CHAPTER II
LITERATURE REVIEW

2.1 Electrospinning Process

Electrospinning is a fabrication process that uses an electrical charge to from a mat of fine fibers. Electrospining is an interesting technique for producing nonwoven fibers with diameter ranging from micrometers down to nanometers. The standard setup for electrospinning consists of a high-voltage power supply, a container for polymer solution with a small opening to be used as a nozzle, a ground collector, and a syringe pump that is used to control the feed rate of polymer solution.

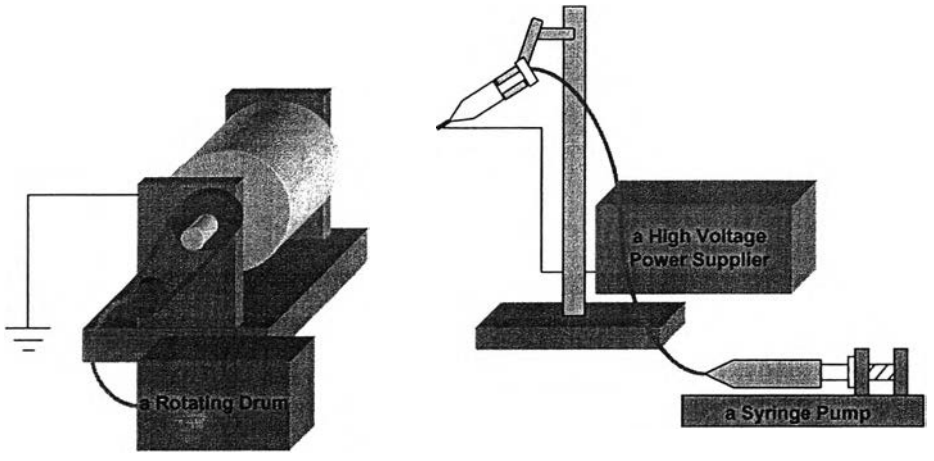

Figure 2.1 A schematic drawing of the electrospinning apparatus.

Polymer solution is passed through syringe feeder system being attached to a metal nozzle across a finite distance between the nozzle and ground collector. A polymer solution is driven to the needle tip by syringe pump, forming a droplet at the tip. When a positive electric potential is applied to the polymer solution, charge are accumulated on the surface of the pendant droplet of polymer solution at the tip of the nozzle. Upon increasing the applied electric field strength up to a critical value, the charges destabilize the partially-spherical shape of the droplet into a conical one.

Beyond a critical value, the electric force overcomes the surface tension of the drop of polymer solution, a jet is produced and travel through the air that solvent evaporates leaving polymer fiber to be collected on the ground collector.

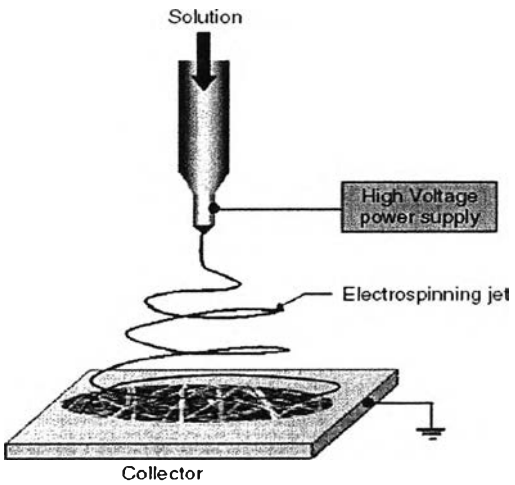

Figure 2.2 Electrospinning jet in electrospinning process.

The formation of fibers from this spinning process can be divided into two parts: 1) the initiation of the jet and 2) the continuous flow of the jet.

2.1.1 The Initiation of the Jet

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 the following equation:

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

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).

When the electric field is applied to the polymer solutions, Charges are accumulated on surface of the droplet. Charges that flow onto liquid surface repel

each other and when the repulsion force on the surface overcomes the surface tension, the polymer droplet becomes unstable. The conditions that are necessary for a charged surface to become unstable are described by the equation as following:

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

Where V_* is the critical potential, r is the droplet radius, and γ is the surface tension of the solutions (Koombhongse, 2001). As the potential is increased and reached the maximum instability of the liquid surface, a charged jet is ejected from the tip of the cone. Taylor, (1969) showed that the critical voltage (V_c) at which the maximum instability develops is given by this eqation:

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

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

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. Cycle of bending instability can be described in three steps (Reneker *et al.*, 2000).

Step1: A smooth segment that was straight or slightly curved suddenly developed an array of bends.

Step2: The segment of the jet in each bend elongated and the array of bends became a series of spiraling loops with growing diameters.

Step3: As the perimeter of the loops increased, the cross-sectional diameter of the jet forming the loop grew smaller; the conditions for step1were established on a smaller scale, and the next cycle of bending instability began.

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 *et al.*, 2001).

2.2 Electrospun Fibers in Tissue Engineering Applications

Over the past years, electrospun fibers are used to develop devices including filters, reinforcing fillers, fibrous scaffolds for tissue engineering, carriers for delivery of drugs, and so on. Tissue engineering of bone regeneration has received much attention as an alternative approach in the treatment of bone tissue defects. In this approach a biocompatible scaffold is required to support seeded cells adhesion to guide and promote controlled cellular growth and differentiation in order to generate new bone tissue. The challenge in tissue engineering is the design of scaffolds that can mimic the structure and functions of the natural extracellular matrix (ECM). ECM plays an important role in mechanical supporting and controlling cell behavior. ECM is composed of a ground substance (i.e. proteoglycan) and fibrous protein (i.e. collagen, elastin).

Hutmacher (2000) reviewed research on tissue engineering of bone and cartilage from the polymeric scaffold point of view. From this paper, it can be concluded that the ideal scaffold for tissue engineering bone and cartilage should have the following characteristics: i) three-dimensional and highly porous with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; ii) biocompatible with a controllable degradation rate to match cell/tissue growth in vitro and/or in vivo; iii) suitable surface chemistry for cell attachment, proliferation, and differentiation and iv) mechanical properties to match those of tissues at the site of implantation. It was found that a porosity greater than 90% is preferable for bone tissue scaffolds (Hu *et al.*, 2002) and the ideal range of pore diameters for bone scaffolds of 100-350 μm has been suggested (Hollinger *et al.*, 1996). By electrospinning process, the as-spun fibrous scaffolds meet the

requirement due to their three dimensional structure with interconnected pores and high porosity that resembles the collagen fiber microstructure in natural ECM.

Laurencin *et al.*, (1999) found that cells can attach and organize well around fibers with diameter smaller than the diameter of cells. Therefore, it is a concept to generate the template or scaffold in form of nanofibrous network which mimic the natural ECM and are preferable for cell attachment. Electrospinning is the well known method which nanofibers can be produced. The important advantages of e-spun fibers are the very high surface area-to-volume or mass ratio, high porosity of the e-spun mats that could promote better cell incorporation, and the morphology and size of the fibers that can be easily controlled. Recently, many research pay attention to fabricate e-spun nano-to-micro fibers for using as tissue scaffolds. Many research reported that cultured cells exhibited a normal phenotype with evidence of filopodia or microvilli on e-spun fibrous scaffolds. Fibrous substrates showed better cell attachment and proliferation than planar structure such as cast films (Bhattarai, 2005; Xu, 2004) and tissue polystyrene plate (TCPS) (Li *et al.*, 2005) and fibrous structure also provided higher uniformity of cells (Bhattarai *et al.*, 2005). It could be due to the greater surface area available for cell attachment and incorporation.

2.3 Poly(butylene succinate)

A variety of biocompatible materials have been investigated for their suitability in tissue engineering application. Almost all the biodegradable scaffolds used in tissue engineering have been made from biodegradable polymers. There are two kinds of biodegradable polymers: synthetic polymers and naturally derived polymers.

Synthetic polymers appeared about more than sixty years ago and medical people realized that this new class of materials was of interest for therapeutic applications. Since then, many polymers have been evaluated as candidate biomaterials. Among these, polyesters are one of the most promising polymers that are often used as tissue scaffolding materials due to their biodegradability and biocompatibility. Polyester biodegradable materials such as polycaprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(3-hydroxybutyrate) (PHB),

poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV), and poly(butylene succinate) (PBS) have attracted a great deal of attention. They can be used as bone scaffolding materials due to their potentially hydrolysable ester bonds and their slow degradation rate that are suitable for bone regeneration.

Poly(butylene succinate) (PBS) is one of biodegradable and biocompatible aliphatic polyesters produced through chemical synthesis based on polycondensation of 1,4-butanediol with succinic acid (Takiyama *et al.*,1994; Doi *et al.*,1996).

PBS is a semicrytalline polymer and flexible as found from the structure. The biodegradability of this polymer depends mainly on polymer structure and especially on the hydrolysable ester bonds in the main chain, which is susceptible to microbial attack. PBS has high melting point and it presents controllable biodegradation rate and high processibility, so can be processed in the field of textile into melt blown, multifilament, monofilament, nonwoven, and flat and split yarn. Due to its excellent biodegradability, melt processability, and thermal and chemical resistance, proposed applications are in areas such as textiles, medical treatment, and environmental engineering.

The mechanical properties are associated with its morphology and crystallinity as well as chemical structure. The mechanical properties such as elongation at break and tensile strength are comparable with those of polypropylene (PP) and low density polyethylene (LDPE) while its crystallization behavior is similar to that of polyethylene (PE) with well formed lamellar morphologies. The crystallization characteristics of PBS were studied by differential scanning calorimetry (DSC) and optical microscopy and were found that they depend closely on the cooling rate. (Miyata *et al.*,1998).

Miyata *et al.*,(1998) examined the crystallization characteristics of PBS films under various thermal conditions with respect to their crystallinity and spherulite morphology. In addition, the melting behavior of isothermally crystallized PBS has been investigated using DSC and wide-angle x-ray analysis by Yoo *et al.*,(1999).

There are many studies have been carried out in order to explain and improve the properties of aliphatic polyesters. (Bikiaris *et al.*, 1996; Kawaguchi *et al.*, 2001; Nikolic *et al.*, 2001). Ahn *et al.*,(2001) investigated the effect of

poly(butylene succinate adipate) (PBSA) copolymer composition on its physical and thermal properties. Aromatic monomer groups were incorporated into the main chain of aliphatic polyesters for improving their mechanical properties.

Jin *et al.*,(2000) introduced phenyl side chains to PBS and poly(ethylene adipate) (PEAd) while Nagata *et al.*,(2000) synthesized network copolyesters from adipic acid, ethylene glycol, and trimesic acid. Many studies on potential degradable aliphatic/aromatic copolyesters and blends based on PBS have also been reported.

It is known that PBS is soluble in chloroform, dichloromethane, o-chlorobenzene, etc. (Gan *et al.*, 2001). Unfortunately, they either have very low boiling temperatures for continuous electrospinning or are unsuitable for commercial electrospinning. Therefore, for the electrospinning of PBS, it is necessary to find solvents with an appropriated evaporation rate.

Jeong *et al.*,(2005) studied the electrospinning of PBS for the first time. In this work, the thermal and structural properties of ultrafine PBS fibers were also investigated. Liu *et al.*,(2007) investigated the morphology of PBS fibers fabricated by electrospinning under different weight concentration and needle-orifice diameters. Moreover, the thermal properties and crystallization of the electrospun PBS fibers were also characterized. Interestingly, PBS was first evaluated *in vitro* for its potential application as a novel material by Li *et al.*,(2005). The *in vitro* biocompatibility of PBS was evaluated by monitoring proliferation and differentiation of osteoblasts cells cultured on PBS films for different period. The result revealed that PBS was biocompatible as the cells could proliferate and differentiate on the PBS films. The biocompatibility of PBS indicated that PBS can be used as a biomaterial for tissue repair

2.4 Bone

Bone is dynamically meabolized connective tissue composed of calcified extracellular matrix and three cell types: 1) Osteocytes, which are found in cavities within the matrix; 2) Osteoblasts, which synthesize the organic components of the matrix; and 3) Osteoclasts, which are multinucleated giants cells involved in the resorption and remodeling of bone tissue (Junqueira *et al.*, 2003).

Bone plays an important role in storing calcium and phosphate in vertebrates. This tissue is maintained by the balance of bone formation and bone resorption (Nakamura, 2007).

Bone consists of 70% inorganic component, 20% organic component, and 10% water. Approximately 90% of organic content is type I collagen whereas the remaining 10% is several noncollagenous proteins (Garant *et al.*, 2003). The organic matrix has ground substance which contains proteoglycan aggregates and several specific structural glycoproteins. Bone glycoproteins may be responsible for promoting calcification of bone matrix (Junqueira *et al.*, 2003).

2.4.1 Bone Cells

Bone cells are classified into four types as following.

2.4.1.1 Osteoprogenitor Cells

Osteoprogenitor cells are spindle-shaped cells, derived from embryonic mesenchyme. Osteoprogenitor cells are capable of differentiating into osteoblasts (Gartner *et al.*, 1993).

2.4.1.2 Osteoblasts

Osteoblasts are generally round in shape and line on the surface of bone. They derived from osteoprogenitor cells which are engaged in bone formation. Some osteoblasts are gradually surrounded by newly formed matrix and become osteocytes. During this process, a space called a lacuna is formed. Osteoblasts become entrapped in lacunae but maintain contact with other cells via their cytoplasmic processes. Once this happens, the cells are known as osteocytes.

Osteoblast also produce cytokines including insulin-like growth factor I, II, transforming growth factor β(TGF-β), and bone morphogenetic proteins (BMPs) (Lian *et al.*, 1999). These growth factors are stored in calcified bone matrix and play an important role in differentiation and function of osteoblasts. Osteoblasts demonstrate intense alkaline phosphatase activity on their plasma membrane. This histochemical feature has been used for a marker of osteoblast-lineage cells (Nakamura, 2007).

2.4.1.3 Osteocytes

Osteocytes are considered to be the terminal differentiation stage of osteoblasts. They are embedded in osteocytic lacunae and are most abundant cells in bone tissue. Osteocytes possess extreamly large surface area because of numerous cytoplasmic processes (Nakamura, 2007).

2.4.1.4 Osteoclasts

Osteoclasts are derived from fusion of monocytes. They are mutinucleated giant cells responsible for bone resorbtion. Bone resorption takes place when osteoclasts secrete acid that creating an acidic environment and decalcifying the surface layer of bone followed by secretion of acid hydrolase, collagenase, and other proteolytic enzymes that degrade the organic portion of the bone (Gartner *et al.*, 1993). Osteoclasts are generally distinguished from other bone cells by their large size and mutiple nuclei.

2.4.2 Osteoblast-like Cell, SaOS-2

The SaOS-2, human osteosarcoma, cell line established from the primary osteogenic sarcoma of an 11-year-old Caucasian woman in 1973 (ATCC HTB 85). The SaOS2 cell line expresses a more limited number of osteoblast phenotypic markers when compared to MG63, another human osteosarcoma cell type, in which MG63 cell line can expresses type I collagen, ALP, osteopontin, bone sialoprotein, and osteocalcin (OC calcitriol dependant) (Bilezikien *et al.*, 2002).