CHAPTER II
LITERATURE REVIEW

2.1 Wound healing process

2.1.1 Wound healing process

The process of wound healing can be divided into four processes; haemostasis and inflammation, migration, cell proliferation and maturation (Debra, 1998). Furthermore, free radical species or infection may be involved in the healing process. Generally, the wound healing stages overlap and traditional plants involved at least two different processes.

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 Other factors related to improved wound healing

2.1.2.1. Antimicrobial characteristics

When open wounds occurred, it is possible to bacteria can infect in wound area. The infection in wound area will be less in the wound healing and also more pleasant exudates resulting in delay wound healing. The test of antibacterial activity was carried out in terms of antimicrobial assay in terms of minimum inhibitory concentration (MIC), and zone of inhibition.

2.1.2.2 Antioxidant properties

The antioxidant property play important role in wound healing. Excess ROS could be killed fibroblasts and other cells. Due to various factors, the generation of ROS will be important in the wound healing process. There are many tests of antioxidant activity in vitro such as DPPH method. DPPH· Radical species reduced into DPPH and change the colored from purple to colorless and can detect by using the intensity of the color.

2.2 Bioactive Thai herbal Substances

2.2.1 Polyphenols

In the ancient times, a wide variety of herbal substances have been used for important biological functions such as treatment of skin disorders, antioxidant, antibacterial, anti-inflammatory. Additionally, these traditional medicinal plants affected as active new lead structures. Polyphenols are compounds occurring in nature and containing phenolic functionalities (Campiani, 1998). Polyphenols have recently received attention as antioxidant. The main sources of polyphenol is dietary such as honey, blackberries, blueberries, grapes and strawberries.(Flight and Clifton, 2006).

2.2.1.1 Gallic Acid

Gallic acid (GA) is a phenolic antioxidant that found in plants such as green tea grapes, cherry, and longan seeds. (Rangkadilok, 2007), and has recently been known to have potential healthy effect. Gallic acid acts as an antioxidant and can be protect oxidative damage of the cells. Moreover, gallic acid has the melanogenesis activity and showed anti-tyrosinase activity ($IC_{50} = 3.59\times10^{-6}$ M) (Kim *et al.*, 2007). It also showed an effective down regulation of reactive species (RS) generation and reduced glutathione of cancer cells. Therefore, gallic acid can be used skin whitening compounds and showed high antioxidant activity (Kim *et al.*, 2007).

2.2.1.2 Caffeic Acid

Caffeic acid is a naturally occurring non-flavonoid compound (Elegir *et al.*, 2008). Caffeic acid has anti-inflammatory and potent antioxidant activity (Dastmalchi *et al.*, 2008). In 2010, Shiu *et al.* successfully immobilized the caffeic acid onto chitosan hybrid scaffolds. These materials were exhibited antibacterial, antioxidant and anti-cancer property. Moreover, the caffeic acid-immobilized scaffolds showed antioxidant activity against *Staphylococcus aureus* and scavenging DPPH activities when compare with chitosan hybrid scaffolds and can inhibited on cell growth and attachment of human osteosarcoma UMR-106 cells.

2.2.1.2 Eupatorium adenophorum Spreng Essential Oil

Eupatorium adenophorum Spreng.(Crofton weed) (Fig. 2.1) belongs to the family Asteraceae (Compositae). There has been much research of these plants that are commonly used in folklore medicine as antimicrobial, antiseptic, blood coagulant, analgesic, antipyretic and enhancer of phenobarbitone induced sleep. *Eupatorium adenophorum* Spreng. is used for their medical properties in India asian antiseptic and blood coagulant. There are some previous reports on the antimicrobial activity of the oil of some species of *Eupatorium adenophorum* Spreng. *Eupatorium adenophorum* extracted from petroleum, chloroform-ether, benzene, and methanol. These crude extract was tested in antimicrobial activity against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes* and *Pseudomonas aeruginosa* by Paper disk diffusion technique. The result found that the antimicrobial activities of *Eupatorium adenophorum* crude extracts show good activity and the extracts from petroleum-ether is the highest in the inhibition zone against *B.subtilis* (Arvind, 2010). The essential oils of *Eupatorium adenophorum* was identified by using GC/MS techniques and the results showed that the major components of the essential oils were amorph-4-en-7-ol 17.7%, bornyl acetate 15.9%, p-cymene 16.6%, 3-acetoxyamorpha-4,7(11)-dien-8-one 16.3%, α-phellandrene 9.6%, camphene 8.9%. Moreover, in 2005, Mandal *et al.* reported *Eupatorium adenophorum* extracted from methanol showed antiimflammatory activity in mouse model.

Figure 2.1 Photograph of *E. adenophorum* Spreng.

2.2.3 Mangosteen

Mangosteen is a tropical fruit with medicinal properties that can be grown mainly in southeast asia (Thai, Malaysia, India and Sri-Lanka). Fruit of the mangosteen is dark purple or reddish, with snow-white, soft and juicy edible pulp with a slightly acid and sweet flavor and a pleasant aroma (Jung *et al.*, 2006). The rind of mangosteen is used for skin infection treatment, infected wounds, dysentery, and diarrhea for many years. (Chaverri, 2008; Balasjbramanian, 1998; Arunrattiyakorn, 2011; Quan, 2010; Jiang, 2010). Morever, the rind of mangosteen has widely used in ayurvedic medicine for anti-imflammatory activity and cholera and dysentery (Balasubramanian and Rajagopalan, 1988, Sen *et al.*, 1980b).

Mangosteen was found to have strong antioxidant because the chemical composition of *G. mangostana* pericarp has been identified to contain complex phenolic compounds, such as tannins, flavonoids, especially, prenylated xanthone derivatives (Naczk, 2011; Zadernowsk, 2009; Chaivisuthangkura, 2009; Chin, 2008; Mahabusarakam, 1987). Xanthones is a major component in mangosteen fruits and found in some plant families (Peres *et al.*, 2000; Vieira and Kijjoa, 2005). Xanthone showed high antioxidant activity and help to heal the cells by free radicals, aging, dieseases, and damages. The rind of mangosteen fruit contains a polyhydroxy-xanthone derivative or mangostin (beta-mangostin), gartanin, beta-disoxygartanin

and *normangostin* (Vieira and Kijjoa, 2005; Pinto *et al.,* 2005; Souza and Pinto, 2005; Gales and Damas, 2005).

Suksamran *et al.*, (2009) successfully to analyze the major components of crude extracts of mangosteen by using reversed-phase high-performance liquid chromatography. The results showed that these are 14 prenylated xanthone in mangosteen fruit and the results are showed in Fig 2.2. *Garcinia mangostana L.* (11-hydroxy-1-isomangostin (1), garcinone C (2), garcinone D (3), c-mangostin (4), 8-deoxygartanin (5), gartanin (6), a-mangostin (7), garcinone E (8), demethylcalabaxanthone (9),1,6-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-60,60-dimethylpyrano(2',3':3,2) xanthone (10), b-mangostin (11), mangostenone A (12), calabaxanthone (13), and tovophyllin B (14)).

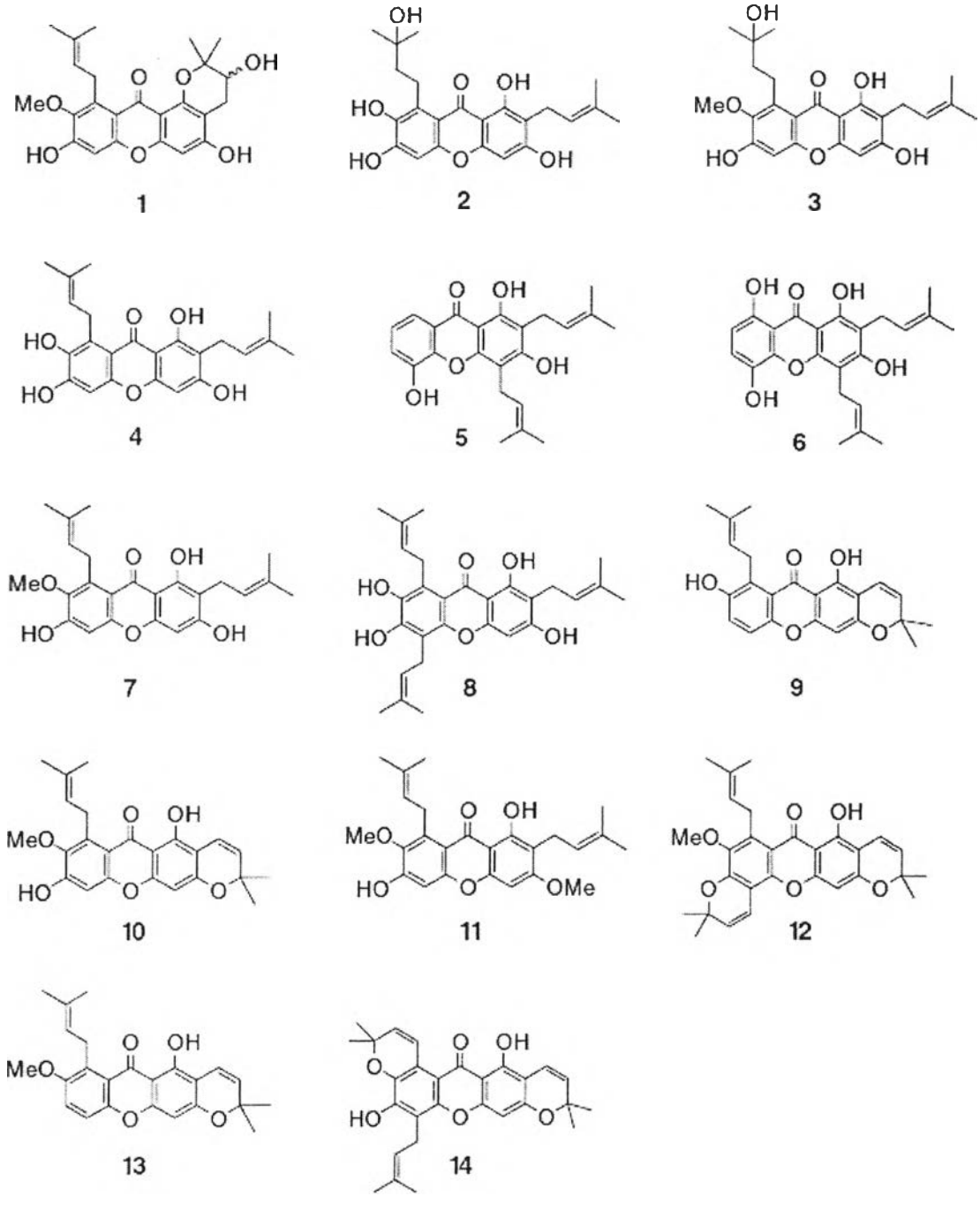


Figure. 2.2 Structures of the xanthones: 1 = 11-hydroxy-1-isomangostin, 2 = garcinone C, 3 = garcinone D, 4 = c-mangostin, 5, 8-deoxygartanin, 6 = gartanin, 7 = a-mangostin, 8 = garcinone E, 9 = demethylcalabaxanthone, 10 = 1,6-dihydroxy-7-methoxy-8-(3-methylbut- 2-enyl)-60,60-dimethylpyrano(20,30:3,2)xanthone, 11 = b-mangostin, 12 = mangostenone A, 13 = calabaxanthone, 14 = tovophyllin B.

In addition, *G. mangostana* exhibits antitumor and antioxidant abilities (Matsumoto, 2003; Matsumoto, 2004; Matsumoto, 2005; Mahabusarakam, 2000; Jung, 2006; Moongkarndi, 2010) as well as antibacterial properties that combat *Staphylococcus epidermidis and Propionibacterium acnes* (Chomnawang, 2005). In 2003, Suksamrarn and coworker studied the antimicrobial activity of the prenylated xanthones from pericarp of mangosteen fruits. They found that prenylated xanthones showed the inhibition zone against *M. tuberculosis* especially mangostin and garcinone B and showed the MIC value of 6.25 mg/ml.

2.3 Polymer Sources

2.3.1 Poly (L-lactic acid) (PLLA)

Poly (L-lactic acid) or PLLA, (Figure 2.3) is biodegradable polyester derived from L-lactic acid. A monomer of PLLA synthesized from sugar-based biomass such as sugarcane, corn by fermentation and polymerized PLLA by using direct condensation or ring opening polymerization. (Tsuji *et al.*, 2005; Gupta *et al.*, 2007) Due to its biocompatibility, and suitable degradation rate is used for tissue scaffolds and suture for biomedical applications (Tsuji *et al.*, 2005; Gupta *et al.*, 2007). PLLA is widely used because of its good biocompatible and robust mechanical properties and also easily to fabricate into scaffolds of different shapes. PLA has a crystallinity around 37%, the glass transition temperature (T_g) 60–70°C and melting behavior 150-170°C (Gupta *et al.*, 2007). PLLA has suitable in the degradation rate and these degradation products are carbondioxide, water and non-toxic compounds. In addition, PLLA degradation rate is suitable for healing process of tissue repair (Cui *et al.*, 2003).

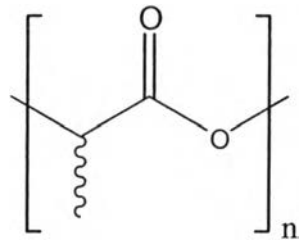


Figure 2.3 Chemical structure of poly(L-lactic acid) (PLLA).

2.3.1.1 Biodegradation of Polymer

The main factors that determine the rate of degradation are:

2.3.1.1.1 Chemical Stability of the Polymer Backbone

The chemical structure of polymers have affected to the degradation rate. For example, anhydride bonds tend to be hydrolyzed faster than ester bonds (Ratner *et al.*, 1996).

2.3.1.1.2 Hydrophobicity

The rate of degradation depends on the ability that can penetrate into polymers. The hydrophobic polymer shows slower rate of degradation than that of the hydrophilic polymer. For example, PCL degrades slower than PLA (Pitt *et al.*, 1981) and PLA degrades slower than PGA (Ratner *et al.*, 1996).

2.3.1.1.3 Morphology of the Polymer

The high crytallinity of polymer is slower rate of hydrolysis than the densely packed of crystalline because of the lower rate of water penetration into polymer. For example, PLLA, which is semicrystalline polymer, tends to degrade slower than PDLLA, which is amorphous polymer (Ratner *et al.*, 1996)

2.3.1.1.4 The Fabrication Process

Polymer in the form of highly porous microspheres degrades faster than the same polymer which is produced in the form of dense microspheres (Ratner *et al.*, 1996).

Due to its biocompatibility, PLLA can be fabricated into films, fibers, and scaffolds for biomedical applications (Tsuji, 2005; Gupta, 2007; Badami, 2006; Peesan, 2006; Xu, 2006; Li, 2006). In recent year, PLLA have been successfully to ultrafine fibers by e-spinning (Zong, 2002). PLLA can be use various solvents to prepare PLLA solutions such as dimethylformamide (DMF) (Zong, 2002), chloroform (Kenawy, 2002), dichloromethane (DCM), or 9:1 v/v DCM/DMF (Xu, 2006). Electrospun PLLA fibers have been reported as carriers for drug delivery system (Zong, 2002; Kenawy, 2002, Cui, 2006; Taepaiboon, 2007; Sikareepaisan, 2008). Recently, Taepaiboon *et al.* (2007) successful to fabricated electrospun cellulose acetate fibers for transdermal delivery of vitamin A and vitamin E. 0.5 % and 5 % wt. of vitamin A and vitamin E were incorporated in cellulose acetate solution and subjected to electrospinning process. Four different types of non-steroidal anti-inflammatory drugs (NSAIDs), i.e., naproxen (NAP), indomethacin (IND), ibuprofen (IBU) and sulindac (SUL), were used as model drugs and incoperated in polyvinyl alcohol power. These solutions were electrospun at 15 kV and collective distance 15 cm Tungprapa *et al.* (2007).

In normally PLLA has been reported for tissue engineering applications. However, due to non-polar group in structure of PLLA, PLLA is hydrophobic resulting in difficult to infiltrate with culture media. In order to compensate the hydrophobic properties, the surface modification of polymer is coated on the surface of material by physical adsorption. However, the drawback of this process is easy to remove when changing the pH of the solution or applied high shear forces (Sikareepaisan, 2008; Tsuji, 2005; Gupta, 2007). Therefore, grafting polymerization is one of the potential techniques that the biomolecule can be chemical grafted onto the surface of materials (Shiu, 2010; Mattanavee, 2009; Cheng, 2004; Wang, 2003). In 2010, Shiu J.C. *et al.* successfully fabricated chitosan and (3-chloropropyl) trimethoxysilane (CPTMS) hybrid scaffolds and further immobilized caffeic acid on the surface of material in order to improve antioxidant, antibacterial, and anticancer activities. Moreover, these materials showed antibacterial activity against *Staphylococcus aureus* when compare with chitosan hybrid scaffolds. They also examined anticancer of human osteosarcoma UMR-106

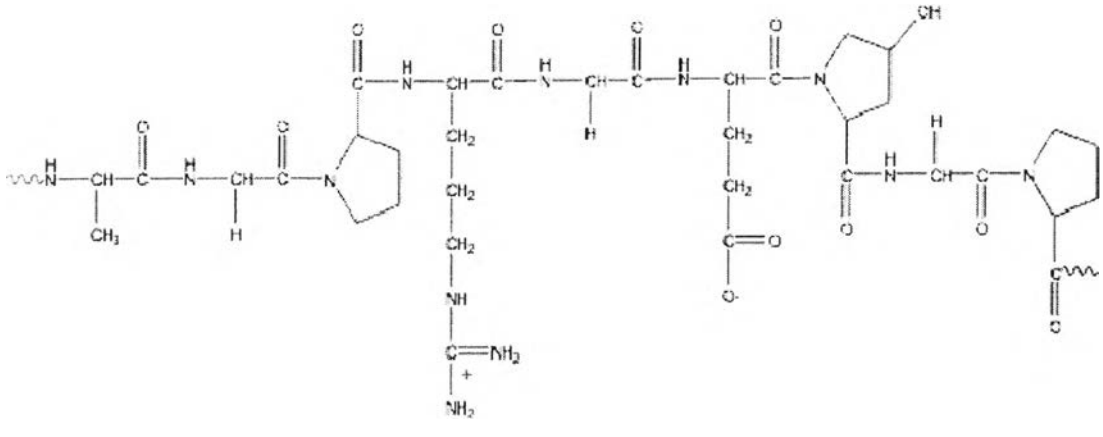
cells and the result showed that caffeic acid immobilized on the surface of hybrid scaffolds can inhibited on cell growth and cell attachment of UMR-106 cells.

2.3.2 Gelatin

Gelatin is a soluble animal protein compounds, consisted of 19 amino acids that joined by peptide linkage and can be obtained by partial hydrolysis of collagen. It is a biopolymer that consisted of 19 amino acids and gelatin found to be the main structure in ligaments, tissue, bone of animal, tendons. Gelatin has been valuably used in food processing, cosmetics, and pharmaceuticals because it has biological origin, biocompatible, excellent fuctional and hydrogel properties and relatively low cost (Choi and Regenstein, 2000).

Structural unit

Gelatin contains single or multible stranded polypeptides 300-4000 amino acid. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.



There are two types of gelatin depending on the pretreatment procedure as type-A and type-B gelatin under acid and alkaline pre-treatment condition, respectively. The alkaline process, the amide groups of asparagine and glutamine hydrolyzed into carboxyl groups and converted into aspartate and glutamate. On the

other hand, pre-treatment with acid has less affected with amide groups resulting in the alkaline treated gelatin treated. An alkaline pre-treatment is differed from acidic process because the alkaline treatment is higher part of carbonyl group and lower isoelectric point (IEP) when compared to acid-treated process. The alkaline process is suitable for more complex collagen while the acidic treatment is for less cross-linked collagens found in pig skin (aonymous, 2007). There are two main methods of crosslinking gelatin which are physical and chemical methods (Kuijpers, 2000). The example of non-zero length crosslinkers are glutaraldehyde, formaldehyde, isocyanates, and polyepoxides. Zero-length crossinkers are directly bonded carboxylic groups and amine groups on adjacent protein molecules such as carbodiimides (Tabata, 1994) and acyl azides (Kuijpers, 2000). To determine the crossink density, a tensile test on gelatin specimens and water content was performed (Iwanaga, 2003). The water content of hydrogels is defined as the amount of water that can penetrate into hydrogel matrix compared to before swelling in 37 °C in phosphate buffer solution. The concentration of glutaraldehyde can affect the crossinking density of hydrogels. Increasing the amount of glutaraldehyde, the crosslink density can be increased.

2.3.3 Poly(acrylonitrile) (PAN)

Poly(acrylonitrile) is a synthetic polymer derived from acrylonitrile polymerization. PAN is commonly produced a large variety of products such as filtration membranes, nano-sensors, textiles and biomedical applications because it has excellent properties such as thermal and mechanical stability, high tensile strength, good solvent resistance (Che, 2005; Shinde, 1999; Nouzaki, 2002). Poly(acrylonitrile) has been reported into fiber forms and used wet or dry spinning process to fabricate into fibers (Mandal, 2004; Frahn, 2004; Kim, 2001; Iwata, 2003; Musale, 1997).

Properties	Details
Chemical structure	$-[CH_2-CH]-$ $\vert$ $C\equiv N$
Glass transition temperature	80-110 °C
Melting temperature	317-330 °C
Amorphous density at 25°C	1.184 g/cm^3
Molecular weight of repeat unit	53.06 g/mole

The PAN fibers with suitable solvent were developed by DuPont in 1940. Various polar solvents that can dissolve PAN such as dimethylformamide, dimethylsulfoxide, dimethylsulfone, tetramethylsulfide.

Polyacrylonitrile (PAN) fibrous membranes have potential properties such as thermal and chemical stability, high tensile strength. Therefore, PAN fibers have widely used in filtration applications. Recently, the electrospun nano-fibrous membrances have been used for filtration applications more polular because the electrospinning process can provide the smaller fiber diameters, large surface to volume and length to diameter ratio (Nam-Wun, 2001, Wang, 2006). There has been numerous research about the filters with antimicrobial functionality due to the qualities of purified water and filtered air. In practical application, the microorganisms can trap on the filters and accumulate into biofilms. The contaminated filters with bacteria biofilms are difficult to clean resulting in the difficulty to fitrate during the operation. Therefore, much attention has been in antibacterial activity of the electrospun fibers. Nanofibers can produce by laser ablation (Ren, 1998) and chemical vapor decomposition (Ijima, 1991) but these methods have high cost and require expensive equipment. Therfore, electrospinning process seem to be a potential methods to produce nanofibers with diameters from 10 nm to 10 μm (Jayaraman, 2004; Formhals, 1934) Fig. 2.4 displays the applications of electrospun fibers in various fields (Moncrieff, 1970; Yang, 2005; Shin, 2001).

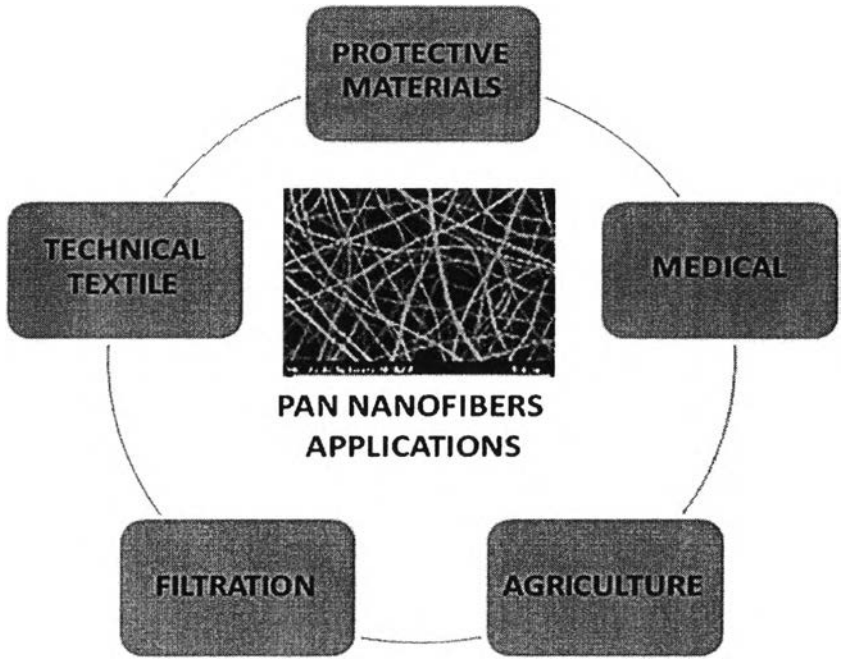


Figure 2.4 PAN-based nanofiber applications

2.4 Methods of Fabrication

2.4.1 Electrospinning

The electrospinning process (Figure 2.5) is an economical and simple technique producing electrospun fibers in submicron to nanometers by using an electrical charge. In the electrospinning process, a polymer solution contained in a syringe or reservoir is subjected to electric fields, the liquid droplet at the tip of the capillary tube is stretched to form a conical shape known as Taylor cone. Further increase the electric field to reach a critical value, the jet is ejected towards from the tip of the Taylor cone. As the jet dried in the air, and finally lays themselves randomly on the grounded collector. If the high molecular cohesion of the polymeric liquid; the jets is formed resulting in non-woven fabric elcetrospun fibers. There are many parameters that should be affected in the characteristics of electrospun fibers such as molecular weight of polymer, viscosity, surface tension, applied voltage, flow rate and distance between syringe and collecting metal screen. Due to the high

surface area, high porosity of the electrospun non-woven fabrics fibers, the elctrospun fibers are excellent characteristics for biomedical applications including 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, Zong *et al.*, 2002 and Kenawy *et al.*, 2002). Recently, cellulose acetate was used as the substrate and fabricated into electrospun cellulose acetate fiber mats for transdermal delivery of various types of drugs (Taepaiboon *et al.* 2007); Tungprapa *et al.* 2007); Suwantong *et al.* 2008). In 2007, Taepaiboon *et al.* prepared the incorporation of vitamin A and vitamin E into electrospun cellulose acetate fibers. Cellulose acetate was dissolved in 2:1 v/v acetone/dimethylacetamide and then added vitamin A and vitamin E in 0.5 and 5% % wt. based on the weight of cellulose acetate, respectively. In addition, hydroxyapatite nanoparticles were incorporated into elctrospun polycaprolactone (PCL) fibers for assessment as a bone scaffolds (Wutticharoenmongkol *et al.*, 2007). The attachment, proliferation, differentiation and minerlization of mouse calvaria-derived pre-osteoblastic cells (MC3T3-E 1) were also examined. The result showed PCL loaded hydroxyapatite are a promising candidate for a bone scaffolds.

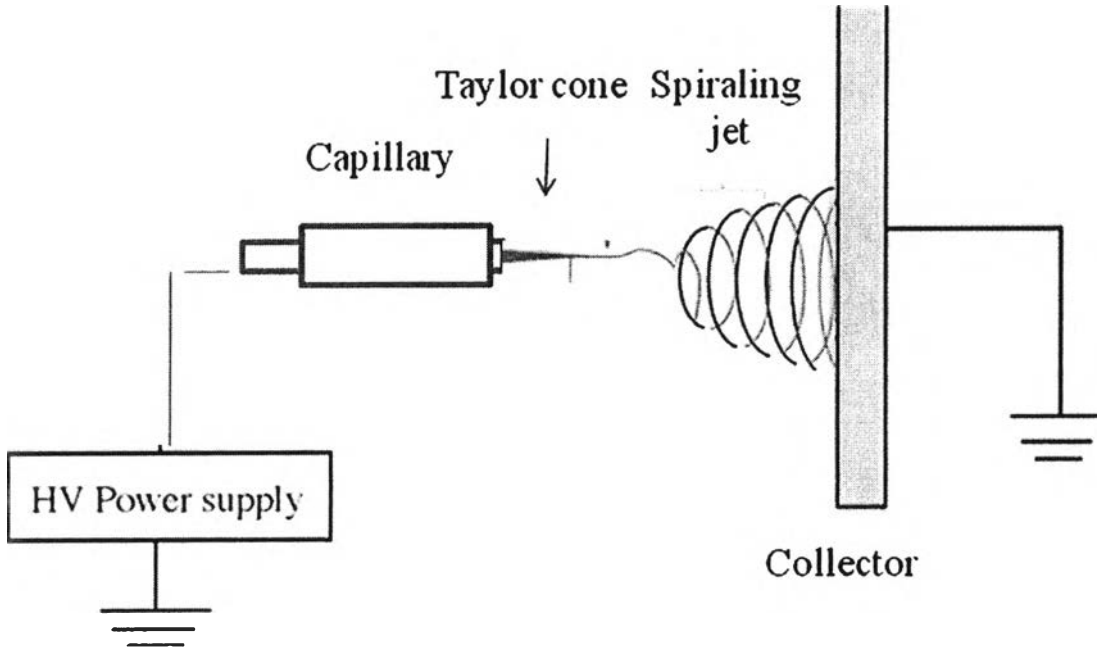


Figure 2.5 Schematic diagram of electrospinning system.

2.4.1.1 Biomedical Applications of Electrospun Fibers

Due to the excellent properties, elctrospun fibers was widely used in biomedical applications including tissue engineering, drug delivery, and wound healing.

2.4.1.1.1 Scaffolds for Tissue Engineering

Electrospun fibers in tissue engineering applications must be considered in term of types of materials, porosity, orientation of fiber and surface modification. There are many types of material used to be fabricated into electrospun fibers, including choices of material, natural and synthetic materials. When tissues injured or damaged, these biocompatible material are fabricated in order to mimic the structure and biological activities of extracellular matrix (ECM). The electrospinning process can be fabricated into fibers in sub-micrometer or nanometer fibers. There are many research reported that fibroblast cells can attach and compatible in electrospun fibers. As a result, nanometer or sub- micrometer fibrous scaffolds could be suitable template for cell seeding, migrating, and proliferating. Additionally, advantages of sub-micro or nano-fibers that have high surface area to volume ratio (ranging from 5 to 100 m^2/g) are useful for fluid absorption and dermal delivery (Haung, 2003).

2.4.1.1.2 Drug Delivery System

The controlled drug delivery systems widely used to release therapeutic agents in proper periods to the site of action. Biodegradable or synthetic polymer used as the matrix to deliver and control therapeutic agents via diffusion and degradation of materials (Kenawy *et al.*, 2002). Examples include poly(lactide-co-glycolide) (PLGA) (Kim *et al.*, 2004), poly(L-lactic acid)(PLLA) fibers (Zeng *et al.*, 2003), Hydroxypropyl methylcellulose (HPMC) (Verreck *et al.*, 2003) and poly (ethylene-co-vinylacetate) (PEVA) (Kenawy *et al.*, 2002). Additionally, there are many of drugs that can be delivered for example; antibiotics, anticancer drugs, proteins, and DNA. Due to the advantages of electrospun fibers compared to cast films methods, the electrospun fibers can be mimic the initial burst

release of drugs. Moreover, the advantages of elctrospinning process over the melt processing are less effect with heat-sensitive drugs.

2.4.1.1.3 Wound Dressing

The electrospun nanofibers are widely used in wound dressing applications due to its high surface area, high porosity, and the ability of electrospun fibers to start signaling pathway. Additionally, the elcetrospun fibers are able to attach and proliferation of fibroblast to skin layer which can release therapeutic agents such as growth factor and cytokines to repair wound area. In 2004, Min *et al.* fabricated silk fibroin by using electrospinning process with diameters of 80 nm. The result concluded that silk fibroin electrospun fiber is a good candidate for wound dressing. Moreover, silk fibroin fibers can attach and proliferate in human keratinocytes (Rho *et al.*, 2006). A composite nanofibrous membrane composed of collagen and chitosan can induce cell attachment and proliferation compared to commercial guaze (Chen *et al.*, 2008).

2.4.1.1.4 Membranes

Membranes are play important role for health and environmental applications including membranes are routinely used for medical care and individual protection, such as wound dressing, dialysis, tissue engineering, and controlled release of drugs. Due to the increasing of population growth, membranes are also used for environmental cleaning and protection, such as filtration media for water purification (e.g., microfiltration, ultrafiltration, nanofiltration and reverse osmosis) and air filtration. There are different kinds of membrane materials, including ceramic, metal, and polymer and hybrid membranes containing both inorganic and organic components. The structures of these membranes can also be different, ranging from non-directional structures (e.g., bi-continuous spinodal and non-woven fibrous structures, or nonporous membranes by means of solution and diffusion mechanism) to directional structures (e.g., microphases in block copolymer and directional cavity in nanocomposites) at different length scales. The two most important properties of a membrane are selectivity and permeation rate. At times, these two properties seem to be at opposing ends, but recent studies indicate that

some membranes with high permeability can also retain good selectivity. Such advances can greatly improve the efficiency of separation leading to breakthroughs in performance and operations. Polyacrylonitrile (PAN) membrane is widely used for filter applications because it has excellent characteristics such thermal stability and tolerance to most solvents, atmosphere, bacteria and photo irradiation. Due to their excellent properties, PAN membrane has attracted much attention in many fields including water treatment, hemodialysis pervaporation and enzyme immobilization (Jindal, 1989).

2.4.2 Hydrogels

The hydrogel is a hydrophilic polymeric network chain of polymers that are water-soluble within its porous structure. The water holding capacity depends on the hydrophilic groups and crosslink density on the polymer chains. The hydrophilic groups are increased leading to the higher of water swelling behavior. If the crosslinking density is high, the swelling behavior will be increased. There are two kinds of hydrogels that occur from the crosslinking reaction which are permanent hydrogels (formation of covalent bonds within the polymer chain) and physical hydrogels (physical interactions in polymer chains) (Hoffman, 2002; Campoccia, 1998).

2.4.2.1 Biomedical Applications of Hydrogels

2.4.2.1.1 Applications of Hydrogels in Drug Delivery

In recent year, there has been used the hydrogels for the development of drug delivery. Diffusion refers to the movement of the molecules or particles along the region of higher to lower concentrations. Diffusion of the drug in the medium is attributed to the Brownian motion. The factor that affect in diffusion of the drug was pH, light, and release sensitivity the hydrogels.

2.4.2.1.2 Applications of Hydrogels in Wound Healing

Hydrogel is crosslinked polymer network structure which can be absorb water and has been most popular in recent years. Hydrogels can absorb and retain the wound exudates in the wound area that can keep the moisture in

the wound area leading to keratinocytes migrate on the surface. Normally, hydrogels should be transparent and give cooling effects in order to easy to handle and healing process. The ideal wound dressing of the hydrogels includes providing protection from further injury or infection, adsorbing fluids draining from the wound, and permitting movement of the body parts proximal to the wound.

2.4.2.3 Applications of Hydrogels in Tissue Engineering

Tissue engineering becomes more popular in bioengineering fields for tissue or organs substitutes. It is the combination of material, medical and biological sciences. There are three basic components namely, cells/tissues, scaffolds and implantation and/or grafting (Hoffman, 2002). The cells are often implanted into a scaffold for tissue reconstruction. A scaffold can be made from ceramic, metal or polymers. The porous should be more than 80 μm (Pal, 2008). The porous is one of the factors that necessary for migration and growth of the cells. There are many people victims of tissue loss and organ failure. Therefore, tissue engineering was used as the tool to substitute the damaged tissues and organs. Biodegradable polymers like poly(lactic acid), polyglycolic acid and their co-polymers are commonly used for tissue engineering.