CHAPTER II
LITERATURE REVIEW

2.1 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:

2.1.1 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 (1969) showed that the critical voltage V_c (expressed in kilovolts) at which the maximum instability develops is given by

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

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.

2.1.2 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 2.1.

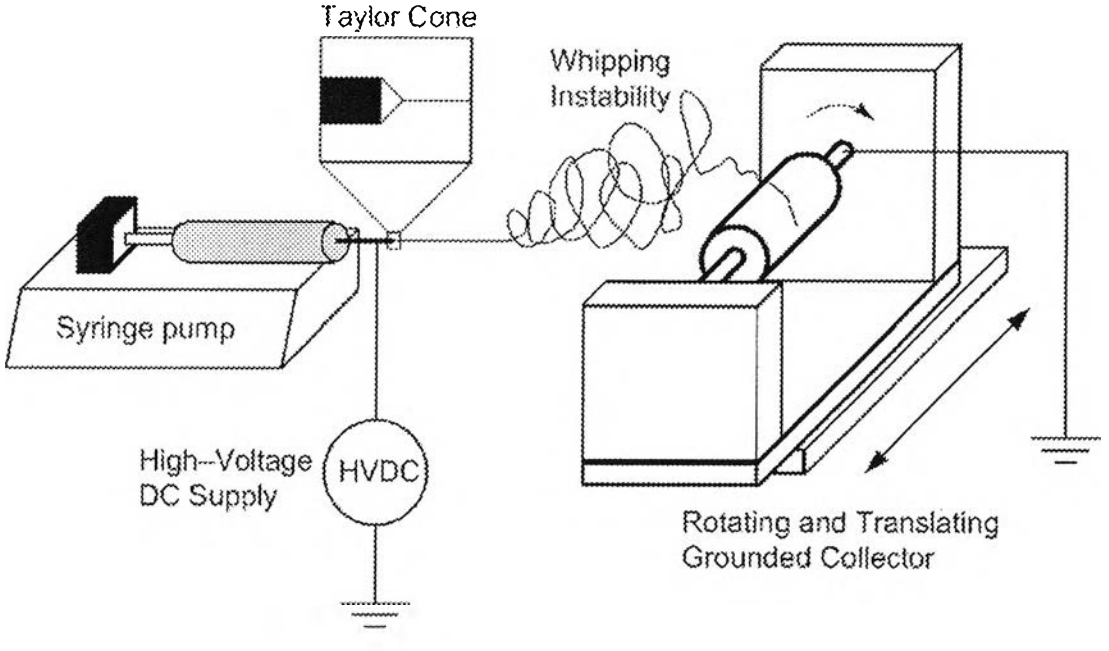


Figure 2.1 Schematic drawing of the electrospinning process.

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).

2.1.3 Biomedical Applications of Electrospun Fibers

Due to the high surface area to volume or mass ratio, high porosity, and light weight of the electrospun fibrous mats, the potential for use of these fibrous materials in biomedical applications is in areas such tissue engineering, drug delivery, and wound healing.

2.1.3.1 Scaffolds for Tissue Engineering

Use of electrospun fibers and fiber meshes in tissue engineering applications often involves several considerations, including choice of material, fiber orientation, porosity, surface modification and tissue application. Choices in materials include both natural and synthetic (biodegradable and non-biodegradable) materials, as well as hybrid blends of the two, which can provide an optimal combination of mechanical and biomimetic properties. 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. 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).

2.1.3.2 Drug Delivery System

Electrospinning affords great flexibility in selecting materials for drug delivery applications. 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 with biocompatible, can be used as delivery matrices to control whether drug release occurs via diffusion alone or diffusion and material degradation, 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). Additionally, due to the flexibility in material selection a number of drugs can be delivered including: antibiotics, anticancer drugs, proteins, and DNA. 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.

2.1.3.3 Wound 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, 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, 2008).

2.2 Drug Delivery System

2.2.1 Mechanisms of Drug Delivery System

There are three primary mechanisms by which active agents can be released from a delivery system: diffusion, degradation, and swelling followed by diffusion (Peppas, 1997). Any or all of these mechanisms may occur in a given release system. Diffusion occurs when a drug or other active agent passes through the polymer that forms the controlled-release device. The diffusion can occur on a macroscopic scale—as through pores in the polymer matrix—or on a molecular level, by passing between polymer chains. Examples of diffusion-release systems are shown in Figures 2.2 and 2.3. In Figure 2.2, a polymer and active agent have been mixed to form a homogeneous system, also referred to as a matrix system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release.

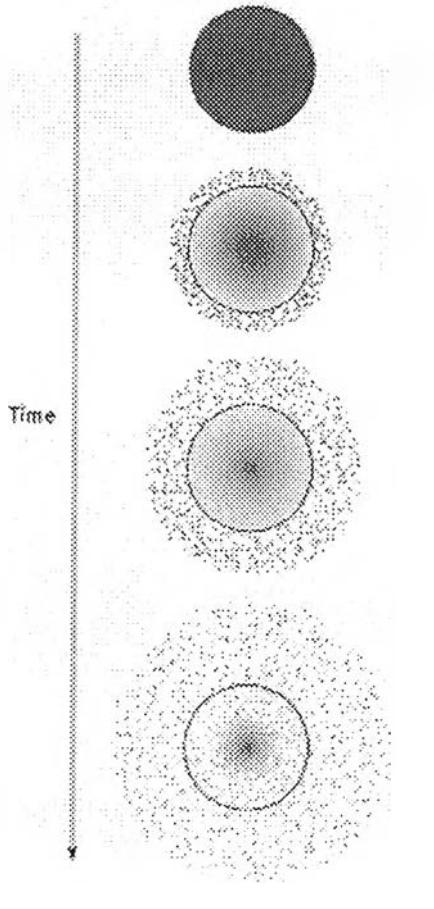


Figure 2.2 Drug delivery from a typical matrix drug delivery system.

For the reservoir systems shown in Figures 2.3a and 2.3b, the drug delivery rate can remain fairly constant. In this design, a reservoir—whether solid drug, dilute solution, or highly concentrated drug solution within a polymer matrix—is surrounded by a film or membrane of a rate-controlling material. The only structure effectively limiting the release of the drug is the polymer layer surrounding the reservoir. Since this polymer coating is essentially uniform and of a non-changing thickness, the diffusion rate of the active agent can be kept fairly stable throughout the lifetime of the delivery system. The system shown in Figure 2.3a is representative of an implantable or oral reservoir delivery system, whereas the system shown in Figure 2.3b illustrates a transdermal drug delivery system, in which only one side of the device will actually be delivering the drug.

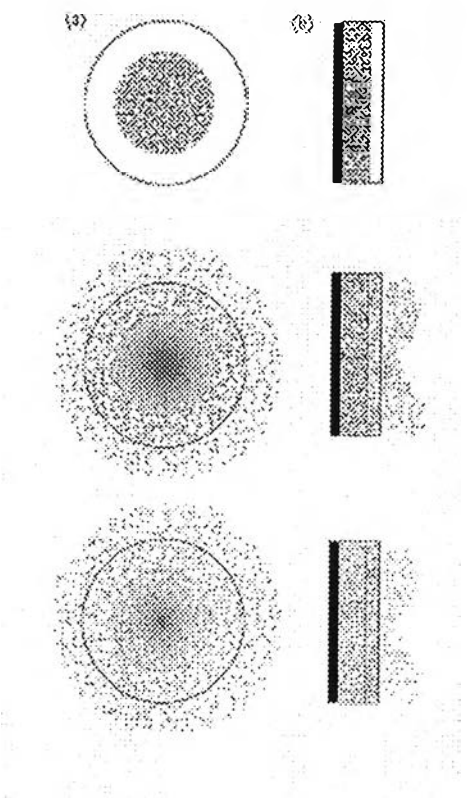


Figure 2.3 Drug delivery from typical reservoir devices: (a) implantable or oral systems, and (b) transdermal systems.

Once the active agent has been released into the external environment, one might assume that any structural control over drug delivery has been relinquished. However, this is not always the case. For transdermal drug delivery, the penetration of the drug through the skin constitutes an additional series of diffusional and active transport steps, as shown schematically in Figure 2.4.

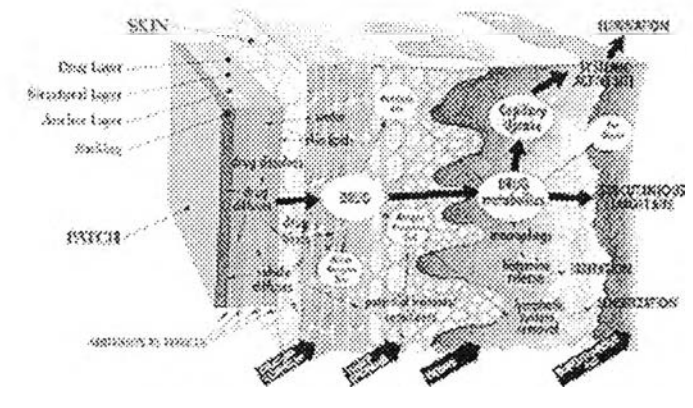


Figure 2.4 Transport processes in transdermal drug delivery (Ishihara, 1983).

For the diffusion-controlled systems described thus far, the drug delivery device is fundamentally stable in the biological environment and does not change its size either through swelling or degradation. In these systems, the combinations of polymer matrices and bioactive agents chosen must allow for the drug to diffuse through the pores or macromolecular structure of the polymer upon introduction of the delivery system into the biological environment without inducing any change in the polymer itself.

2.2.2 Electrospun Fibers Used in Drug Delivery System

Electrospinning affords great flexibility in selecting materials for drug delivery applications. Either biodegradable or non-degradable materials can be used to control whether drug release occurs via diffusion alone or diffusion and scaffold degradation. Additionally, due to the flexibility in material selection a number of drugs can be delivered including: antibiotics, anticancer drugs, protein, and DNA.

Release of tetracycline from electrospun mats of poly (ethylene-co-vinylacetate) (PEVA), poly(lactic acid) (PLA) and their blend was studied by Kenawy *et al.* (2002). Electrospinning was carried out using 14% w/v solution of PEVA, PLA and a 50/50 blend in chloroform. The concentration of tetracycline hydrochloride was 5 wt%. It was found that release of tetracycline hydrochloride from electrospun mats of PEVA, PLA and 50/50 PLA/PEVA gave relatively smooth release of drug over about 5 days. Moreover, electrospun PEVA showed a higher release rate than the mats derived from blend or PLA. For comparison purpose, cast

film with equal amount of drug were prepared and tested. The results showed that the total percent released from the cast film were lower than that of the electrospun mats, as would be expected due to the much lower surface area of film samples.

Hydroxypropyl methylcellulose (HPMC), a water-soluble polymer, was selected to electrospun in the presence of itraconazole (poorly water-soluble drug) by Verreck *et al.* (2003). When a 40:60, itraconazole:HPMC ratio was used, the diameter of obtained fiber was about 1-4 μm (applied voltage =16 kV) and 300-500 nm (applied voltage =24 kV). They concluded that there were a number of potential applications of electrospun fibers in drug delivery based on the following observations: 1) complete release of highly poorly water-soluble drugs can be achieved and 2) the rate of drug release can be tailored by a variety of parameters: for example, the drug/polymer ratio and the diameter of electrospun fibers.

The successful incorporation and sustained release of a hydrophilic antibiotic drug (Mefoxin®, cefoxitin sodium) from electrospun poly(lactide-co-glycolide) (PLGA)-based nanofibrous scaffolds without the loss of structure and bioactivity was demonstrated by Kim *et al.* (2004). PLGA or its mixture (PLGA/PEG-b-PLA diblock copolymer/PLA = 80:15:5 by wt.%) was dissolved in 3.5 g of *N,N*-dimethyl formamide (DMF) solvent to produce a 33 wt.% solution. The antibiotic drug (cefoxitin sodium) in the mass range between 10 and 90 mg was dissolved in 0.11 g of Milli-Q water and then was added to polymer solution. When cefoxitin sodium was added, the morphology of electrospun PLGA/PLA/PEG-b-PLA scaffolds changed from bead-and-string to a completely fibrous structure. In addition, the average fiber diameter and the fiber diameter distribution decreased from 360 ± 220 nm (without drug) to 260 ± 90 (with 5 wt.% of drug). The introduction of PEG-b-PLA block copolymer in the polymer matrix reduced the cumulative amount of the released drug at earlier time points and sustained the drug release profile to longer time (up to 1 week).

Xu *et al.* (2006) examined the incorporation of the anticancer drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) into electrosun poly(ethylene glycol)--poly(L-lactic acid) (PEG–PLLA) diblock copolymer fiber mats. Both the polymer and the BCNU were dissolved in chloroform and electrospun, giving BCNU

embedded in the PEG-PLLA fibers. The average fiber diameters depended on drug loading with values ranging from 690 to 1350 nm for drug loading of 5 and 30 wt.%, respectively. Both the release rate and the initial burst release increased with increasing BCNU loading. The effect of BCNU release from electrospun PEG-PLLAmats on the growth of rat Glioma C6 cells was also examined. BCNU loaded PRG-PLLA mats exhibited anticancer activity over a period of 72 h, while free BNCU began to lose its anticancer activity after 48 h. Thus, by embedding the drug in the polymer fiber were able to protect it from degradation and preserve its anticancer activity.

Heparin was incorporated into electrospun poly(ε-caprolactone) (PCL) fiber mats for assessment as a controlled delivery device by Luong-Van *et al.* (2006). Fibers with smooth surfaces and no bead defects could be spun from polymer solutions with 8%w/v PCL in 7:3 dichloromethane:methanol. A significant decrease in fiber diameter was observed with increasing heparin concentration. Assessment of drug loading and imaging of fluorescently labeled heparin showed homogenous distribution of heparin throughout the fiber mats. A total of approximately half of the encapsulated heparin was released by diffusional control from the heparin/PCL fibers after 14 days. The fibers did not induce an inflammatory response in macrophage cells in vitro and the released heparin was effective in preventing the proliferation of VSMCs in culture. These results suggest that electrospun PCL fibers are a promising candidate for delivery of heparin to the site of vascular injury.

In 2006, Taepaiboon *et al.* have been studied the electrospun poly(vinyl alcohol) (PVA) fiber mats as carriers of drugs for a transdermal drug delivery system. Four types of non-steroidal anti-inflammatory drug with varying water solubility property, i.e. sodium salicylate (freely soluble in water), diclofenac sodium (sparingly soluble in water), naproxen (NAP), and indomethacin (IND) (both insoluble in water), were selected as model drugs. PVA powder was dissolved in distilled water at 80 °C to prepare a PVA at a fixed concentration of 10% w/v. The drugs were loaded at either 10 or 20 wt.% (based on the weight of PVA powder). The drug-loaded polymer solutions were electrospun at an electrical potential of 15 kV and collective distance of 15 cm. The morphological appearance of the drug-loaded electrospun PVA mats depended on the nature of the model drugs. The

molecular weight of the model drugs played a major role on both the rate and the total amount of drugs released from the as-prepared drug-loaded electrospun PVA mats, with the rate and the total amount of drugs released decreasing with increasing molecular weight of the drugs. Lastly, the drug-loaded electrospun PVA mats exhibited much better release characteristics of the model drugs than drug-loaded as-cast films.

2.3 Wound Dressing

2.3.1 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).

2.3.1.1 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.

2.3.1.2 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.

2.3.1.3 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.

2.3.1.4 Maturation

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

2.3.2 Types and Properties of Wound Dressing

Skin plays an important role in haemostasis and the prevention of invasion by microorganisms. Skin generally needs to be covered with a dressing immediately after it is damaged. An ideal dressing should maintain a moist environment at the wound interface, allow gaseous exchange, act as a barrier to microorganisms and remove excess exudates. It should also be non-toxic, non-allergenic, non-adherent and easily removed without trauma, and it should be made from a readily available biomaterial that requires minimal processing, possesses antimicrobial properties and promotes wound healing. At present, there are three categories of wound dressing: biologic, synthetic and biologic–synthetic. Alloskin and pigskin are biologic dressings commonly used clinically, but they have some disadvantages, such as limited supplies, high antigenicity, poor adhesiveness and risk

of crosscontamination. Synthetic dressings have long shelf life, induce minimal inflammatory reaction and carry almost no risk of pathogen transmission. . In recent years, researchers have focused on biologic–synthetic dressings (Suzuki, 1990; Matsuda, 1990), which are bilayered and consist of high polymer and biologic materials.

Wound dressing and devices form an important segment of the medical and pharmaceutical wound care market worldwide. In the past traditional dressing such as natural or synthetic bandages, cotton wool, lint and gauzes all with varying degree of absorbency were used for the management of wounds. Their primary function was to keep the wound dry by allowing evaporation of wound exudates and preventing entry of harmful bacteria into the wound. Effective wound management depends on understanding a number of different factors such as the type of wound being treated, the healing process, patient conditions in terms of health (e.g. diabetes), environment and social setting, and the physical chemical properties of the available dressing (Morgan, 2002). In addition, recent advances in dressings are discussed within the context of the formulations for delivery therapeutic agents to moist wound surfaces. Finally, general physical characterization of topical dressings, for application to wounds, in terms of their fluid affinity water uptake, rheological properties (gel and tensile strength, elasticity), compressive and bioadhesive properties are specifically discussed. There are two opposite requirements for wound dressing to meet: it should prevent loss of fluids, electrolytes and other biomolecules from the wound and obstruct bacterial entry, but it should also be permeable enough to allow the passage of discharge through pores or cuts. It addition it should be able to adhere to the wound surface, and be easy to peel from the skin without disturbing new tissue growth.

2.3.3 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.

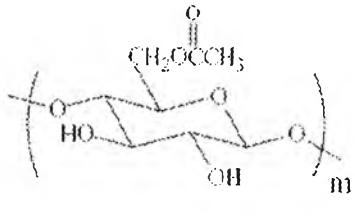
The best biomaterials for wound dressing should be biocompatible and promote the growth of dermis and epidermis layers. Chen *et al.* (2008) have successfully produced a composite nanofibrous membrane composed of collagen and chitosan, which are known for their beneficial effects on wound healing. The 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.

2.4 Cellulose Acetate

2.4.1 Properties of Cellulose Acetate

Cellulose acetate (CA) is the acetate ester of cellulose, the primary structural component of the cell wall of green plants and is one of the most common biopolymers on earth (Anonymous, 2006). CA is prepared from cellulose by a solution process employing sulfuric acid as the catalyst with acetic anhydride in an acetic acid solvent. The acetylation reaction is heterogeneous and topochemical (Mark, 1985). General properties of CA are shown in Table 2.1.

Table 2.1 General properties of cellulose acetate (40.4% acetyl content) (Mark, 1985)

Properties	Details
Chemical structure	
Melting point (°C)	306
Char point (°C)	315
Density (g/ml)	1.28
Tensile strength (MPa)	71.6

The properties of cellulose acetate are affected by the number of acetyl groups per anhydroglucose unit of cellulose and the degree of polymerization. Fewer acetyl groups per anhydroglucose unit (increased hydroxyl content) increase the solubility in polar solvent and decrease moisture resistance (Mark, 1985).

Solubility characteristics of cellulose acetates with various acetyl contents are given in Table 2.2.

Table 2.2 Solubility characteristics of cellulose acetate (Mark, 1985)

Acetyl, %	Soluble in	Insoluble in
43.0-44.8	Dichloromethane	Acetone
37-42	Acetone	dichloromethane
24-32	2-methoxymethanol	Acetone
15-20	Water	2-methoxymethanol
<13	None of the above	All of the above

Cellulose acetate is used in textile fibers, plastics, film, sheeting and lacquers. In biomedical application, cellulose acetate had been used as a semipermeable coating on tablets (Makhija, 2003; Lu, 2003), especially on osmotic pump-type tablets and implants, which allows for controlled, extended release of actives (Santus, 1995).

2.4.2 Electrospinning of Cellulose Acetate

Liu and Hsieh (2002) reported the preparation of ultra-fine CA fiber mats as well as regenerated cellulose membranes by electrospinning. Three solvents (acetone, acetic acid, and dimethylacetamide (DMAc)) were used electrospinning of cellulose acetate. Their results showed that none of these solvents alone can produce fibers. An improvement in the electrospinning of CA was achieved when 2:1 v/v acetone/dimethylacetamide (DMAc) was used as the solvent system. This mixture allowed the resulting CA solutions with concentration in the range of 12.5-20 wt.% to be continuously spun into fibers with diameters ranging between ~100 nm and ~1 μm.

Electrospun ultrafine fibers of CA were successfully prepared by using a mixture of acetone/water with the water content in the range of 10-15 wt.%. Moreover, Son *et al.* (2004) found that the electrospinning of a CA solution in acetone/water in an acidic condition produced larger fibers, while that of the solution in a basic condition produced much finer ones.

In 2005, Ma *et al.* have prepared the affinity CA fibrous membranes from cellulose acetate (CA) solution (0.16g/ml) in mixture solvent of 3:1:1 v/v/v acetone/dimethylformamide (DMF)/trifluoroethylene (TFE) by electrospinning (applied electrical potential = 25 kV; collection distance = 15 cm; polarity of emitting electrode = positive; solution flow rate = 4 ml h^{-1}). The CA nanofiber mesh (fiber diameter ranging from 200 nm to 1 μm) was heat treated under 208 °C for 1 h to improve structural integrity and mechanical strength, and then treated in 0.1 M NaOH solution in H_2O/ethanol (4:1) for 24 h to obtain regenerated cellulose (RC) nanofiber mesh, in which Cibacron Blue F3GA, a sulfonated triazine dye, was coupled on their surface.

Son *et al.* (2006) prepared the antimicrobial CA fibrous membranes from 10 wt.% CA (acetyl content = 39.8%; M_w = 30 000 Da) solution in 80:20 w/w

acetone/water containing $AgNO_3$ in the amount of 0.5% (based on the weight of CA) by electrospinning (applied electrical potential = 17 kV; collection distance = 10 cm; polarity of emitting electrode = positive; solution flow rate = 3 ml h^{-1}). Ag+ ions were photoreduced into Ag nanoparticles, an active antimicrobial agent, by irradiating the as-spun fibers (average diameters = 610-1910 nm) with UV light (the maximum wavelength = 245 or 365 nm) that resulted in the Ag nanoparticles with the average diameters ranging between 3 and 21 nm.

A new solvent system for the electrospinning of cellulose acetate (CA) nanofibers was studied by Han *et al.* (2008). The CA solutions were electrospun at a positive voltage of 25 kV, a tip-to-collector distance of 10 cm, and a solution flow rate of 3 mL/h. Long uniform CA nanofibers with an average diameter of 180 nm were electrospun from a 17 wt.% CA solution in mixed solvent containing acetic acid/water at a ratio of 75:25 by weight. The average diameters of the CA nanofibers could be controlled from 160 nm to 1280 nm by changing the composition of the mixed solvent.

2.4.3 Biomedical Applications of Cellulose Acetate

2.4.3.1 Drug Delivery System

Cellulose acetate is a widely use and investigated material both in the industry and in research. However, cellulose acetate, when used as rate controlling membrane material for transdermal drug delivery systems, generally requires plasticizers to improve its mechanical property. A plasticizer is supposed to weaken the intermolecular forces between the polymer chains resulting in a softened and flexible polymer matrix. Thus, drug permeability through the membranes may also be affected by the addition of plasticizer. The commonly used plasticizers in membrane formulations include phthalate ester, phosphate ester, fatty acid and glycol derivatives (Rama Rao and Diwan, 1997). Polyethylene glycol (PEG, MW 600) was used as a plasticizer as well as a pore-forming agent and incorporated into cellulose membranes. Due to its hydrophilicity, PEG can be easily removed by putting the membranes into an aqueous solution before they are assembled into transdermal patches. The in vitro release rates of scopolamine base as a model drug through the membranes were evaluated in phosphate buffer solution (PBS, pH 7.4) at 32 °C. It was observed that no drug flux from the cellulose acetate membranes prepared

without PEG and the drug permeation through the cellulose acetate membranes was affected by the incorporated PEG content and formed membrane morphology (Wang, 2002).

Cellulose acetate was selected as hydrophobic coating to entrap the hydrophilic chitosan microcores. During the preparation of chitosan/cellulose acetate multimicropheres (CCAM), no chemical crosslinking was used. Model drugs with different hydrophilicity were selected to investigate the delivery system. With the increasing of hydrophobicity of drug, the holes in the holes in the appearance of microspheres became smaller and the loading efficiency increased. The loading efficiency of different model drugs increased with the increasing of hydrophobicity of drug (Zhou, 2006).

2.4.3.2 Wound Dressing

Cellulose acetate (CA), an acetylated derivative of cellulose polymer, is a good candidate, because this polymer is non-toxic, biodegradable, and renewable with good processability (Ding, 2004). A unique fabric with non-adherent characteristics making it suitable for use as a wound dressing, and particularly as a dressing for burn, is disclosed. The fabric comprises cellulose acetate fibers and a siloxane finishing on the fibers. In a preferred embodiment, the dressing comprises cellulose acetate fibers, cellulose acetate fiber having an anti-biologic incorporated into the fiber resin, and a siloxane finishing on the fibers. The fabric of the invention was found to be less adherent to burn than dressing made from cotton or having a nylon net (Chen and Soden, 2002). In 2008, Luong *et al.* developed an appropriate co-solvent system to be used for the development of a cellulose acetate nanofibrillar aerogel structure, which gives highly branched continuous nanofibrils. This structure provides a highly-porous and self-sustaining robust structure with a large surface area, which is essential for the facile incorporation of the silver ions in the metallization process to give a high loading content of silver. The CA nanoporous structure acted as an effective nanoreactor for the in situ synthesis of silver nanoparticles. This method developed herein could be used for various biomedical applications, especially antimicrobial membranes.

2.5 Herbal Substances

2.5.1 Curcumin

Curcumin (see chemical structure in Figure 2.5a) is a naturally-occuring compound found in the plant *Curcuma longga* L. Its major constituents are curcuminoids, which are polyphenols normally exist in at least two tautomeric forms, keto and enoal. The enol form is more energetically stable, both in the solid phase and in solution (Anonymous, 2007). Curcumin is widely known for its anti-tumor, antioxidant, and anti-inflammatory properties (Sharma, 2005; Jayaprakasha, 2005; Jayaprakasha, 2006; Maheshwari, 2006).

Wound healing is a complex multifactiorial process that results in the contraction and closure of the wound and restoration of a functional barrier (Hunt, 2000). Wound related non-phagocytic cells also generate free radicals by involving non-phagocytic NAD(P)H oxidase mechanism (Griendling, 2000). Thus, the wound site is rich in both oxygen and nitrogen centered reactive species along with their derivatives. The presence of these radicals will results in oxidative stress leading to lipid peroxidation, DNA breakage, and enzyme inactivation, including free radical scavenger enzymes (Wiseman, 1996).

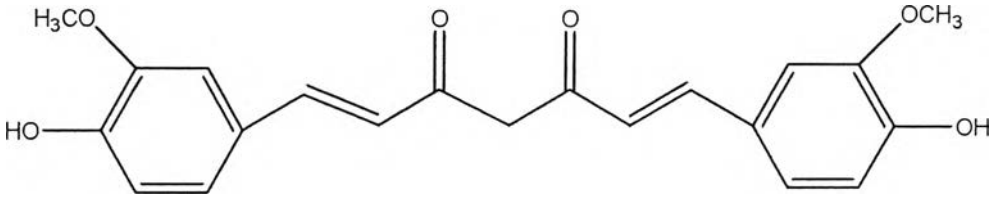
Due to curcumin has been shown to possess antioxidant (free radical scavenging activity) Thus, it can enhance cutaneous wound healing in rats and guinea pigs. Sidhu *et al.* (1999) have evaluated the efficacy of curcumin treatment by oral and topical applications on impaired wound healing in diabetic rats and genetically diabetic mice using a full-thicknesss cutaneous punch wound model. Wounds of the animals treated with curcumin showed early re-epithelialization, improved neovasculatization, increased migratory activity of various cells including dermal myofibroblasts, fibroblasts, and macropages into the wound bed, and a higher collagen content. The wound healing process involves extensive oxidative stress to the system, which generally inhibits tissue remodeling.

In 2004 Gopinath *et al.* have improved the quality of wound healing by slow delivery of antioxidants like curcumin from collagen, which also acts as a supportive matrix for the regenerative tissue. They found that the presence of curcumin helped increase wound reduction, enhance cell proliferation, and provide

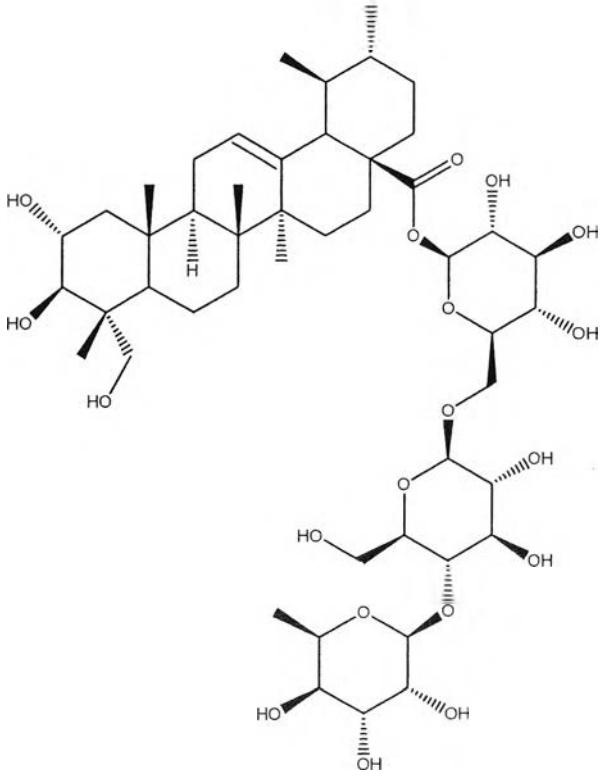
efficient free radical scavenging activity. Panchatcharam *et al.* (2006) have determines the role of curcumin on changes in collagen characteristics and antioxidant property during cutaneous wound healing in rats. Full thickness excision wounds were made on the back of rat and curcumin was administered topically. They found that curcumin increase cellular proliferation and collagen synthesis at the wound sits, as evidenced by increase in DNA, total protein and type III collagen content of wound tissues.

2.5.2 *Centella Asiatica* and Asiaticoside

Centella asiatica (L.) Urban, also known as Asiatic Pennywort or Buabok (in Thai), is a poorly water-soluble drug. It has been reported to heal wounds, burn, and ulcerous abnormalities of the skin, cure stomach and duodenal ulcers, and are effective in the treatment of leprosy, lupus, scleroderma, and diseases of the veins (Kartnig, 1988). Among the four major triterpenoid components of C. asiatica (i.e., asiatic acid, asiaticoside, madecassic acid and madeassoside), asiaticoside (see chemical structure in Figure 2.5b), 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. Moreover, Cheng *et al.* (2003) have investigated the healing effects of *Centella asiatica* water extract and asiaticoside on acetic acid induced gastric ulcers (kissing ulcers) in rats. They found that these herbs can reduce the size of the ulcer and also promote epithelial cell proliferation and angiogenesis.



(a)



(b)

Figure 2.5 Chemical structures of (a) curcumin (keto form) and (b) asiaticoside (AC).