# CHAPTER II
# LITERATURE REVIEW

## 2.1 Chitin and Chitosan

### 2.1.1 Source of Chitin and Chitosan

Chitin occurs in a wide variety of species, from fungi to the lower animals. Arthropod shells (exoskeletons) are the most easily accessible sources of chitin. These shells contain 20-50% chitin on dry weight basis. From a practical viewpoint, shells of crustaceans such as crabs and shrimps are conveniently available as wastes from seafood processing industries and are used for the commercial production of chitin. Other potential sources of chitin production include krill, crayfish, insects, clams, oysters, jellyfish, algae, and fungi. Krill is likely to be the most promising source in the future.

Squid pens also contain chitin that is classified as β-chitin. This material is distinguished from the ordinal α-chitin in the crustacean shells according to the difference in the crystalline structure. β-chitin has weaker intermolecular forces and is quite attractive as another form of chitin having some characteristics considerably different from those of α-chitin. The chemistry of β-chitin is rapidly advancing, although this starting material is less abundant and is not yet produced commercially. The cell walls of some fungi (Zygomycetes) contain chitosan as well as chitin and these may be used as sources of chitosan. Practically, however, chitosan is more easily prepared by the deacetylation of chitin.

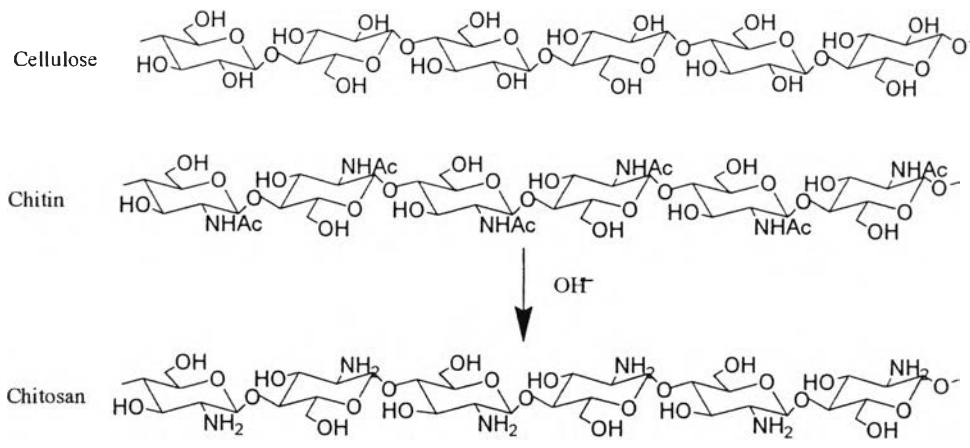



**Figure 2.1** Structure of cellulose, chitin, and chitosan.

2.1.2 Preparation of Chitin

α-chitin is produced commercially from crab and shrimp shells, which contain calcium carbonate and protein as the two other major components. Pigments are also contained in small quantities. Furthermore, the chitin molecules are assumed to have polypeptide side chains attached covalently to some of the C-2 amino groups through amide linkages. Chitin is the most stable substance against acid and alkali among these components and not is soluble in ordinary solvents. Accordingly, it can be isolated as a residue, which remains after decomposing the other substances present in the shell with acid and alkali.

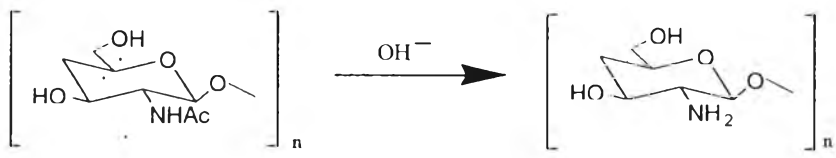
The shells are first cleaned and treated with diluted hydrochloric acid at room temperature to remove calcium carbonate. The decalcified shells are then cut into small flakes or are pulverized and heated in 1-2 mol/l sodium hydroxides near 100°C to decompose the proteins and pigments. Chitin is obtained as almost colorless to off-white powdery material. There are some free amino groups besides acetamide groups, and the degree of deacetylation for the isolated chitin is around 0.1. Proteases may be used to remove some of the proteins under mild conditions.

β-chitin can be isolated from squid pens in a similar but simpler manner, since squid pens are composed almost exclusively of chitin and proteins

with only trace amount of metal salts. Moreover, the molecular packing of β-chitin is less tight. Squid pens can be treated with hydrochloric acid and sodium hydroxide under mild condition to give β-chitin. When the isolated chitin is pulverized within an ultra-centrifugal mill, a white fluffy cotton-like material is obtained. During the isolation procedure, some of the acetyl groups are removed, and the resulting chitin has a degree of deacetylation near 0.1.

2.1.3 Preparation of Chitosan

Chitosan itself occurs in some fungi and can be isolated from their cell wall. It is formed by the action of the chitin deacetylase on the precursor chitin. Further detailed studies, however are necessary for the practical production of chitosan, although the feasibility of its preparation has been demonstrated.




Chitin Chitosan

**Figure 2.2** Schematic route for deacetylation of chitin.

Chitosan is commonly prepared by deacetylating α-chitin using 40-50% aqueous alkali at 100-160°C for a few hours (see Figure 2.2). The resulting chitosan has a degree of deacetylation up to 0.95. For complete deacetylation, the alkaline treatment can be repeated. Chitosans with various extents of deacetylation and grades are now commercially available. The deacetylation of β-chitin isolated from squid pens proceeds much more rapidly under similar conditions, but this results in the formation of heavily colored chitosan. Since β-chitin can be deacetylated at a much lower temperature than α-chitin, a reaction near 80°C is adequate for deacetylation as well as for the suppression of coloration processes, giving almost colorless chitosan products.

### 2.1.4 Applications and Properties of Chitosan in Biomedical Field

The main driving force in development of new applications for chitosan lies in the reason that this polysaccharide is not only naturally abundant, but it is also nontoxic, biocompatible, biodegradable, and easy for membrane casting. One of the most useful properties of chitosan is its possible use in biomedical applications.

#### *2.1.4.1 Biocompatibility and Biodegradability*

Chitin and chitosan are substrate for lysozyme, an enzyme found invarious mammalian tissues, and for chitinase and chitosanase, respectively. The enzymatic degradation of chitin and chitosan leads to the production of N-acetyl-D-glucosamine and D-glucosamine which play an important role in in vivo biochemical processs. Chitosan lacks irritant or allergic effect and is biocompatible with both healthy and infected human skin (Malette, 1986)

#### *2.1.4.2 Chitosan as Biomaterials for Wound Healing*

Because of the biocompatibility, exudate absorbability, and film forming properties of chitosan products, they are good candidates for burn and wound management uses (Hon, 1996). Chitosan may be used to inhibit fibroplasia in wound healing and promote tissue growth and differentiation in tissue culture. Chitosan also shows a biological aptitude to stimulate cell proliferation and histoarchitectural tissue organization (Porporatto, 2003).

#### *2.1.4.3 Chitosan as Matrix for Drug Delivery System*

As a pharmaceutical excipient, chitosan has been added for sustained release and to improve dissolution of poorly soluble drugs (Illum, 1998). Chitosan hydrochloride and chitosan glutamate are potent absorption enhances in acidic environments (Kotze, 1999).

#### *2.1.4.4 Chitosan as Scaffolds*

It is known that glycosaminoglycans are involved in cell-cell and cell-matrix interactions, and probably act as modulators of cell morphology, differentiation, movement, synthesis, and function (Muzzarelli, 1988; Hirano, 1989; Ma, 2001). Chitosan, having structural similarity to glucosaminoglycans, was used to fabricate scaffolds for tissue regeneration of various tissues (Kawase, 1997; Sechriest, 2000; Park, 2000; Marippan, 1999; Ma, 2001). It was found that chitosan-

based material could support cell adhesion, growth, differentiation, and function both in vitro and in vivo.

2.1.5 Limitation of Chitosan

Solubility is a very important criterion for the different uses of chitosan in any fields of applications. Although chitosan combined a number of useful properties, it was known to be insoluble in common solvents. Chitosan is insoluble in water, alkali and organic solvents but soluble in solutions of organic acids when the pH of the solution is less than 6. In this case the free amino groups become protonated to form cationic amino groups. Acetic and formic acid are two of most widely used solvent for dissolving chitosan. These solvents offer only very limited utility for chemical derivertizations in homogeneous solution. Poor solubility of chitosan in common organic solvents was due to its rigid crystalline structure through intra- and intermolecular hydrogen bonding.

2.1.6 Chemical Modification of Chitosan

Chitosan, being a high molecular weight biopolymer, is a linear polyelectrolyte whose reactive amino groups and primary and secondary hydroxyl groups are readily available for chemical reactions. The amino groups of chitosan can act as a nucleophile and undergo the reaction as the reactive primary amine while both hydroxyl groups of chitosan can react as the alcohols. Chitosan can undergo etherification, esterification, cross-linking and graft copolymerization reactions. Much emphasis has been paid on the chemical modification of chitin and chitosan in developing new useful materials from the viewpoint of the high potential of this amino polysaccharide. In many cases, the chemically modified chitosans show greater solubility than the original polymer.

*2.1.6.1 Water Soluble Derivatives*

In 1983, Tokura *et al.* prepared water soluble chitin derivatives which were carboxymethyl chitin and dihydroxy-propyl chitin. The initial alkylation sites were investigated (Tokura, 1983). The result showed that C-6 hydroxyl group of chitin was attacked predominantly.

N-alkylation of chitosan with various types of carbohydrates was reported by Yalpani and Hall (Yalpani, 1983). Specific attachment of carbohydrates to the amino functional groups of chitosan transformed this water

insoluble linear polymer into branched water-soluble derivatives. Facile conversion can be achieved by reductive alkylation using sodium cyanoborohydride and any aldehydo or keto sugar, by Schiff's base formation or amidation reaction using carboxylic acid or lactone derivatives. The synthetic chitosan derivatives exhibited a number of useful and specific properties in terms of their solution characteristics.

Water-soluble N-acylated chitosans were prepared via ring opening reactions with various cyclic anhydrides in aqueous in methanol system by Sashiwa and Shigemasa (Sashiwa, 1999). N-acylated chitosans were soluble in aqueous media at all pH regions except at the isoelectric point, varied from pH 3.5-7, of these derivatives. N-alkylation of chitosan was also performed in aqueous methanol with various aldehydes, monosaccharides and disaccharides. Most of these derivatives displayed solubility in water at all pH regions.

*2.1.6.2 Organo-Soluble Derivatives*

*N*-phthaloylchitosan was prepared by the reaction of chitosan with phthalic anhydride in DMF at 130°C (Nishimura, 1991). The resulting phthaloylchitosan exhibited improved solubility in common organic solvents such as DMF, *N,N*-dimethylacetamide, dimethyl sulfoxide, and pyridine. Since the conventional N-phthaloylation of chitosan in DMF was accompanied by partial phthaloylation of the hydroxyl groups, the addition of a small amount of hydroxyl-containing compounds effectively suppressed the O-phthaloylation (Kurita, 2001). The addition of water during synthesis gave the complete N-phthaloylated chitosan without any *O*-phthaloyl groups. This product exhibited some degree of crystallinity and could dissolve in *m*-cresol, dichloroacetic acid, *N,N*-dimethylacetamide/8%LiCl, and methanol/$CaCl_2$. The *N*-phthaloylchitosan could also be synthesized in reduced reaction time using microwave irradiation instead of conventional heating technique (Liu, 2004).

*2.1.6.3 Acylated Chitosan Derivatives*

In 1981, Kaifu *et al.* studied the hexanoylation, decanoylation and dodecanoylation of chitin by the acyl chloride-methane sulfonic acid method (Kaifu, 1981). O-hexanoylated chitin (DS=2) was successfully obtained by a standard procedure with 5 equivalent molar of hexanoyl chloride. The reaction proceeded more completely with shorter aliphatic chain of acyl chlorides due to the

smaller steric hindrance. The introduction of a long alkyl chain into chitin molecules resulted in the remarkable enhancement of the solubility of chitin in organic solvents. Highly substituted hexanoyl, decanoyl, and dodecanoyl chitins were soluble in benzene, m-cresol, tetrahydrofuran, methylene chloride, and dichloroacetic acid. Hexanoyl chitin was additional soluble in butanol, dioxane, acetic acid, ethyl acetate.

The N-acylated chitosans were prepared from chitosan with various carboxylic anhydredes e.g. acetic, propionic, n-butyric, n-valeric and n-hexanoic anhydrides, in methanol (Lee, 1995). In vitro blood compatibility tests of these derivatives revealed that N-acyl chitosans were more blood compatible than N-acetyl chitosan, and particularly, N-hexanoyl chitosan was the most blood compatible. Although the N-acyl chitosans showed blood compatibility, the N-hexanoyl chitosan was less than N-butyryl and pure chitosan in another reported (Lee, 2004). The differences in these results might come up with different evaluation methods being used. Fully substituted acyl chitosans were also synthesized (Zong, 2000). A series of acylated chitosans were prepared by reacting chitosan with corresponding acyl halides via a heterogeneous acylation reaction in $CHCl_3$/pyridine. The degree substitution was 4 per monosaccharide ring. These derivatives exhibited excellent solubility in organic solvents such as chloroform, benzene, pyridine, and THF. Zong *et al.* found that the reaction condition used did not alter the chain length of the original chitosan. Biodegradability of acyl chitosan was intensively studied (Lee, 1995; Hutadilok, 1995; Hirano, 2000; Muzzarelli, 2004). They found that the enzymatic degradation rate strongly depended on the acyl chain length and degree of substitution. The longer chain gave the lower degradation rate. Hirano *et al.* found that lysozyme could not hydrolyse fibers of *N*-acyl chitosan with the acyl chain longer than propionyl group. Muzzarelli *et al.* reported that dibutyryl chitin were not susceptible to degradation by lipase and lysozyme. Hirano and Yagi also found that chitinase did not hydrolyse N-acyl chitosan and lysozyme did not hydrolyse N-butyryl chitosan (Hirano, 1980). In contrary to those results, Lee *et al.* (1995) claimed that lysozyme could hydrolyse the N-acyl chitosans (up to $C_6$) with degree of substitution less than 0.5. Enzymatic degradation of fully substituted hexanoyl chitosan (DS=4) film were studied by Peesan *et al.* They found that hexanoyl

chitosan film was not degraded by neither lysozyme, lipase, nor proteinase (Peesan, 2007).

In 2003, Tien *et al.* studied N-acylated chitosans ($C_6$-$C_{16}$) as hydrophobic matrices for controlled drug release. They revealed that N-acylated chitosan, especially palmitoyl chitosan, can increase acetaminophen release time up to 90 hours (Tien, 2003). Microspheres of N-acylchitosans were prepared by water-in-oil interfacial N-acylation method for controlled release of 6-mercaptourine (6-MP) (Mi, 2005). The release tests showed that the introduction of long acyl chains onto chitosan not only increased hydrophobicity, but also interfered crystallization of the polymer chains, leading to affecting the swelling and drug release properties of prepared N-acylchitosan microspheres.

## 2.2 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:

2.2.1 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 $\gamma$ is the surface tension of the liquid of density $\rho$, 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 $\gamma$ 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 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 $\gamma$ is the surface tension.

### 2.2.2 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 2.3.

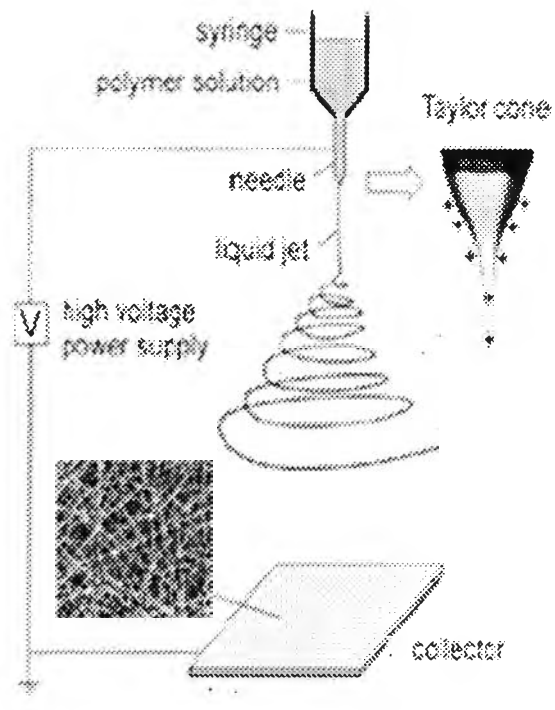



**Figure 2.3** Schematic drawing of the electrospinning process (Dan, 2004).

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).

2.2.3 Applications of Electrospun Fibers

Due to the high surface area to volume ratio, high porosity, and light weight of the electrospun fibrous mats, a number of applications have been sought out (Jayaraman, 2004).

*2.2.3.1 Filters*

Filtration is a necessary process in various engineering applications. Filtration efficiency or capture efficiency of filter media has been shown to be inversely proportional to the diameters of the fibers in filters. Because of the very high surface area-to-volume ratio and the resulting high surface cohesion of nanofibers, tiny particles on the order of less than 0.5 μm are easily trapped in the nanofiber mats.

*2.2.3.2 Protective Clothing*

Protective clothing for military personnel is expected to help maximize the survivability, sustainability, and combat effectiveness of soldiers against extreme weather conditions, ballistics, and nuclear, biological, and chemical warfare. So a lightweight, breathable fabric, permeable to both air and water vapor, insoluble in all solvents, and highly reactive to chemical agents, is desirable. Polymer nanofibers had been developed for various protective clothing applications. It was found that compared with conventional textiles, electrospun nanofiber mats provide minimum impedance to moisture vapor diffusion and maximum efficiency in trapping aerosol particles.

*2.2.3.3 Reinforcement in Composite Materials*

Publications on nanofiber-reinforced composite materials are limited in the literature because of the difficulty of producing these fibers. However, their higher surface-to-volume ratio may improve the interlaminar toughness and interfacial adhesion in nanofiber-reinforced composites.

*2.2.3.4 Sensors*

Polymer nanofibers have been used in the development of functional sensors possessing high sensitivity due to the high surface area of nanofibers. Polymer used in this applications were poly(lactic acid-*co*-glycolic acid) and poly(acrylic acid)-poly(pyrene methanol).

*2.2.3.5 Drug Delivery System*

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 buy biocompatible, can be used as delivery matrices, 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). 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.

*2.2.3.6 Scaffolds for Tissue Regeneration*

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. Due to similarity in the structure, electrospun fibers are easily found to be prospective materials to be used as templates for tissue scaffold applications, controlled release fibers for would dressing, pharmaceutical, and cosmetic applications. 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 fibers that are smaller than cellular size. 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 $m^2/g$) are efficient for fluid absorption and dermal delivery (Haung, 2003).

## 2.3 Tissue Engineering

Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences to the development of biological substitutes that restore, maintain, or improve tissue function (Langer, 1993). In general terms, the goal of tissue engineering is to develop materials and approaches which can be used to facilitate repair, regeneration, or replacement of damaged or diseased tissues.

2.3.1 Polymers for Tissue Engineering

At the most fundamental level, biocompatible polymers used in engineered implants can be broadly classified as either natural or synthetic. There are advantages and disadvantages associated with both types of materials. For example, natural polymers provide the clear advantage of possessing specific molecular recognition features which generally translate into specific biological activities. They are usually enzymatically degradable to nontoxic subunits which can be utilized by adjacent cells. The advantages of such natural materials include source-associated

variability and contamination; limited control over parameters such as molecular weight; and the potential for adverse immunological responses; variations in degradation rates due to differences in host enzyme levels. In contrast, synthetic polymers can be synthesized in pure form with complete control over molecular and physical properties. These polymers also usually permit a wider range of processing options. However, they generally lack intrinsic biological activity. The degradation products may be toxic or may drastically alter the local microenvironment (e.g. pH) (Matthew, 2002).

2.3.2 Materials for Scaffolding Applications

The tissue engineer typically seeks to restore tissue specific architecture, and the use of a scaffold provides the means to this end. In order to achieve restoration of tissue architecture, the tissue scaffold may be required to perform a variety of tasks. A porous micro structure which allows cellular in-growth and scaffold colonization is almost a universal requirement. Enhancements to the microstructure may include spatial variations in pore morphology to help orient cells or variations in material surface properties to facilitate selective cell adhesion and migration. In contrast, inhibition of cell adhesion may be an important performance characteristic for certain locations. The scaffold material may be required to deliver biologically active agents (for example, growth factors or genes) to target tissue. The material for scaffolding application should be biocompatible, non-toxic and should not release toxic substance to the surrounding. It should facilitate cell attachment, proliferation, and migration. The cells should retain phenotypic functions when associated with these materials.

2.3.3 Electrospun Fibrous Mats for Tissue Scaffolding Applications

As mammalian anchorage-dependent cells were found to attach and organize well around fibers with diameter smaller than the diameter of the cells themselves (Laurencin, 1999), the fibrous network composed of several fibers with small diameters produced from electrospinning processed emerged to have potential in tissue scaffolding application. The use of electrospun fibers 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 materials, as well as hybrid blends of the

two, which can provide an optimal combination of mechanical and biomimetic properties. By varying the processing and solution parameters the fiber orientation (aligned vs. random) and porosity/pore size of the electrospun scaffold can be controlled and optimized for each individual application. After fabrication the surface of the scaffold can be modified with a high density of bioactive molecules due to the relatively high scaffold surface area. Due to the flexibility in material selection as well as the ability to control the scaffold properties, electrospun scaffolds have been employed in a number of different tissue applications including: vascular, bone, neural, tendon/ligament, and skin.

In 2004, Xu et al. produced electrospun aligned fibrous scaffolds of poly(L-lactid-co-ε-caprolactone) [P(LLA-CL)] (75:25) copolymer and evaluated the possibility to develop the scaffold for blood vessel engineering. They found that human coronary artery smooth muscle cells (SMCs) attached and migrated along the axis of the aligned nanofibers, and expressed a spindle-like contractile phenotype. The distribution and organization of smooth muscle cytoskeleton proteins inside the cells were parallel to the direction of the nanofibers. The adhesion and proliferation rate of SMCs on the aligned nanofibrous scaffold was significantly improved than on the plane polymer films.

Another tissue for which electrospun nanofiber meshes have emerged as potential scaffolds is bone (Yoshimoto, 2003; Wutticharoenmongkol, 2007). Wutticharoenmongkol and her colleages fabricated polycaprolactone/hydroxyapatite (PCL/HAp) nanofiber nanocomposites via electrospinning process. The fibrous scaffold was tested with mouse calvaria-derived pre-osteoblastic cells, MC3T3-E1. Their obtained results revealed that the scaffold supported the attachment and growth of the cells cultured on the scaffold. In addition, the attached and proliferated cells showed high alkaline phosphatase activity and high expression of osteocalcin protein. The mineralization was extensively observed.

Electrospinning has also been investigated as a polymer processing technique for skin tissue engineering applications. The non-woven with random fiber orientation of the electrospun fibrous scaffold was necessary for skin tissue. In 2003, electrospinning of type I collagen in 1,1,1,3,3,3-hexafluoro-2-propanol to fabricate a biomimetic nanofibrous extracellular matrix for tissue engineering was investigated

by Rho et al. The as-spun 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. The crosslinked scaffold showed lower porosity but higher tensile strength than the uncrosslinked scaffold. With evalution with the cells in vitro and with open wound healing in rats, the groups found that the nanofibrous matrix was very effective as wound-healing accelerators in early-stage wound healing. Powell et al. studied the electrospun collagen scaffolds as a skin substitute in comparison with collagen scaffolds produced from freeze-drying technique. In vitro evaluation of freeze-dried collagen skin substitutes (FCSS) and electrospun collagen skin substitutes (ECSS) revealed no significant differences in cell proliferation, surface hydration, or cellular organization. Both groups exhibited excellent stratification with a continuous layer of basal keratinocytes present at the dermal–epidermal junction. After grafting to full thickness wounds in athymic mice, both skin substitutes had high rates of engraftment: 87.5% in the FCSS group and 100% in the ECSS group. Moreover, the electrosun scaffold could be used to fabricate skin substitutes with optimal cellular organization and can potentially reduce wound contraction compared to the freeze-dried scaffold (Powell, 2008).