded up to 2000 frames per second with a time resolution of approximately 0.0125 ms (Reneker, 2000).

There are two kinds of electrical forces that act on the jet: the external field that tries to pull the jet toward collector and the self-repulsion between the charges carried by adjacent segments of the jet that try to push each other apart. The self-repulsion can also cause different types instability such as bending instability and splitting instability.

In bending instability, or whipping instability, the jet rotates in a conical region, whose vertex is the end of the straight jet. The other end of the jet, which is highly stretched and reduced in diameter, is deposited on the collector as a result of the fast whipping motions (Shin, 2001).

After some time, segment of a loop suddenly developed a new bending instability, but at a smaller scale than the first. Each cycle of bending instability can be described in three steps (Reneker, 2000).

Step (1) A smooth segment that was straight or slightly curved suddenly developed an array of bends.

Step (2) The segment of the jet in each bend elongated and the array of bends became a series of spiraling loops with growing diameters.

Step (3) As the perimeter of the loops increased, the cross-sectional diameter of the jet forming the loop grew smaller; the conditions for step (1) were established on a smaller scale, and the next cycle of bending instability began.

The schematic drawing of the electrospinning process is shown in figure 2.8.

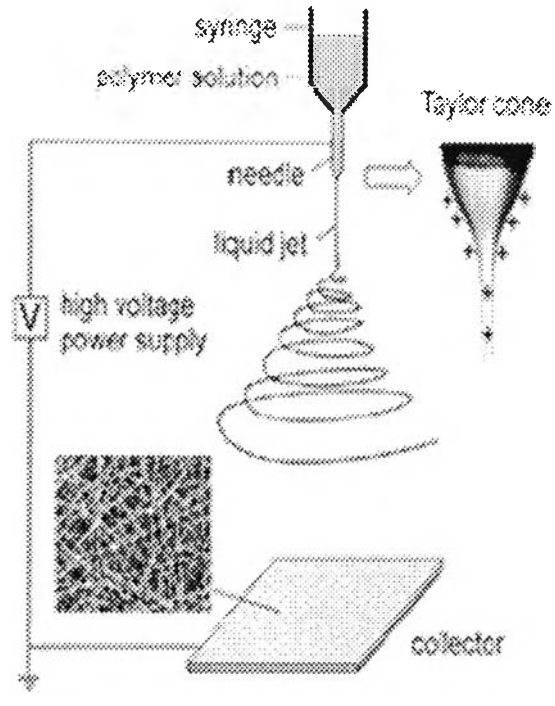


Figure 2.8 Schematic drawing of the electrospinning process (Dan, 2004).

The other instability of the charged jet is the splitting instability. It occurs when the charge density of the charged jet increases as the solvent evaporates. The charged jet can reduce its charge per unit surface area by ejecting a smaller jet from the surface of the primary jet, or by splitting apart to form two smaller jets (Koombhongse, 2001).

2.7.2 Applications of Electrospun Fibers

Due to the high surface area to volume ratio, high porosity, and light weight of the electrospun fibrous mats, a number of applications have been sought out (Jayaraman, 2004).

2.7.2.1 Filters

Filtration is a necessary process in various engineering applications. Filtration efficiency or capture efficiency of filter media has been shown to be inversely proportional to the diameters of the fibers in filters. Because of the very high surface area-to-volume ratio and the resulting high surface cohesion

of nanofibers, tiny particles on the order of less than 0.5 μm are easily trapped in the nanofiber mats.

2.7.2.2 Scaffolds for Tissue Engineering

Almost all of the human tissues and organs have fibrous network to provide mechanical integrity to them. These tissues and organs are, for examples. Bone, dentin, collagen, cartilage, and skin. Due to similarity in the structure, electrospun fibers are easily found to be prospective materials to be used as templates for tissue scaffold applications, controlled release fibers for would dressing, pharmaceutical, and cosmetic applications. For the treatment of injured or defective tissues or organs, biocompatible materials are designed and fabricated to form structure that mimic the structure and biological functions of extracellular matrix (ECM). Human cells can attach and organize well around the fivers that are smaller them. As a result, nanometer or sub- micrometer fibrous scaffolds could be suitable template for cell seeding, migrating, and proliferating. It has been reported that scaffolds having high surface area to mass ratio (ranging from 5 to 100 m^2/g) is efficient for fluid absorption and dermal delivery (Haung, 2003).

2.7.2.3 Protective Clothing

Protective clothing for military personnel is expected to help maximize the survivability, sustainability, and combat effectiveness of soldiers against extreme weather conditions, ballistics, and nuclear, biological, and chemical warfare. So a lightweight, breathable fabric, permeable to both air and water vapor, insoluble in all solvents, and highly reactive to chemical agents, is desirable. Polymer nanofibers had been developed for various protective clothing applications. It was found that compared with conventional textiles, electrospun nanofiber mats provide minimum impedance to moisture vapor diffusion and maximum efficiency in trapping aerosol particles.

2.7.2.4 Reinforcement in Composite Materials

Publications on nanofiber-reinforced composite materials are limited in the literature because of the difficulty of producing these fibers. However, their higher surface-to-volume ratio may improved the interlaminar toughness and interfacial adhesion in nanofiber-reinforced composites.

2.7.2.5 Sensors

Polymer nanofibers have been used in the development of functional sensors possessing high sensitivity due to the high surface area of nanofibers. Polymer used in this applications were poly(lactic acid-*co*-glycolic acid) and poly(acrylic acid)-poly(pyrene methanol).

2.7.2.6 Drug Delivery System

Controlled delivery systems are used to improve therapeutic efficiency and safety of drugs by delivering them a rate dictated by the need of the physiological environment over a period of treatment to the site of action (Kenawy, 2002). A wide variety of polymeric materials, either biodegradable or non-biodegradable buy biocompatible, can be used as delivery matrices, for example; poly(lactide-co-glycolide) (PLGA) (Kim, 2004), poly(L-lactic acid)(PLLA) fibers (Zeng, 2003), Hydroxypropyl methylcellulose (HPMC) (Verreck, 2003) and poly (ethylene-co-vinylacetate) (PEVA) (Kenawy, 2002). The advantages of the electrospun fibers over the convention cast film are the electrospun fiber has higher surface area and high porosity than film resulting in minimization of the initial burst release of drug and higher amount of drug release was obtained. Moreover, the electrospinnning process is the better alternative compare to the melt processing which is especially important for heat-sensitive drugs.

2.8 Literature surveys

2.8.1 Silver with Anti-Bacterial and Anti-Fungal

Tian *et al.* (2007) investigated the wound-healing properties of silver nanoparticles in an animal model and found that rapid healing and improved cosmetic appearance occur in a dose-dependent manner. Furthermore, through quantitative PCR (polymerase chain reaction), immunohisto-chemistry, and proteomic studies, we showed that silver nanoparticles exert positive effects through their antimicrobial properties, reduction in wound inflammation, and modulation of fibrogenic cytokines. These results have given insight into the actions of silver and have provided a novel therapeutic direction for wound treatment in clinical practice.

Feng *et al.* (2000) investigated the mechanism of inhibition of silver ions on microorganisms, two strains of bacteria, namely Gram-negative *Escherichia*

coli (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*), were treated with $AgNO_3$ and studied using combined electron microscopy and X-ray microanalysis. Similar morphological changes occurred in both *E. coli* and *S. aureus* cells after Ag^+ treatment. The cytoplasm membrane detached from the cell wall. Comparing the $AgNO_3$ treated *E. coli* and *S. aureus* with the control setting, it was found that:

- The free state of deoxyribonucleic acid (DNA) molecules changed to a condensed form in the center of the electron-light region in the cells;
- Many electron-dense granules appeared surrounding the cell wall or electron-light region;
- X-ray microanalysis demonstrated the existence of silver in electron-dense granules, cytoplasm, and DNA molecules.

The above results lead to the following suggestions about the bactericidal mechanism of silver ions against *E. coli* and *S. aureus*:

1. As a reaction against the denaturation effects of silver ions, DNA molecules become condensed and lose their replication abilities;

2. Silver ions interact with thiol groups in protein, which induce the inactivation of the bacterial proteins.

The antimicrobial activity of silver nanoparticles against *E. coli* was investigated as a model for Gram-negative bacteria by Sondi *et al.* (2004). Bacteriological tests were performed in Luria–Bertani (LB) medium on solid agar plates and in liquid systems supplemented with different concentrations of nanosized silver particles. The results confirmed that the treated *E. coli* cells were damaged, showing formation of "pits" in the cell wall of the bacteria, while the silver nanoparticles were found to accumulate in the bacterial membrane. A membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell. These nontoxic nanomaterials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials.

Fungal infections of burn wounds have become an important cause of burn-associated morbidity and mortality. The nature of fungal infections dictates

aggressive treatment to minimize the morbidity associated with these infections. Persons with large total body surface area burns are particularly susceptible to fungal infections and are treated in such a manner as to minimize their risk of infection. Wright *et al.* (1999) examined the in vitro fungicidal efficacy of a variety of different topical agents. By placing fungal inocula in contact with mafenide acetate, silver nitrate, silver sulfadiazine, and a nanocrystalline silver-coated dressing, we determined the kill kinetics of these topical agents against a spectrum of common burn wound fungal pathogens. The topical antimicrobials that were tested demonstrated varying degrees of efficacy against these pathogens. In conclusion, the nanocrystalline silver-based dressing provided the fastest and broadest-spectrum fungicidal activity and may make it a good candidate for use to minimize the potential of fungal infection, thereby reducing complications that delay wound healing.

Over the past decade, a variety of advanced silver-based dressings have been developed. There are considerable variations in the structure, composition, and silver content of these new preparations. Burd *et al.* (200) examined five commercially available silver-based dressings (Acticoat™, Aquacel® Ag, Contreet® Foam, PolyMem® Silver, Urgotul®SSD). We assessed their cytotoxicity in a monolayer cell culture, a tissue explant culture model, and a mouse excisional wound model. The results showed that Acticoat™, Aquacel® Ag, and Contreet® Foam, when pretreated with specific solutes, were likely to produce the most significant cytotoxic effects on both cultured keratinocytes and fibroblasts, while PolyMem® Silver and Urgotul®SSD demonstrated the least cytotoxicity. The cytotoxicity correlated with the silver released from the dressings as measured by silver concentration in the culture medium. In the tissue explant culture model, in which the epidermal cell proliferation was evaluated, all silver dressings resulted in a significant delay of reepithelialization. In the mouse excisional wound model, Acticoat™ and Contreet® Foam indicated a strong inhibition of wound reepithelialization on the post-wounding-day. These findings may, in part, explain the clinical observations of delayed wound healing or inhibition of wound epithelialization after the use of certain topical silver dressings. Caution should be

exercised in using silver-based dressings in clean superficial wounds such as donor sites and superficial burns and also when cultured cells are being applied to wounds.

2.8.2 Electrospun Poly(acrylonitrile) Nanofibers

Li *et al.* (2007) fabricated poly(acrylonitrile) (PAN) nanofibers by electrospinning and using N,N'-dimethylformamide (DMF) as a solvent. Poly(acrylonitrile) (PAN) electrospun nanofibrous membrane (NFM) with uniform fiber diameter below 300 nm could be fabricated by ES and used for lipase immobilization after activation by amidination reaction. Enzyme molecules could be covalently bound to the nanofiber and formed aggregates on the fiber surface, which also became more hydrophilic and robust after enzyme immobilization. With the huge specific surface area provided by the nanofiber, protein loading could reach as high as 2.1% (w/w) in the NFM while the immobilized enzyme still retains high activity at 81.3%. After enzyme immobilization, the storage stability was substantially improved over that of free enzyme. This simple but effective enzyme immobilization system shows improvements in enzyme properties over previous immobilized lipase systems using the same enzyme and substrate.

Im *et al.* (2008) prepared Poly(acrylonitrile) (PAN)-based electrospun nanofiber webs containing TiO_2 for photocatalytic degradation. PAN nanofibers web containing TiO_2 catalysts were prepared by electrospinning method and using N,N'-dimethylformamide (DMF) as a solvent. The prepared fibers were nano-scale sized and contained TiO_2 catalysts on the surface of the fibers. The degradation of dye rhodamine B was more effective in case of the suspending nanofibers when comparing immersed nanofibers. The nanofibers immersed in the bottom of dye solution, suffers on hindrance and blocking of UV light penetration. The use of this kind of supported photocatalyst might improve the exposure to light and avoid the need of secondary operations like liquid–solid separation.

Oh et al. (2008) synthesized the novel manganese/PAN-based carbon nanofiber composites by stabilizing, carbonizing and activating the electrospun fiber, prepared from a composite solution containing manganese acetate and 10% PAN solution (N,N'-dimethylformamide as a solvent). The average diameter of ACNF was approximately 250 nm, ranging from 200 nm to 400 nm. The specific surface area and micropore volume of the carbon nanofibers (CNs) were 853 m^2/g, and

0.280 cm^3/g, respectively, but those of Mn2-CN were 1229 m^2/g, and 0.416 cm^3/g, respectively. The enhancement of the micropore characteristics by embedding Mn particles was attributed to the formation of pore channels by physical phenomena and the creation of pore by catalytic activation during the burn-off process. The O/C ratios of all samples were non-polar, approximately 0.1. This means that the carbon surface has an affinity to toluene adsorption because of its nonpolarity. The adsorption capacity was increased to 68 gtoluene/ 100 g-composite by loading the fibers with 0.23 wt.% Mn particles because the pore characteristics of the Mn/carbon fiber were largely enhanced by the synergic effect of the proper metal loading to the carbon fiber and electrospinning.

2.8.3 Poly(acrylonitrile) Used in Filtration Application

Wang et al. (2006) prepared nanofiltration membranes from poly(acrylonitrile). They have shown that by using the PAN/$ZnCl_2$ system one can easily prepare nanofitration (NF) membranes with highly dense pore surface functional groups. The phenomenon of pore collapse during the drying of ultrafiltration (UF) membranes could be used to prepare NF membranes when the right template was chosen. This method could be extended to other polymer systems such as polystyrene or poly (styrene-co-acrylonitrile). In these systems, UF membranes could be precipitated from non-solvents besides water by the phase separation technique. These non-solvents can cause functional groups of the polymers to migrate onto the mesomacro continuous pore surface as in the case of water and nitrile groups of PAN. Also, the large pore size in the UF membrane allows easier modification of the pore surface chemistry. With the help of templates that interact with the functional groups on the meso-macropore surface, as in the case of $ZnCl_2$ with nitrile groups, continuous micropores with highly dense pore surface functional groups could be formed during the heating and evaporation of the solvent of the template, e.g. water of $ZnCl_2$, once the surface tension force exceeds the modulus of the polymers.

2.8.4 Eletcropsun Gelatin Nanofibers

Gelatin solutions in 2,2,2-trifluoroethanol (TFE) with concentration ranging from 5 to 12.5% w/v were electrospun into ultrafine fibers with an average diameter ranging between 100 and 340 nm by Huang *et al.* (2004)

Gelatin solutions in 98% formic acid with concentration ranging from 7 to 12 wt.% were electrospun into ultrafine fibers with an average diameter ranging between 70 and 170 nm by Ki *et al.* (2005)

Choktaweesap *et al.* investigated the effect of solvent systems to prepare electrospun gelatin fibers. Gelatin solutions were prepared in either single solvent system [i.e., glacial acetic acid (AA)] or mixed solvent systems [i.e., AA/2,2,2-trifluoroethanol (TFE), AA/dimethyl sulfoxide (DMSO), AA/ethylene glycol (EG), and AA/formamide (F)]. The electrospinning was carried out under fixed electrostatic field strength of 7.5 kV/7.5 cm and the polarity of the emitting electrode was positive. Electrospinning of 15–29% w/v gelatin solutions in AA produced beads, beaded fibers, and smooth fibers, depending on the concentration range. Only smooth fibers were observed at the concentration range of 21–29% w/v, with their average diameter ranging between 214 and 839 nm. The addition of TFE as a co-solvent or another modifying liquid of DMSO, EG, or F helped improve the electrospinnability of the resulting gelatin solution. Among the three modifying liquids, DMSO and EG contributed to the formation of smooth gelatin fibers with reduced diameters when compared with those obtained from the solution in pure AA.

2.8.5 Crosslinking Electrospun Gelatin Nanofibers

Physical methods include dehydrothermal treatment and UV-irradiation by Bottoms *et al.* (1966) and Fujimori *et al.* (1965), however, they are generally less efficient.

Many chemicals such as formaldehyde, glutaraldehyde, carbodiimide and dextran dialdehyde, have been used to chemically modify gelatin for biomedical applications. Amongst, glutaraldehyde (GTA) is by far the most widely used chemical, due to its high efficiency in stabilizing collagenous materials by Khor *et al.* (1997).

Zhang *et al.* (2006) prepared the as-electrospun gelatin nanofibers water insoluble through a GTA crosslinking treatment so as to preserve their fibrous morphology and enhance their thermal and mechanical performance. The electrospun gelatin nanofibers were crosslinked with saturated glutaraldehyde (GTA) vapor at room temperature. An exposure of this nanofibrous material in the GTA vapor for 3 days generated a crosslinking extent sufficient to preserve the fibrous

morphology tested by soaking in 37 8C warm water. The crosslinking also led to improved thermostability and substantial enhancement in mechanical properties. Cytotoxicity was evaluated based on a cell proliferation study by culturing human dermal fibroblasts (HDFs) on the crosslinked gelatin fibrous scaffolds for 1, 3, 5 and 7 days. It was found cell expansion took place and almost linearly increased during the course of whole period of the cell culture. The initial inhibition of cell expansion on the crosslinked gelatin fibrous substrate suggested some cytotoxic effect of the residual GTA on the cells.

2.8.6 Gelatin Used in Wound Dressing Material

Tucci *et al.* (2001) investigated chitosan and gelatin as engineered dressing for wound repair. A therapeutic support for the less effective steps in skin's recovery should improve reparative capacity. A morphological and immuno-histochemical evaluation of tissue repair in rats in the presence of chitosan glycolate gel and cross-linked gelatin wound dressings was conducted and compared. In chitosan-treated animals, the repaired dermis preserved high cellularity with mesenchymal features associated with a rich vascular network. These aspects were less marked in the presence of Phytostimuline gauze or gelatin. The hydrophilic properties of chitosan are clearly demonstrated by the high level of hydration observed in the neoformed tissue. The chitosan gel was able to modulate the early steps of wound healing. Cross-linked gelatin represents an active dressing for the more dystrophic wounded tissues often associated with aging and systemic disease.

Chang *et al.* (2003) studies *in vitro* and *in vivo* of the genipin-crosslinked gelatin membrane as wound-dressing material. A naturally occurring crosslinking agent (genipin) was used in this study to crosslink gelatin hydrogel to develop a wound-dressing membrane. The study was to investigate the *in vitro* characteristics of the genipin-crosslinked gelatin membrane. The glutaraldehyde-crosslinked counterpart, at a similar crosslinking degree, was used as control. Additionally, an *in vivo* experiment was undertaken to study the wound healings covered with the glutaraldehyde- and genipin-crosslinked dressings in a rat model. The *in vitro* results obtained suggested that crosslinking of gelatin membranes with glutaraldehyde or genipin may produce distinct crosslinking structures. The differences in crosslinking structure can significantly affect the mechanical property,

water-vapor-transmission rate, swelling ratio, degradation against enzyme and cellular compatibility of the crosslinked membranes. In the *in vivo* study, it was found that the degree of inflammatory reaction for the wound treated with the genipin-crosslinked dressing was significantly less severe than that covered with the glutaraldehyde-crosslinked dressing throughout the entire course of the study. Additionally, the healing rate for the wound treated with the genipin-crosslinked dressing was notably faster than its glutaraldehyde-crosslinked counterpart.

US Patent 4767619-Burn wound dressing material. A burn wound-adherent dressing material in the form of a continuous, wound-adherent, preformed film comprising a complex of gelatin of a Bloom # of 80 to 350 and a water-soluble polyethylenimine of molecular weight of 10,000 to 100,000, in a weight ratio of gelatin to polyethylenimine of 9:1 to 3:7.

2.8.7 Electrospun Fibers Containing Silver Nanoparticles with Antibacterial Activity

Son *et al.* (2004) prepared the antimicrobial ultrafine cellulose acetate (CA) fibers with silver nanoparticles by electrospinning process. The average diameters of ultrafine CA fibers electrospun from a 10 wt.-% CA solution in acetone/water (80:20, w/w) with 0, 0.05, 0.3, and 0.5 wt.-% $AgNO_3$ were 1910, 680, 640, and 610 nm, respectively. Silver ions in ultrafine CA fibers were photoreduced into silver nanoparticles even in a general laboratory environment, which were stabilized by interactions with carbonyl oxygen atoms in the CA. The average diameters of the silver nanoparticles were in the range of 3–16 nm. The ultrafine CA fibers showed very strong antimicrobial activity attributable to the silver nanoparticles and nonreduced silver ions.

Lee *et al.* (2005) prepared the ultrafine poly(acrylonitrile) (PAN) fibers containing Ag nanoparticles. Ag+ ions in a PAN solution were directly reduced to produce Ag nanoparticles and the resulting solution was electrospun into ultrafine PAN fibers. *N,N*-dimethylformamide (DMF) was used as a solvent for PAN as well as a reducing agent for the Ag^+ ions. The numbers of Ag nanoparticles in the ultrafine PAN fibers were increased as the amount of $AgNO_3$ was increased from 0.05 to 0.5 wt.%. The Ag nanoparticles were all sphere shaped with an average diameter of less than 5.8 nm.

Wang *et al.* (2005) prepared poly(acrylonitrile) (PAN) nanofibrous film containing silver ions by electrospinning. Ag nanoparticles with average diameter of 10 nm were dispersed homogeneously in PAN nanofibrous film. The structure of PAN has been changed after Ag nanoparticles are dispersed in PAN. D and G peak observed in SERS spectrum revealed that PAN embedded Ag nanoparticles had been partly converted to graphite structure at room temperature. The mechanism of structural changes of PAN embedded Ag nanoparticles is under investigation. SERS spectrum indicates that

Jin *et al.* (2005) prepared the electrospun PVP nanofibers containing Ag nanoparticles directly from 47 wt.-% PVP solutions containing 0.5 wt.-% of $AgNO_3$ 1 h and 7 d after preparation. The average size of the Ag nanoparticles was 3.4 and 4.5 nm, respectively. The PVP nanofibers containing Ag nanoparticles could be prepared via a simple one-step method using DMF. PVA nanofibers were electrospun with a 5 wt.-% solution of PVP containing 15 wt.-% Ag nanoparticles. The Ag nanoparticles were evenly distributed in the PVA nanofibers and their average size was 6.0 nm. The PVAnanofiber mats were strong enough for antimicrobial separation filters. The PVP containing Ag nanoparticles can be used to introduce Ag nanoparticles to other polymer nanofibers that are miscible with PVP.

Xu *et al.* (2006) prepared Biodegradable PLA ultrafine fibers containing silver nanoparticles via electrospinning and are characterized by SEM, TEM and XRD. The fiber diameter increases with increasing amount of AgNO3 added. After hydrogenation reduction of the AgNO3 into Ag nanoparticles, the average diameter of the Ag particles is about 30 nm and does not depend on the AgNO3 content in the fibers. These fibers show strong antimicrobial activities against *S. aureus* and *E. coli*. Their antibacterial efficacy in 12 h is as high as 94–98% and the duration is longer than 20 days. Therefore, the Ag/PLA composite fibers may find practical clinic applications such as wound dressings or anti-adhesion membranes.

Son *et al.* (2006) found for the first time that polymer nanofibers containing Ag nanoparticles on their surface could be produced by UV-irradiation of polymer nanofibers electrospun with small amounts of silver nitrate ($AgNO_3$). When the CA nanofibers electrospun from CA solutions with 0.5 wt% of $AgNO_3$ were

irradiated with UV light at 245 nm, the number and size of the Ag nanoparticles generated increased continuously up to 240 min, but their size distribution did not broaden. The Ag nanoparticles were generated only on the surface of the CA nanofibers and their average size was 21 nm after UV-irradiation for 240 min. It is considered that Ag^+ ions and Ag clusters diffused and aggregated on the surface of the CA nanofibers during the UV-irradiation. When the CA nanofibers were irradiated with UV light at 365 nm, the photoreduction of the Ag^+ ions was delayed and the average size of the Ag nanoparticles was 12 nm. The CA nanofibers incorporating Ag nanoparticles with an average size of 21 nm exhibited strong antimicrobial activity.

Hong *et al.* (2006) prepared polyvinyl alcohol (PVA) nanofibers containing Ag nanoparticles by electrospinning PVA/silver nitrate ($AgNO_3$) aqueous solutions, followed by short heat treatment, and their antimicrobial activity was investigated for wound dressing applications. Since PVA is a water soluble and biocompatible polymer, it is one of the best materials for the preparation of wound dressing nanofibers. After heat treatment at 155 °C for 3 min, the PVA/$AgNO_3$ nanofibers became insoluble, while the Ag^+ ions therein were reduced so as to produce a large number of Ag nanoparticles situated preferentially on their surface. The residual Ag^+ ions were reduced by subsequent UV irradiation for 3 h. The average diameter of the Ag nanoparticles after the heat treatment was 5.9 nm and this value increased slightly to 6.3 nm after UV irradiation. It was found that most of the Ag^+ ions were reduced by the simple heat treatment. The PVA nanofibers containing Ag nanoparticles showed very strong antimicrobial activity.

Hong *et al.* (2007) prepared a novel wound dressing material by electrospinning poly(vinyl alcohol) (PVA)/AgNO3 aqueous solution into nonwoven webs and then treating the webs by heat or UV radiation. It was observed that the silver (Ag) nanoparticles were generated and existed in the near surface of the electrospun nanofibers. It was found that heat treatment as well as UV radiation reduced the Ag^+ ions in the electrospun PVA/$AgNO_3$ fiber web into the Ag nanoparticles. Also the heat treatment improved the crystallinity of the electrospun PVA fiber web and so it made the web unsolved in moisture environment. Therefore, it was concluded that the only heat treated electrospun PVA/$AgNO_3$ fiber web was a

good material as wound dressings because it had structural stability in moisture environment as well as excellent antimicrobial ability and, quick and continuous release of the effectiveness.