CHAPTER II
THEORITICAL BACKGROUND AND LITERATURE SURVEYS

2.1 Wound Dressing

2.1.1 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).

2.1.1.1 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 exudates 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 colored 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 mechanism.

2.1.1.2 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.

2.1.1.3 Proliferation

The proliferative phase occurs almost simultaneously or just after the migration phase (Day 3 onwards) and basal cell proliferation, which lasts between 2 and 3 days. Granulation tissue 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 edema recedes.

2.1.1.4 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 a cellular mass from several months up to about 2 years.

2.1.2 Types and Properties of Wound Dressings

2.1.2.1 Ideal Wound Dressings

New occlusive dressing materials concentrate on creating the correct environment for wound healing to occur. The ideal dressing is described by Griffiths (Griffiths, *et al,* 1991) as being one that provides a moist environment; is comfortable for the patient; removes any necrotic material; promotes the production of granulation tissue; stimulates re-epithelialization; and is cost-effective. Bolton and Rijswijk (Bolton and Rijswijk, 1991) state that for optimal results the wound dressing must not only meet the clinical needs of both patient and nurse, but also the wound's physiological and biochemical needs. They believe that a dressing should fulfill the following functions: conformability, particularly with uneven body surfaces, pain control, odor control, cost effectiveness, safety, aid healing, convenience, environmental acceptability, quality of life, and restores normal daily activities. Not only these clinical requirements have to be met but the specific physiological and biochemical requirements of a wound should be addressed, such as

exudates management, debridement, microbial barrier, antimicrobial, compression and adherence (Bolton and Rijswijk, 1991).

No single dressing is suitable for all types of wounds. Often a number of different types of dressings will be used during the healing process of a single wound. Briefly, dressings should perform one or more of the functions showed in Table 2.1. Table 2.2 shows classification of wound dressings.

Table 2.1 Properties of ideal wound dressings

Properties
- Maintain a moist environment at the wound/dressing interface
- Absorb excess exudates without leakage to the surface of the dressing
- Provide thermal insulation and mechanical protection
- Provide bacterial protection
- Allow gaseous and fluid exchange
- Absorb wound odor
- Be non-adherent to the wound and easily removed without trauma
- Provide some debridement action (remove dead tissue and/or foreign particles)
- Be non-toxic, non-allergenic and non-sensitizing (to both patient and medical staff)
- Sterile

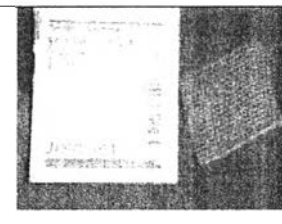
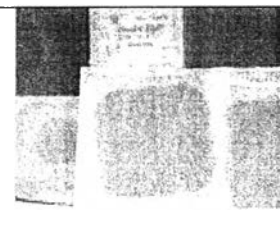
Table 2.2 Classification of wound dressings

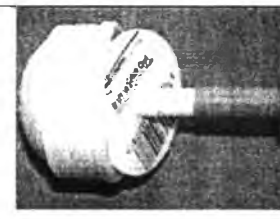
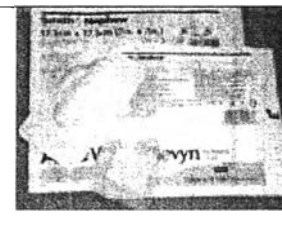
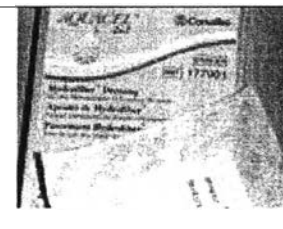
Type	Properties
Passive products	Traditional dressings that provide cover over the wound, e.g. gauze and tulle dressings
Interactive products	Polymeric films and forms which are mostly transparent, permeable to water vapor and oxygen, non-permeable to bacteria, e.g. hyaluronic acid, hydrogels, foam dressings
Bioactive products	Dressings which deliver substances active in wound healing, e.g. hydrocolloids, alginates, collagens, chitosan

2.1.2.2 Types of Wound Dressings and Their Main Properties

Table 2.3 describes different types of wound dressings and their main properties.

Table 2.3 Types of wound dressings and their main properties (Ngan, *et al,* 2007)

Dressing type	Properties	Example
Gauze	- Dressings can stick to the wound surface and disrupt the wound bed when removed - Only use on minor wounds or as secondary dressings	
Tulle	- Dressing does not stick to wound surface - Suitable for flat, shallow wound - Useful in patient with sensitive skin	 Jelonet®, Paranet®
Semipermeable film	- Sterile sheet of polyurethane coated with acrylic adhesive - Transparent allowing wound checks - Suitable for shallow wound with low exudates	 OpSite®, Tegaderm®
Hydrocolloids	- Composed of carboxymethylcellulose, gelatin, pectin, elastomers and adhesives that turn into a gel when exudate is absorbed. This creates a warm, moist environment that promotes debridement and healing - Depending on the hydrocolloid dressing chosen, it can be used in wounds with light to heavy exudate, sloughing or granulating wounds	 DuoDERM®, Tegasorb®

	- Available in many forms (adhesive or non-adhesive pad, paste, powder) but most commonly as self-adhesive pads	
Hydrogels	- Composed mainly of water in a complex network or fibers that keep the polymer gel intact. Water is released to keep the wound moist - Used for necrotic or sloughy wound beds to rehydrate and remove dead tissue. Do not use for moderate to heavily exudating wounds	 Tegagel®, Intrasite®
Alginates	- Good for exudating wounds and helps in debridement of sloughing wounds - Do not use on low exudating wounds as this will cause dryness and scabbing - Dressing should be changed daily	 Kaltostat®, Sorbsan®
Polyurethane or silicone foams	- Designed to absorb large amounts of exudates - Maintain a moist wound environment but are not as useful as alginates or hydrocolloids for debridement - Do not use on low exudating wounds as this will cause dryness and scabbing	 Allevyn®, Lyofoam®
Hydrofiber	- Soft non-woven pad or ribbon dressing made from sodium carboxymethylcellulose fibers - Interact with wound drainage to form a soft gel - Absorb exudate and provide a moist environment in a deep wound that needs packing	

2.1.3 Moist Wound Healing

Aside from the advent of sterile technique and materials promulgated by Semmelweiss and Pasteur in the 19th century, perhaps one of the most significant ideas to change the nature of wound dressing materials is the concept of moist wound healing. 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, reduced pain, reduced infection, and reduced overall healthcare costs.

2.1.4 Moist Wound Healing Dressings

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 the early, inflammatory phase of healing, wound tissues may be overly moist and not only lose fluid through evaporation, but also through exudate production. Chronic or nonhealing wounds are often stalled in the inflammatory process and therefore produce exudate for long periods. Excessive wound exudate levels must be managed to prevent maceration or 'water logging' of the tissues as well as potential soiling of patient clothes and linens. In addition, certain wound etiologies may be associated with very high exudate levels such as venous leg ulcers or wounds in a setting of lymphedema. In this case, the dressing must be able to manage the exudate by absorption to establish optimal tissue moisture levels. If the wound tissues are adequately moist with minimal exudate production, then the dressing should be capable of maintaining the tissue hydration status without too much absorption that could desiccate the wound. Alternatively, if tissue moisture levels are already depleted, the dressing must be able to restore optimal tissue hydration by donating moisture to the wound.

2.1.4.1 Hydrocolloid Dressings

Hydrocolloid dressings are among the most widely used dressings. The term 'hydrocolloid' describes the family of wound management products obtained from colloidal (gel forming agents) materials combined with other materials such as elastomers and adhesives. Typical gel forming agents include carboxymethylcellulose (CMC), gelatin and pectin. Examples of hydrocolloid

dressings include GranuflexTM and Aquacel TM (Conva Tec, Hounslow, UK), Comfeel TM (Coloplast, Peterborough, UK) and Tegasorb TM (3M Healthcare, Loughborough, UK). They occur in the form of thin films and sheets or as composite dressings in combination with other materials such as alginates. Hydrocolloid dressings are clinically useful because unlike other dressings, they adhere to both moist and dry sites.

Hydrocolloid dressings are used for light to moderately exuding wounds such as pressure sores, minor burns and traumatic injuries. As they do not cause pain on removal, they are particularly useful in pediatrics wound care for management of both acute and chronic wounds.

2.1.4.2 Alginate Dressings

Alginate dressings are produced from the calcium and sodium salts of alginic acid, a polysaccharide comprising mannuronic and guluronic acid units. Alginate dressings occur either in the form of freeze-dried porous sheets (foams) or as flexible fibers, the latter indicated for packing cavity wounds. The use of alginates as dressings stems primarily from their ability to form gels upon contact with wound exudates (high absorbency). The high absorption occurs via strong hydrophilic gel formation, which limits wound secretions and minimizes bacterial contamination. Alginates rich inmannuronate, such as SorbsanTM (Maersk, Suffolk, UK) form soft flexible gels upon hydration whereas those rich in guluronic acid, like Kaltostat TM (Conva Tec), form firmer gels upon absorbing wound exudate. Some contain calcium alginate fiber such as SorbsanTM and TegagenTM (3M Healthcare). Comfeel PlusTM is a hydrocolloid/alginate combination dressing. When applied to wounds, ions present in the alginate fiber are exchanged with those present in exudate and blood to form a protective film of gel. This helps to maintain the lesion at an optimum moisture content and healing temperature. The gelling property of the alginates is attributed to the presence of calcium ions which help to form a cross-linked polymeric gel that degrades slowly. The ability of calcium ions to form cross-links with the alginic acid polymer makes calcium alginate dressings ideal materials as scaffolds for tissue engineering. A comparative study of hydrocolloid dressings and alginates showed that alginates gels remain on the wound for a longer period than hydrocolloids. Alginate dressings, as well as forming gels, have a

pharmacological function due to the action of the calcium ions present in the dressing.

Alginate dressings are useful for all stages of wound healing described above. Calcium ions present in alginate dressings, when released into the wound, also play a physiological role aiding in the clotting mechanism (haemostat) during the first stage of wound healing. Alginate dressings are useful for moderate to heavily exuding wounds. Alginate dressings in the form of fibers when trapped in a wound are readily biodegradable and can be rinsed away with saline irrigation. Subsequent removal therefore, does not destroy granulation tissue, making dressing change virtually painless. The ease of biodegradation is exploited in making alginate sutures used in surgical wound closures. A study of different brands of alginate dressings showed significant differences in characteristics such as fluid retention, adherence and dressing residues. Since alginate dressings require moisture to function effectively, they cannot be used for dried wounds and those covered with hard necrotic tissue. Alginate dressings function by interacting with exudate to form a gel on the wound surface. In this way they produce the moist wound healing conditions.

Alginate dressings have a number of properties which appear to promote wound healing. These dressings are highly absorbent, with the dressing picking up exudate through an ion exchange reaction. The alginate dressing is an occlusive dressing, intended to cover the whole wound. In addition to absorbing fluids which seep from the wound, these dressings also create a barrier which resists bacteria and other organisms. This reduces the risk of infection at the wound site and limits complications which may emerge during the course of recovery from the wound.

2.1.4.3 Hydrogel Dressings

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (Peppas and Mikos, 1986). The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical cross-links (tie-points, junctions), or physical cross-links, such as entanglements or crystallites (Peppas and Merrill, 1976). The latter provide the network structure and physical integrity. These

hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media (Peppas and Mikos, 1986). There are numerous applications of these hydrogels, in particular in the medical and pharmaceutical sectors (Peppas, 1994). Hydrogels resemble natural living tissue more than any other class of synthetic biomaterials. This is due to their high water contents and soft consistency which is similar to natural tissue (Peppas, 1994). Furthermore, the high water content of the materials contributes to their biocompatibility. Thus, hydrogels can be used as contact lenses, membranes for biosensors, linings for artificial hearts, materials for artificial skin, and drug delivery devices (Peppas, 1997).

Some dressings such as Nu-gel TM (Johnson & Johnson, Ascot, UK) and PurilonTM (Coloplast) are hydrogel/alginate combinations. Hydrogels can be applied either as an amorphous gel or as elastic, solid sheet or film. To prepare the sheets, the polymeric components are cross-linked so that they physically entrap water. The sheets can absorb and retain significant volumes of water upon contact with suppurating wounds.

Hydrogel dressings contain significant amounts of water (70–90%) and as a result they cannot absorb much exudate, thus they are used for light to moderately exuding wounds. Hydrogels possess most of the desirable characteristics of an 'ideal dressing' They are suitable for cleansing of dry, sloughy or necrotic wounds by rehydrating dead tissues and enhancing autolytic debridement. Hydrogel dressings are nonreactive with biological tissue, permeable to metabolites and are nonirritant. Hydrogels also promote moist healing, are non-adherent and cool the surface of the wound, which may lead to a marked reduction in pain and therefore have high patient acceptability.

2.2 Biomaterial of Choice

2.2.1 Gelatin

2.2.1.1 Source

Gelatin 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.

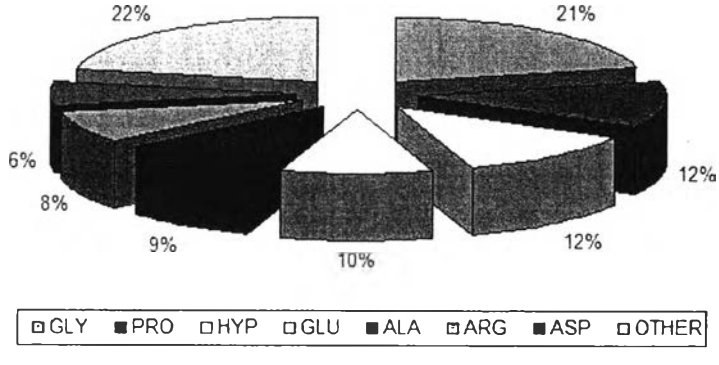


Figure 2.1 Amino acid composition of gelatin.

Two different types of gelatin can be produced depending on the method in which collagen is pretreated, prior to the extraction process (Ikada, 1998). All manufacturing operations extract and hydrolyze collagen found in fish skins, bovine bone, and porcine skin with subsequent purification, concentration, and drying operations. Various gelatin applications will require certain specific sources, or processing steps, to achieve certain functionalities or grades. Some may be based on religious preference e.g., porcine gelatin is forbidden for Halal or Kosher. Others

depend on additional processing steps that provide an appropriate type, strength, viscosity, and water-absorption capacity. Comestible grades are selected based on (neutral) flavor and texture (Choi and Regenstein, 2000).

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 2.2).

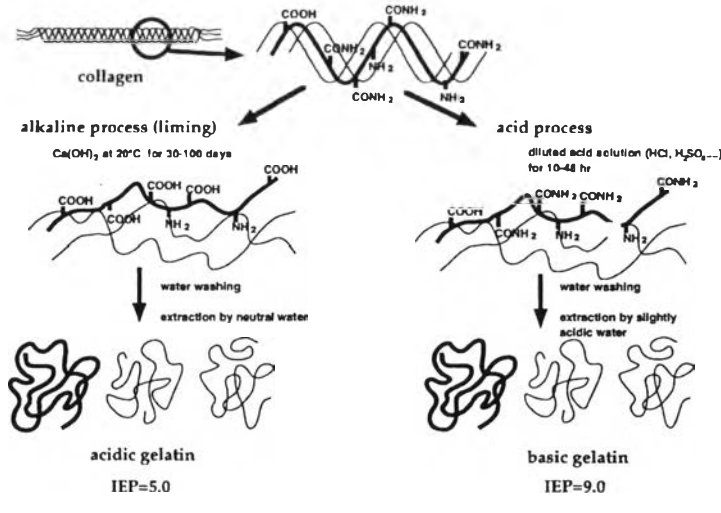


Figure 2.2 Preparative processes for acidic and basic gelatins from collagen.

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).

2.2.1.2 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 2.3)

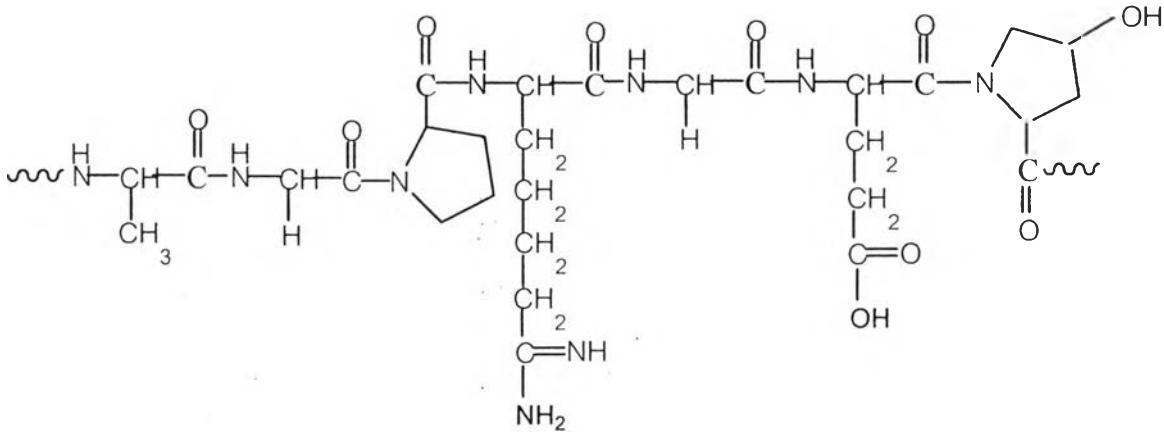


Figure 2.3 The general structure of gelatin is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly.

2.2.1.3 Biomedical Application 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). Its flexibility in processing that has allowed gelatin-based controlled-release systems to find diverse applications in fields ranging from tissue engineering, to drug delivery and gene therapy.

2.2.2 Alginate

"Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself; in some publications the term "algin" is used instead of alginate. Alginate is present in the cell walls of brown algae such as the seaweeds *Laminaria* sp. and *Ascophyllum* sp. (Clare, 1993) as the calcium, magnesium and sodium salts of alginic acid. The

calcium and magnesium salts do not dissolve in water while the sodium salt does. That is the reason why, the goal of the extraction process is to obtain dry, powdered, sodium alginate and sodium alginate is the main form of alginate in use.

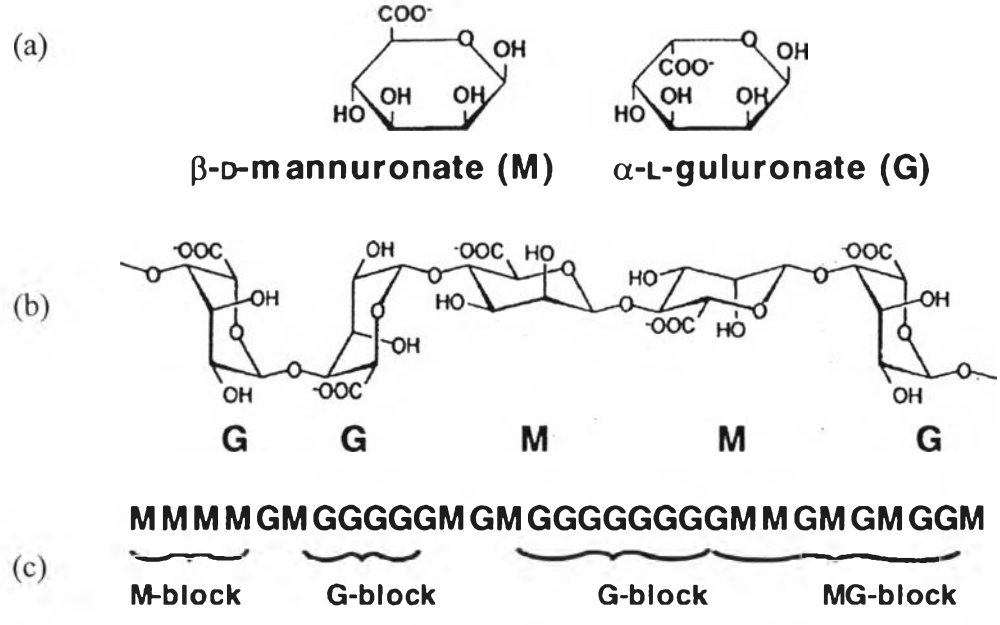


Figure 2.4 Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, (c) block distribution.

2.2.2.1 General Properties of Alginate

Alginate is a linear block copolymer consisting of uronic acid residues, namely β-D-mannuronic and α-L-guluronic acid, linked by (1→4)-linkages. For simplicity, alginate molecules are long chains that contain two different acidic components, abbreviated here to M and G. The way, in which these M and G units are arranged in the chain and the overall ratio, M/G, of the two units in a chain can vary from one species of seaweed to another. The chemical structure of alginate/alginic acid is displayed in Figure 2.4 (Collins, 1998).

2.2.2.2 Alginate Uses

The uses of alginates are based on three main properties. The first is their ability, when dissolved in water, to thicken the resulting solution (technically described as their ability to increase the viscosity of aqueous solutions).

The second is their ability to form gels; in the presence of multivalent cations such as Ca^{2+}, an aqueous solution of alginate will become a gel. Gel formation occurs due to the ionic interaction between guluronic acid residues from two or more alginate chains and cations, yielding a three-dimensional network of alginate molecules well described by the "Egg-Box Model" (see Figure 2.5) (Goth *et al,* 2004).

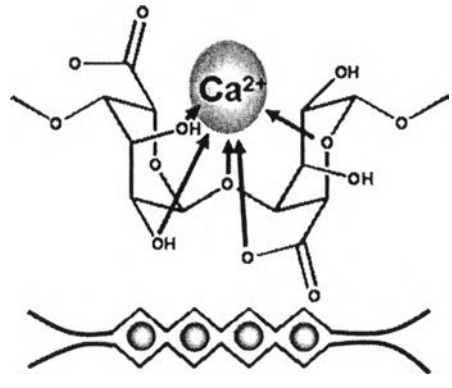


Figure 2.5 Egg-Box Model.

No heat is required and the gels do not melt when heated. This is in contrast to the agar gels where the water must be heated to about 80°C to dissolve the agar and the gel forms when cooled below about 40°C. The third is the ability to form films of sodium or calcium alginate and fibers of calcium alginates.

2.2.2.3 Alginate as Wound Dressing

Among various fibrous and hydrogel products, alginate-based products are currently the most popular ones used in wound management, since they offer many advantages over traditional cotton and viscose gauzes (Horncastle, 1995; Qin and Gilding, 1996). They are biocompatible and form a gel on absorption of wound exudate. This eliminates fiber entrapment in the wound, which is a major cause of patient trauma/discomfort during dressing removal. Such gelation prevents the wound surface from drying out, which is beneficial since a moist wound environment promotes healing and leads to a better cosmetic repair of the wound (Winter, 1962). Performance requirements for such gel led dressings (which often aim to replicate the inherent permeability/water content of natural skin) are obviously

higher than mere absorbent coverings in order for the wound to remain moist during the contact period (which could be more than several days) (Thomas, 1990). Hence, it is also reported that alginate-based dressings have haemostatic properties and can enhance the rate of healing of wounds (Attwood, 1989; Jarvis *et al,* 1987).

2.3 Bioactive Substances

2.3.1 Botanical Medicine in Wound Healing

2.3.1.1 Herbal Substance: Centella Asiatica and Asiaticoside

Centella asiatica is a tropical medicinal plant with a long history of therapeutic uses, e.g., dermal disorders, venous insufficiency and microangiopathy (Incandela, *et al,* 2001). *Centella asiatica* also known as Asiatic Pennywort or Buabok (in Thai), is a poorly water-soluble drug. Traditionally, the leaves may be squeezed or ground and the juice or powder used as an ointment. It is also used in traditional medicine for the treatment of leprosy, phlebitis and slow healing wounds (Hausen, 1993).

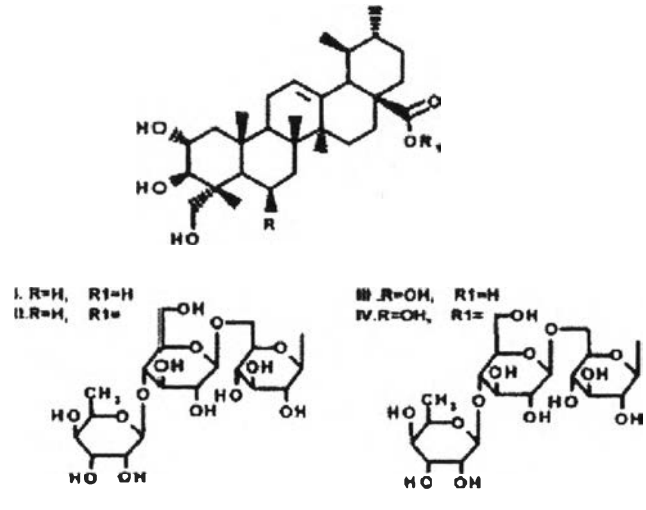


Figure 2.6 Structure of Asiatic acid (I), asiaticoside (II), madecassic acid (III) and madecassoside (IV).

The active constituents present in the Centella asiatica extract are triterpenes namely asiatic acid (I), asiaticoside (II), madecassic acid (III) and madecassoside (IV) as shown in Figure 2.6. Among the four major triterpenoid components of *Centella asiatica*, asiaticoside, a trisaccharide triterpene, is supposedly the most active compound associated with the healing of wounds in rats (Shukla, 1999), the observed increase in the proliferation and the production of type I and III pro-collagen mRNA and protein levels of human dermal fibroblast (Maquart, 1990; Shim, 1996), and the stimulation of extracellular matrix accumulation in rat experimental wound (Suguna, 1996; Maquart, 1999) in response to the presence of this substance.

Continued use of this plant as healing agents has led to scientific investigation of their efficacy as wound healing agents. *Centella asiatica* has been documented to aid wound healing in several scientific studies. *Centella asiatica* is claimed to possess anti-inflammatory (Suguna, *et al,* 1996), memory improvement (Veerendrakumar, *et al,* 2002), anticancer activity (Yoshinori, *et al,* 1982 and Babu, *et al,* 1995) and collagen synthesizing activities (Suguna, *et al,* 1996, Kumar, *et al,* 1998 and Maquart, *et al,* 1990). Studies have shown that *Centella asiatica* increased collagen synthesis *in vitro* and extracellular matrix accumulation *in vivo* (Maquart, *et al,* 1990; Maquart, *et al,* 1999). It enhanced tensile strength in wound tissue (Suguna, *et al,* 1996), and facilitated the wound healing process. The latter activity was attributed to the active ingredient, asiaticoside, presented in *Centella asiatica* (Shukla, *et al,* 1999).

A number of studies have demonstrated the effectiveness of *Centella asiatica* triterpenes, in particular the glycoside asiaticoside, in promoting wound healing (Macquart, *et al,* 1999 and Shukla, *et al,* 1999). Shukla, *et al,* showed a 0.2% asiaticoside solution applied topically twice daily for seven days to punch wounds in guinea pigs resulted in 56-percent increase in hydroxyproline, 57% increase in tensile strength, increased collagen content, and better epithelialization compared to controls. Using the same punch wound model the researchers demonstrated an oral dose of 1 mg/kg for seven days produced a 28 percent reduction in wound area and a significant increase of tensile strength and hydroxyproline content of the wound. Enhanced healing activity has been attributed

to increased collagen formation and angiogenesis. (Shukla, *et al,* 1998) *Centella asiatica* is also useful in vascular diseases such as venous hypertension and atherosclerosis (DeSanctis, *et al,* 2001; Incandela, *et al,* 2001). Based on the hypothesis of free radical mediated toxicity, *Centella asiatica* has been recently reported to have anti-lipid peroxidative (Katare, *et al,* 2001) and free radical scavenging activities (Jayashree, *et al,* 2003).

2.3.2 Metallic Silver as Antimicrobial Agent

For centuries silver has been use for the treatment of burns and chronic wounds. The antimicrobial property of silver is related to the amount of silver and the rate of silver released. Silver in its metallic state is inert but it reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is highly reactive, as it binds to tissue proteins and brings structural changes in the bacterial cell wall and nuclear membrane leading to cell distortion and death. Silver also binds to bacterial DNA and RNA by denaturing and inhibits bacterial replication (Lansdown, 2002; Castellano, *et al,* 2007)

2.3.2.1 Mechanism of Action of Silver Nanoparticles

The mechanism of action of silver is linked with its interaction with thiol group compounds found in the respiratory enzymes of bacterial cells. Silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process (Klasen, 2000). The silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Feng, *et al,* 2000; Sondi and Salopek-Sondi, 2004; Morones, *et al,* 2005; Song, *et al,* 2006).

2.3.2.2 Effect of Size and Shape on the Antimicrobial Activity of Nanoparticles

The surface plasmon resonance plays a major role in the determination of optical absorption spectra of metal nanoparticles, which shifts to a longer wavelength with increase in particle size. The size of the nanoparticle implies that it has a large surface area to come in contact with the bacterial cells and hence, it will have a higher percentage of interaction than bigger particles (Kreibig and Vollmer, 1995; Mulvaney, 1996; Morones, *et al,* 2005; Pal, *et al,* 2007). The nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which enhance the reactivity of nanoparticles. Thus, it is corroborated that the bactericidal effect of silver nanoparticles is size dependent (Raimondi, *et al,* 2005; Morones, *et al,* 2005). The antimicrobial efficacy of the nanoparticle depend on the shapes of the nanoparticles also, this can be confirmed by studying the inhibition of bacterial growth by differentially shaped nanoparticles (Morones, *et al,* 2005). According to Pal, *et al.* (Pal, *et al*, 2007) truncated triangular nanoparticles show bacterial inhibition with silver content of 1 μg. While, in case of spherical nanoparticles total silver content of 12.5 μg is needed. The rod shaped particles need a total of 50 to 100 μg of silver content. Thus, the silver nanoparticles with different shapes have different effects on bacterial cell.

2.4 Radiation Chemistry

2.4.1 Sterilization and Cross-linking

Two types of ionizing radiations are used for radiation sterilization and cross-linking: gamma rays emitted from the artificial radioactive isotopes ^{60}Co and ^{137}Cs and beams of energetic electrons from electron accelerators. The absorption of radiation energy from both types of sources occurs on a subatomic level. Electrons injected into matter from an electron accelerator enter into Coulombic interactions with atomic electrons of the medium, which results in numerous electronic excitations and ionizations of atoms along the tracks of energetic electrons. The principal mechanisms of gamma ray interactions also involve the ionization of the interacting atom and the ejection of a high-energy

electron in the first step; high-energy electrons ejected in the primary ionization continue to produce numerous electronic excitations and ionizations along their tracks quite in the same way as they would do if they were injected directly from an accelerator. The only difference is that the probability of gamma ray interactions decreases exponentially with depth, while the probability of electron interactions decreases in a much steeper fashion as function of depth. The fraction of gamma ray energy deposited in the primary ionization is negligible in comparison with the energy deposited by the subsequent generations of secondary electrons. Energy deposition mechanisms of these two types of radiation being the same, the same amount of energy absorbed by matter, irrespectively whether irradiated by gamma rays or fast electrons will produce the same kind and amount of chemical change. This is the rationale for the use of the two types of radiation sources, isotopes and accelerators on an equal footing in practice. Qualitatively different effects observed with gamma ray as compared to electron beam irradiation mainly arise because of the dose-rate effects, particularly in the presence of oxygen or other scavenger molecules. Large dose-rate irradiation of liquids producing high local concentrations of free radicals favors mutual reactions of free radicals (recombination) over their reactions with scavengers in the tracks of the respective impinging radiations. Because principal interactions involve atomic electrons, the distribution of energy deposited in individual components of irradiated matter depends on the contribution made by that component to the atomic composition. In solution the main contribution to the total mass is made by a solvent. Irradiation of aqueous solutions gives rise to oxidizing (hydroxyl radical OH) and reducing (hydrated electron e_{aq}^-) reactive species produced by the radiolysis of water, their relative amounts depending on pH and presence of solutes. These species may disappear through recombination with other reactive species of water radiolysis or they may diffuse some distance away from the site of their original formation, which increases the probability of their reaction with dissolved substances. Irradiation of solid substances in the absence of water does not, of course, lead to the formation of diffusible oxidizing and reducing aqueous radiolysis species but, due to the restricted mobility in solids, the consequences of excitation and ionization remain localized on the affected molecules or confined to the immediate vicinity of the site of primary interaction.

Intramolecular redistribution of localized charge and excitation energy may then lead to the fragmentation of affected molecules according to the interplay of electron affinities, ionization potentials and bond dissociation energies among the subunits of complex molecules.

2.4.2 Radiation Formation of Hydrogels

Use of radiation for the formation of hydrogels for biomedical purposes has some general advantages. First of all it solves the problem of sterilization of products and in a few cases allows to establish a more simple and compact technology than a "conventional" one. Secondly, it allows to fabricate a pure product non-contaminated with ballast materials or the residuals of toxic initiators. Last, but not least, the application of ionizing radiation originated from electron accelerators or gamma facilities is safe for human beings and the environment, and can lead to formation of human-friendly products.

2.4.3 Radiation Synthesis of nAg

A number of production techniques have been reported for preparation of metallic colloids using metal salts as starting materials, such as chemical, photochemical, electro-chemical, radiolytic, and sonochemical reduction. Of these techniques, the radiation-induced synthesis is one of the most promising strategies because there are some important advantages to the use of the irradiation techniques, as compared to conventional chemical and photochemical methods: (1) the process is simple and clean, (2) the γ-ray irradiation has harmless feature, (3) controlled reduction of metal ions can be carried out without using excess reducing agent or producing undesired oxidation products of the reductant, (4) the method provides metal nanoparticles in fully reduced, highly pure and highly stable state and (5) no disturbing impurities like metal oxide are introduced.

Radiolytic reduction generally involves radiolysis of aqueous solutions that provides an efficient method to reduce metal ions and form homo- and heteronuclear clusters of transition metals. In the radiolytic method, aqueous solutions are exposed to γ-rays creating solvated electrons, e_{aq}^-. These solvated electrons, in turn, reduce the metal ions and the metal atoms eventually coalesce to form aggregates as depicted by following reactions:

$$H_2O \longrightarrow OH^{\bullet}, e^{-}_{aq}, H^{\bullet}, H_2O_2, H_2, H^{+}, \ldots$$

$$Ag^{+} + e^{-}_{aq} \longrightarrow Ag^{0}$$

$$Ag^{0} + Ag^{+} \longrightarrow Ag_2^{+}$$

$$Ag_2^{+} + Ag^{+} \longrightarrow Ag_3^{+}$$

$$\dashrightarrow \dashrightarrow Ag_n \text{ (aggregation to form Ag nanoparticles)}$$

2.5 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).

Electrospinning process (Figure 2.7) has been recognized as an efficient method for the fabrication of submicron-sized fibers with a large surface area to volume ratio. It is well-accepted in the research community because of its simplicity, adaptability, and inexpensive tooling costs. Due to interesting characteristics of the electrospun (e-spun) non-woven fabric (such as high surface area to mass or volume ratio, high porosity, etc.), e-spun fiber mats are excellent candidates for various biomedical applications, such as tissue engineering (Still, *et al*, 2008), wound dressing (Noh, *et al*, 2006 and Zhou, *et al*, 2008), and carriers for delivery of drugs (Cui, *et al*, 2006; Taepaiboon, *et al*, 2007 and Sikareepaisan, *et al*, 2008).

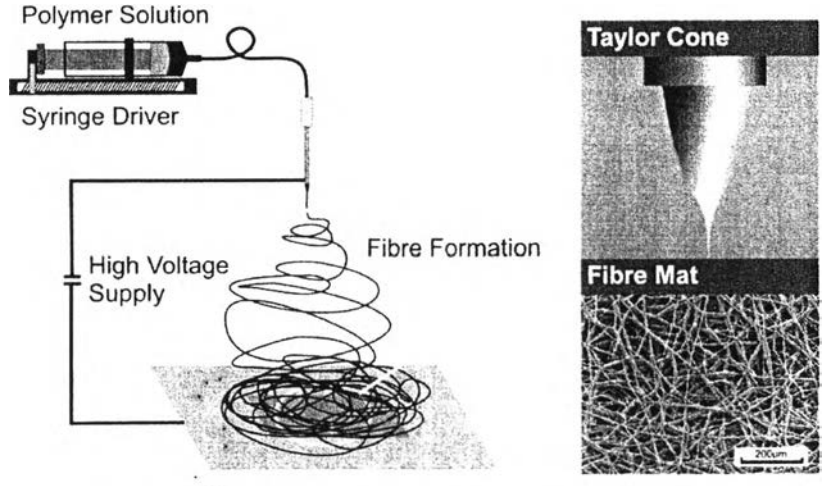


Figure 2.7 Schematic diagram of electrospinning system.