CHAPTER II
LITERATURE REVIEW

2.1 Wound Healing

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. It is a complex biological process involving hemostasis and inflammation, migration, proliferation, and maturation (Boateng *et al.*, 2008)

2.1.1 Hemostasis 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 hemostasis 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. Hemostasis therefore, plays a protective role as well as contributing to successful wound healing.

The inflammatory phase occurs almost simultaneously with hemostasis, 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.

2.1.2 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.

2.1.3 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 edema recedes.

2.1.4 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.

2.2 Wound Dressing

2.2.1 Ideal Wound Dressing

New occlusive dressing materials concentrate on creating the correct environment for wound healing to occur. The ideal dressing is described by Griffiths (Griffiths, 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 *et al.*, 1991) state that for optimal results the wound dressing must not only meet the clinical needs of both patient and nurse, but also the wounds 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 do these

clinical needs 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 *et al.*, 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 the category synthetic 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

2.2.2 Hydrogel Dressing

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (Peppas, 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 *et al.*, 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, 1986). There are numerous applications of these hydrogels, in particular in the medical and pharmaceutical sectors (Peppas *et al.*, 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 *et al.*, 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.

2.2.3 Nanofibrous Dressing

Wound dressing from electrospun nanofibrous membranes potentially offers many advantages over conventional processes. With its high surface area and microporous structure, the nanofibrous membranes could quickly start signaling pathway and attract fibroblast to the derma layer, which can excrete important extracellular matrix components such as collagen and several cytokines, to repair damaged tissue. Moreover, the nanofibrous membranes should not only serve as a substrate of tissue regeneration, but also may deliver suitable bioactive agents, including drugs (e.g. antibiotic agent), within a controlled manner during healing. In 2004, Min *et al.* have prepared silk fibroin (SF) electrospun scaffolds with fiber diameters of around 80 nm. They found that normal human keratinocytes and fibroblast seeded on SF nanofibers were able to attach and grow, indicating that SF nanofibers may be a good candidate for wound dressing. A collagen nanofibrous matrix produced by the electrospinning process was also used for the application of wound dressing (Rho *et al.*, 2006). A composite nanofibrous membrane composed of collagen and chitosan 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 (Chen *et al.*, 2008).

2.3 Radiation Chemistry

2.3.1 Sterilization and Crosslinking

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.

2.3.2 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.

2.3.3 Radiation Synthesis of Ag Nanoparticles

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:

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

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

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

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

$\dashrightarrow \dashrightarrow Ag_n$ (aggregation to form Ag nanoparticles)

2.4 Electrospinning

The electrospinning process (Figure 2.1) is an economical and simple approach which involves the application of a strong electrical potential to a polymeric liquid contained in a reservoir (e.g., syringe) attached to a metal nozzle (e.g., needle) across a finite distance between the nozzle and a grounded collector (i.e., collection distance). Upon increasing the applied electrostatic field strength (i.e., electrical potential divided by collection distance) to a critical value, a pendant droplet of the polymeric liquid at the tip of the nozzle gradually changes its shape from partially-spherical into conical. Further increase in the electrostatic field strength causes an ejection of a stream of charged liquid (i.e., charged jet) from the apex of the liquid cone. The jet accelerates towards and finally rests on the grounded collector. Owing to the high enough viscosity of the polymeric liquid, the ejected, charged jet remains stable and does not break up into spherical droplets as commonly found in the electrospraying of low molecular weight liquids. This results in the deposition of ultrafine polymeric fibers with extremely long length and high specific surface area on the collector as a non-woven mat. 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, wound dressing (Noh *et al.*, 2006, Zhou *et al.*, 2007), and carriers for delivery of drugs (Cui *et al.*, 2006, Taepaiboon *et al.*, 2006, Sikareepaisan *et al.*, 2008). As previously mentioned, a number of e-spun polymeric fiber matrices have been developed as carriers for delivery of drugs (Kenawy *et al.*, 2002, Zong *et al.*, 2002). Recently, e-spun CA fiber mats have been developed as carriers for topical/transdermal delivery of various types of drugs (Taepaiboon *et al.*, 2006, Tungprapa *et al.*, 2007, Suwantong *et al.*,

2008). Taepaiboon *et al.*(2007) prepared e-spun CA fiber mats containing all-trans retinoic acid or vitamin A acid (Retin-A) and α-tocopheral or vitamin E (Vit-E) from CA solutions in 2:1 v/v acetone/dimethylacetamide (DMAc) containing Retin-A and Vit-E in the amount of 0.5 and 5 wt.% (based on the weight of CA), respectively. Wutticharoenmongkol et al. (2007) and Chen *et al.* (2007) prepared electrospun scaffolds, which are similar to that of the natural extracellular matrix (ECM). In addition, fiber mats fabricated by electrospinning have many other advantages, including the high porosity, interconnected pores and relatively large surface areas. All of these structural characteristics promote favorable responses of seeded cells in vitro, such as enhanced cell attachment and proliferation (Yoshimoto *et al.*, 2003, Venugopal *et al.*, 2008).

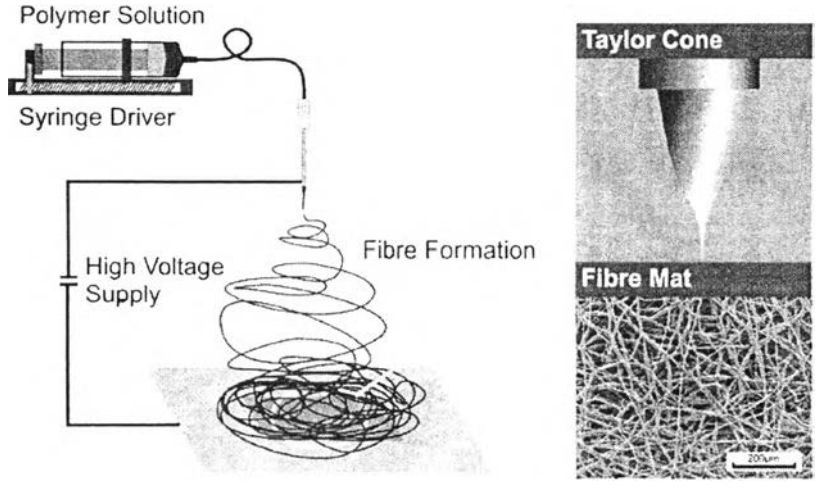

Figure 2.1 Schematic diagram of electrospinning system.

2.5 Biomaterial of Choice

2.5.1 Poly(Vinyl Pyrrolidone)

Poly(N-vinyl pyrrolidone) (PVP) is a synthetic polymer with good biocompatibility and can be used as main component of temporary skin covers and wound dressings. Under ionizing radiation, PVP undergoes crosslinking to form transparent hydrogels with good biocompatibility (Rosiak *et al.*, 1995). However, for the sake of its inferior fragile mechanical properties and low swelling capability, the

application of PVP hydrogels is limited. Blended with other polymer, such as polysaccharides (Maolin *et al.*, 2000, Risbud *et al.*, 2001, Nho *et al.*, 2002, Abad *et al.*, 2003, Zhao *et al.*, 2006). PVP hydrogels can get obviously increased properties, and play a significant role in a series of hydrogels as biomedical materials (Huglin *et al.*, 1986).

2.5.2 Poly(Acrylic Acid)

PAA is a synthetic high molecular weight polymer of acrylic acid, which behaves like an anionic polymer according to its carboxylate group (Atchison *et al.*, 2012). To fabricate PAA nanofibers, the operating parameters, solution concentration and solvent, applied voltage, distance between needle and collector, and flow rate of the polymer solution have to be optimized (Theron *et al.*, 2004, Thompson *et al.*, 2007). In order to prepare water insoluble PAA nanofibers, the electrospun PAA nanofibers are then crosslinked by thermally-induced esterification (Xiao *et al.*, 2010).

2.5.3 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 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.

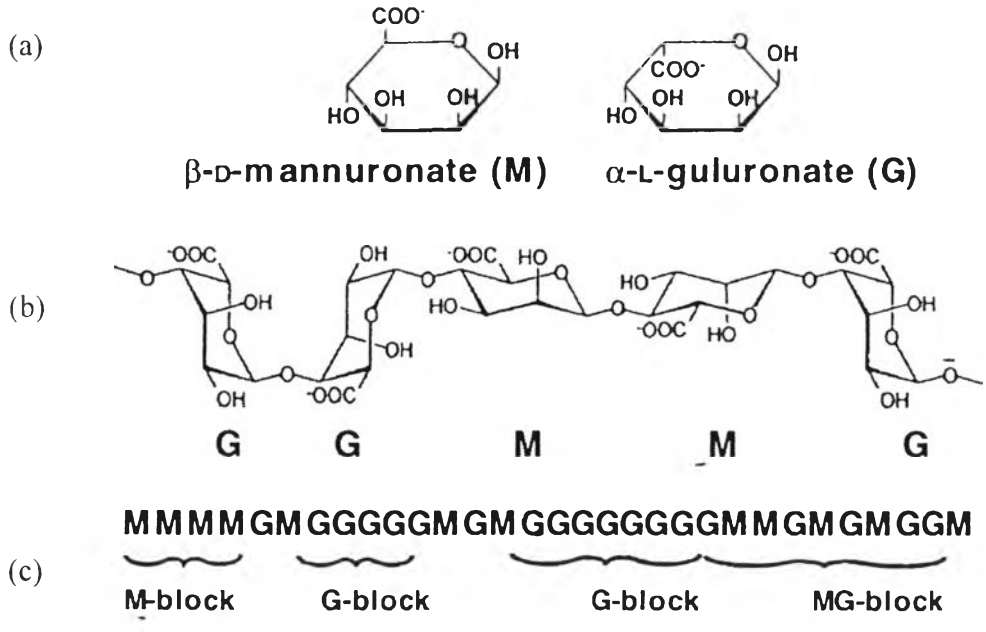

Figure 2.2 Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, (c) block distribution.

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, 1997).

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) (Grant *et al.*, 1973).

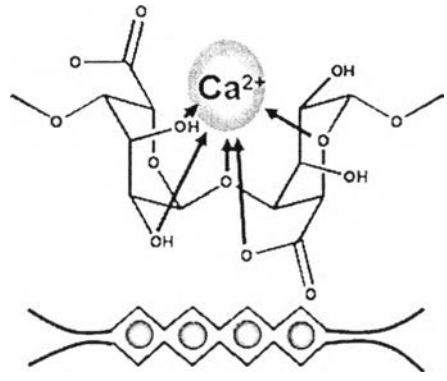

Figure 2.3 Egg-Box Model.

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.

Among the 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 *et al.*, 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) Hence, it is also reported that alginate-based dressings have haemostatic properties and can enhance the rate of healing of skin wounds (Attwood, 1989).

2.6 Bioactive Substances

2.6.1 Silver Nanoparticles

Metal nanoparticles and nanostructured materials are novel classes of materials, which have attracted great attention in catalysis (Lewis, 1993, Daniel *et al.*, 2004), optics (Hayward *et al.*, 2000, Ispasoiu *et al.*, 2000), electronics (Poizot *et*

al., 2000, Kiesow *et al.*, 2003) and biomedicine (Daniel *et al.*, 2004, Geckeler *et al.*, 2006) as well as quantum-size domain applications (Wang *et al.*, 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 *et al.*, 1993), co precipitation (Chen *et al.*, 2002), carbon nanotubes (Kim *et al.*, 2006) and polymer protection (Yanagihara *et al.*, 2001, Gao *et al.*, 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. 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.

2.6.1.1 Release and Mechanism of Silver Ions Against Bacteria

Pal (2002) showed that, in an aqueous medium containing a nucleophile (e.g., $NaBH_4$, SCH^-, and I^-), the dissolution of silver is possible due to the significant decrease in the reduction potential and the redox reaction for silver dissolution can be written as

$$4Ag + O_2 + 2H_2O \leftrightarrow 4Ag^+ + 4OH^- \quad (2.1)$$

Here, it is postulated that the as-formed elemental Ag^0 dissolved readily upon the contact with the releasing medium and both the remnant and the dissolved Ag^+ ions were released into the medium during the release studies (Figure 2.3).

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^0) appears to have no antibacterial, whereas its cation (Ag^+) is highly reactive (Lansdown, 2002, Brett, 2006), particularly at a concentration between 5 and 40 mg/l (Burrell, 2003), and its low concentration component means it retains efficacy even when dilute.

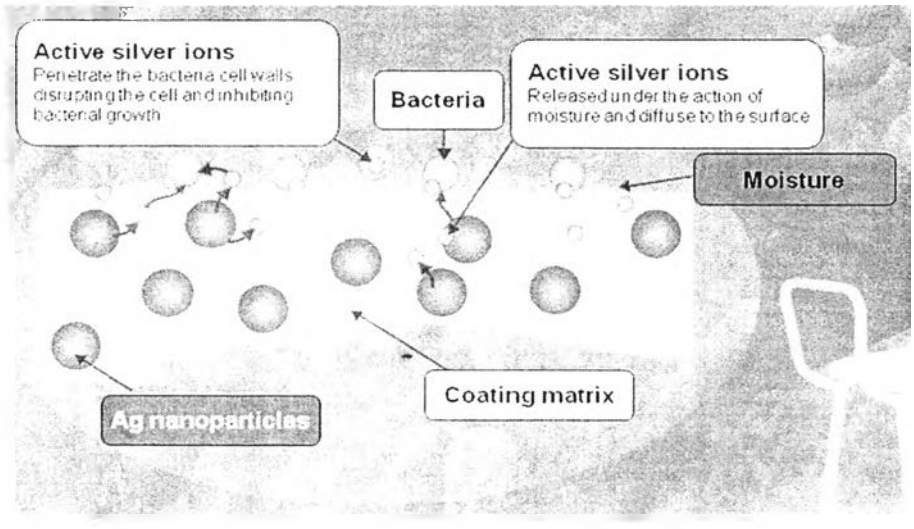

Figure 2.4 Release of active silver ions.

The antimicrobial effect of silver can be explained by various mechanisms:

(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, 2002, Lansdown, 2002). (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) Silver has also been shown to interact with structural proteins and preferentially bind with DNA nucleic acid bases to inhibit replication(Lansdown, 2002, Lansdown, 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 *et al.*, 1990).

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. 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 *et al.*, 2006). Systemic toxicity, argyria, is unlikely as absorption from dressings is so small and probably depends on wound size (Lansdown, 2002). This systemic risk is probably overstated, just as the risk of thyroid disorder is after the use of povidone–iodine in chronic wounds. 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.

2.6.2 Doxycycline Hyclate

The doxycycline hyclate is a broad spectrum antibiotic used in several countries to treat infectious diseases and as an additive in animal nutrition to facilitate growth. Doxycycline hyclate has been studied as an inhibitor of matrix metalloproteinases (intercellular substance), an action unrelated to its effects on bacterial protein synthesis (Skulason *et al.*, 2003, Brunton *et al.*, 2006). Doxycycline is more active than tetracycline against many species of bacteria including *Streptococcus pyogenes*, enterococci, anaerobic, and various *Nocardia* spp. Cross-resistance is common, although some *Staphylococcus aureus* resistant to tetracycline

respond to doxycycline. Doxycycline is also more active against protozoa, particularly *Plasmodium* spp. (Sweetman, 2011).

2.6.3 Silk Sericin

Sericin is one type of protein which is obtained from the silk cocoons. These silk cocoons contain two type of protein one is sericin and other is fibroin. Amount of sericin is 25-30%, rest is fibroin (PK *et al.*). There are 18 kinds of amino acids in sericin, among which, serine and aspartate have the highest contents. Besides, it has the other amino acids necessary to human body, it is an excellent protein. Because in sericin, about 80% amino acid has hydrophilic lateral group, about 1/3 of which is serine which water absorption is 50 times high than that of glycerin.

Sericin, as the raw material of cosmetic, has excellent moisture absorption and preservative ability. Sericin protein can form a film on the surface of skin and hair so that the water in skin can be preserved, and then the harm to skin cutin can be avoided. By applying it, the skin can be soft and smooth, and the hair can be soft and flexible, and it also benefits for the shaping of hair. Meanwhile, it will not adhere to hair.

Nowadays, there are many researches shown that SS has good wound healing properties. Its component also improves the attachment of cultured human skin fibroblast (Tsubouchi *et al.*, 2005) as well as enhanced effect in promoting corneal wound healing (Nagai *et al.*, 2009). According to SS cream, it can heal the wound by activating collagen synthesis and wound size reduction without any allergic reaction or inflammation (Aramwit *et al.*, 2007, Aramwit *et al.*, 2009). Recent studies have found unique characteristics of sericin, such as induction of accelerated proliferation of mammalian cells (Terada *et al.*, 2002) and heterogeneous nucleation of apatite (Takeuchi *et al.*, 2003). Hence it is anticipated that sericin is a promising natural resource for developing novel protein-based materials (Zhu *et al.*, 1995). Sericin could suppress lipid peroxidation, inhibit tyrosinase (polyphenol oxidase) activity in vitro and contributes its antioxidant activities to hydroxyl group chelation with trace elements such as copper and iron. The result implied that sericin is a valuable ingredient for cosmetics because it can inhibit tyrosinase activity and this enzyme is responsible for biosynthesis of skin melanin (Kato *et al.*, 1998).

In addition that sericin exerts inhibitory activity on ultraviolet radiation induced acute damage and tumor promotion by reducing oxidative stress in the skin of hairless mouse (Zhaorigetu *et al.*, 2003). Sericin possesses the biological activity of preventing cell death and promoting cellular growth after acute serum deprivation. Moreover, sericin has been shown to be useful as a degradable biomaterial, biomedical material and polymers for forming articles, functional membranes, fibers and fabrics (Takahashi *et al.*, 2003).