CHAPTER II
REVIEW OF THEORETICAL BACKGROUND AND LITERATURES

2.1 Fundamental of Electrospinning Process and Their Challenges

Electrospinning or electrostatic spinning is an old fiber formation technique that has recently been rediscovered, use for fabricating continuous ultrafine fibers with diameters down to a few nanometers to form non-woven or alignment structure. This process involves the application of a strong electrostatic field across a conductive capillary attaching to a reservoir containing a polymer solution or melt and a screen collector. Upon increasing the electrostatic field strength up to a critical value, charges on the surface of a pendant drop destabilize its shape from partially spherical into conical shape known as the Taylor cone. Beyond a critical value at which the electrostatic field strength overcomes the surface tension of polymer solution or melt, a charged polymer jet is eventually ejected from the apex of the cone. The fiber jet undergoes an instability and elongation process, which allows the jet to become very long and thin. During the jet travelling, the solvent evaporates or solidifies to finally leave ultrafine fibers on the collector. For a typical electrospinning set-up, it requires; (1) a polymer reservoir attach with a capillary or needle, (2) a high voltage power supply, and (3) a screen collector as shown in Figure 2.1.

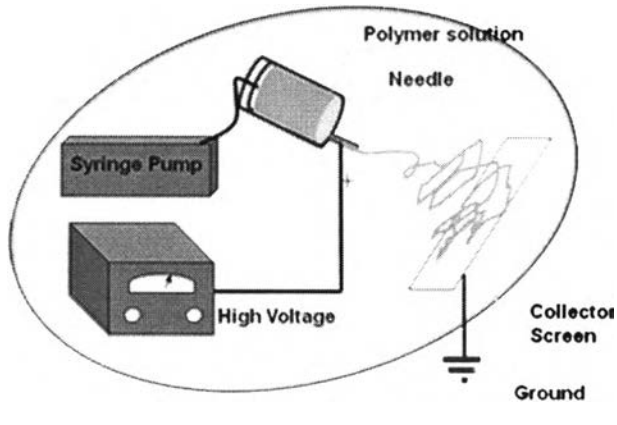

Figure 2.1 Schematic drawing of electrospinning apparatus.

Although conceptually a simple process, electrospinning has significant challenges. A number of parameters can greatly influence on the formation and structure of the obtained fibers. In principal, these parameters can be divided into 2 majors parameter include system parameters and process parameters. By appropriately varying all or some of these parameters, fibers are successfully electrospun. To understand the electrospinning process, the different parameters that affect the process have to be considered.

2.1.1 Process Parameters

Despite the electrospinning technique is relative ease of use; there are a number of process parameters that can greatly affect fiber formation and structure. Grouped in order of relative impact to the electrospinning process involves the applied voltage, polymer flow rate, and capillary-collector distance. All three parameters can influence on the formation of bead defect nanofibers.

2.1.1.1 Applied Voltage

The fiber diameters can be controlled by the applied voltage which the achieved results vary strongly with the polymer system. The strength of the applied electric field controls formation of fibers from several microns in diameter to tens of nanometers. Suboptimal field strength could lead to bead defects in the electrospun fibers or even failure in jet formation. Based on the various previous works (Deitzel *et al.*, 2001; Meechaisue *et al.*, 2006), it is evident that there is an optimal range of electric field strengths for a certain polymer/solvent system, as either too weak or too strong a field will lead to the formation of beaded fibers.

2.1.1.2 Polymer Flow Rate

Polymer flow rate also has an impact on fiber size, and additionally can influence fiber porosity as well as fiber shape (Still *et al.*, 2008; Taylor *et al.*, 1969; Megelski *et al.*, 2002). Additionally, at high flow rates significant amounts of bead defects were noticeable, due to the inability of fibers to dry completely before reaching the collector. Incomplete fiber drying also leads to the formation of ribbon-like (or flattened) fibers as compared to fibers with a circular cross section (Still *et al.*, 2008; Lannutti *et al.*, 2007).

2.1.1.3 Capillary to Collector Distance

While playing a much smaller role, the distance between capillary tip and collector can also influence fiber size. The fiber diameter decreased with increasing distances from the Taylor cone to the collector (Jaeger *et al.*, 1998). Additionally, morphological changes can occur upon decreasing the distance between the syringe needle and the substrate by forming beaded on as-spun fibers, which can be attributed to inadequate drying of the polymer fiber prior to reaching the collector (Megelski *et al.*, 2002).

2.1.2 System Parameters

In addition to the process parameters a number of system parameters play an important role in fiber formation and structure. System parameters involve molecular weight, molecular weight distribution, architecture of polymer and solution properties. Especially, the solution properties play an important role on the electrospinning process. In relative order of their impact on the electrospinning process these include polymer concentration, solvent volatility and solution conductivity.

2.1.2.1 Polymer Concentration

The polymer concentration influences both the viscosity and the surface tension of the solution. The formation of nanofibers through electrospinning is based on the uniaxial stretching of a viscoelastic solution. For instance, the polymer solution must have a concentration high enough to cause polymer entanglements. However, the viscosity dose not so high that prevents polymer motion induced by the electric field and not too be low to prevent the charged jet from collapsing into droplets before the solvent has evaporated (Venugopal *et al.*, 2005). Further investigations on polymer concentration and viscosity are observed that fibers become more uniform and assume a cylindrical shape with increasing polymer concentration in solution and fiber diameters also increase significantly with increasing polymer concentration. At lower concentrations, increasingly thinner fibers are formed with additional beads along the fiber axis. However, at very high dilution, the fiber formation no longer takes place (Greiner *et al.*, 2007). It is not possible to make a general recommendation for

particular concentrations and the resulting viscosities, because the ideal values of these parameters vary considerably with the polymer–solvent system.

2.1.2.2 Solvent Volatility

Choice of solvent is also critical as to whether fibers are capable of forming, as well as determining surface topography (Still *et al.*, 2008; Lannutti *et al.*, 2007). If the fluid jet is collected prior to complete solvent evaporation, the deformable fibers may either flatten upon impact with the surface of a collector or adhere to other fibers. If the arriving jet or fiber lands on previously collected fibers the still fluid material can merge and coalesce at crossing point to create interconnected network which appear to be useful in some situations where a well established network is desirable.

2.1.2.3 Solution Conductivity

Solution conductivity can influence fiber size within 1 to 2 orders of magnitude (Still *et al.*, 2008). Solutions with high conductivity will have a greater charge carrying capacity than solutions with low conductivity. Therefore, the fiber jet of highly conductive solutions will be subjected to a greater tensile force in the presence of an electric field than will a fiber jet from a solution with a low conductivity. The highly conductive solutions were extremely unstable in the presence of strong electric fields, which led to a dramatic bending instability as well as a broad diameter distribution (Hayati *et al.*, 1987). However, semi-conducting and insulating liquids such as paraffinic oil produced relatively stable fibers. By adding a small amount of salt or ion, the electric force exerted on the jet also increase and attributed to decrease in the mean fiber diameter. Generally, the radius of the fiber jet is inversely related to the cube root of the solution conductivity (Baumgarten *et al.*, 1971).

The above description of the process suggests that many parameters can influence on the electrospinning apparatus. By appropriately varying all or some of these parameters, fibers are successfully electrospun.

2.2 Biocompatible Polymers and Surface Functionalization for Enhancement Biological and Chemical Activity

The field of biomedical application often requires an interdisciplinary approach combining life science and medicine, materials science, and engineering. For a successful application, the material must be biocompatible, meaning that the ability of a material to perform with an appropriate host response in a specific application. Due to the complicate interaction between materials and biological system, there is no precise definition or accurate measurement of biocompatibility. Nevertheless, whether the materials will be accepted by a living body should be the criterion to evaluate the biocompatibility of materials (Chena *et al.*, 2008). The requirements of this response will vary from application to application; however, toxicity as well as inflammatory and possibly immune responses should typically be minimized. Initial studies in biocompatibility materials relied upon the potential use of bioinert substrates which attempted to reduce host specific interactions. While processes such as protein adsorption and the inflammatory response will occur to some degree with any implant, a bioinert material will form no or very little specific interactions with the surrounding environment, including the extracellular fluid or surrounding tissues. Since the interaction of materials with biological environment such protein, proteoglycan receptors on cell surfaces and biological molecules normally present in the extracellular matrix (ECM), through their interfaces are largely dependent on the surface chemistry and topography of the materials. Protein adsorption on the material surface is believed to be the initial state when a material contact with a biological environment. These mechanisms will influence the subsequent biological reactions including cell adhesion and proliferation. However, polymeric materials with different surface properties such as hydrophilicity or hydrophobicity, smooth or roughness surfaces and random or alignment direction may provide the different of cell response in vitro and in vivo. Therefore, understanding the influence of surface properties is critical, and control of protein–surface interactions continues to be an important factor for consideration in the design of biocompatible surfaces.

Recently, current research has been investigating the incorporation of bioactive materials which can intentionally interact with the biological environment and influence such things as cell function (Kim *et al.*, 2006). These interactions are often accomplished through surface modifications and functionalization with bioactive molecules such as extracellular proteins such as laminin fibronectin etc. In addition, the materials must exhibit suitable physical and mechanical properties closely matching the desired requirements such as modulus of elasticity, strength, structural integrity etc. need to match that of the neighboring tissue. The material selection plays a key role in the biomedical application. Synthetic polymers provide many advantages over natural polymers because they can be tailored to give a wider range of properties with predictable lot-to-lot uniformity and reliable source of raw materials. However, naturally occurring polymers normally exhibit better biocompatibility and low immunogenicity. Within the subdivision of synthetic materials, they can further divided into biodegradable and non-degradable materials. Biodegradable materials have been the more popular choice due to the elimination of a second surgery to remove the implanted scaffold (Yang *et al.*, 2005). It is imperative that the rate of degradation coincide with the rate of new tissue formation. If the rate of degradation is too slow then new tissue formation will be impeded; however, if the rate of degradation is too fast then the mechanical stability of the scaffold and developing tissue will be compromised. The rate of degradation can be controlled to some extent by altering parameters such as polymer blends (Kim *et al.*, 2003), and ratio of amorphous to crystalline segments (Still *et al.*, 2008). In order to more accurately mimic the natural ECM, research has also examined the electrospinning of natural materials such as: collagen (Zhong *et al.*, 2006), chitosan (Matsuda *et al.*, 2007), gelatin (Song *et al.*, 2007), fibrinogen (McManus *et al.*, 2007), chitin (Noh *et al.*, 2006), and hyaluronic acid (Ghosh *et al.*, 2006). However, these materials often lack the desired physical properties or are difficult to electrospin on their own, which has led to the development of hybrid materials, which consist of a blend of synthetic and natural materials (Stitzel *et al.*, 2006; Sui *et al.*, 2007).

2.3 Importance Aspect of Biocompatible Polymers in Tissue Engineering

As defined by Langer and Vacanti in 1993, tissue engineering is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" (Langer *et al.*, 1993). The importance aspect of tissue engineering has been the design of polymeric scaffolds with specific mechanical and biological properties similar to native extracellular matrix (ECM). The ECM is defined as any material that is known broadly as tissue but it is not part of a cell. The main components that make up the ECM are glycoproteins which the most abundant being collagens, proteoglycans, hyaluronic acid and other molecules depending on the specific tissue, such as fibrin, elastin, fibronectins, laminins, hydroxylapatite (HA), and even fluids such as serum and bound adhesive motifs (Han *et al.*, 2006). The ECM can also influence cellular function by the physical arrangement of the network of molecules that constitute it (Murugan *et al.*, 2007; Berthiaume *et al.*, 1996). Because of fibril arrangements are tissue specific, it has proven difficult to replicate the physical features of the ECM for regenerative medicine (Teo *et al.*, 2006). The complexities of temporal environment influence phenotypic and other cellular behavior by providing indirect and direct informational signaling cues (Behonick *et al.*, 2003). These interactions between cells and ECM can modulate cellular activities such as adhesion, migration, proliferation, differentiation and gene expression. Thus, the more closely the in vivo environment can be created, the more likely the successes of the tissue engineering scaffold (Le *et al.*, 2002; Mo *et al.*, 2004; Smith *et al.*, 2004).

The electrospun fibers mats function as temporary support for cells to regenerate new extra cellular matrix which has been destroyed by disease, injury or congenital defects. Although the desired characteristics of a scaffold vary slightly with native tissue, there are general properties that are desirable. First and foremost, the scaffold should be biocompatible, meaning that it will integrate with the host tissue without stimulating any immune response. The scaffold should also be porous to allow for cell attachment and in-growth, as well as exchange of nutrients during in vitro or in vivo culture (Liu *et al.*, 2004; Sharma *et al.*, 2004). Also, because the

scaffold acts as a temporary support for the cells to adhere and proliferate, it should mimic native ECM both architecturally and functionally (Liu *et al.*, 2004; Rosso *et al.*, 2005). In addition, a tissue engineering scaffold should be biodegradable so that a second surgery is not required to remove the implant. The rate of degradation should coincide with the rate of new tissue formation. For these properties, a number of requirements must be concerned involve types of materials, fiber orientation, porosity, surface modification and types of tissue that will be replaced. Types of materials such as natural or synthetic materials as well as polymer blend which can provide an optimal mechanical and biological property of fibrous scaffolds by varying the processing and solution parameters. For the fiber orientation, by using a stationary or rotating collector either randomly oriented or aligned fibers can be formed, respectively. The degree of anisotropy within an electrospun fibrous mat can greatly affect not only the mechanical properties but also cell adhesion, proliferation, and alignment. In many applications it is desirable to develop an aligned fibrous mats to replace highly oriented tissue such as the medial layer of a native artery of smooth muscle cells (Xu *et al.*, 2004) and axons and glial cells in peripheral nerve system (Schnell *et al.*, 2007). These experiments demonstrate the potential applications in which it is desirable to develop a nanofibrous scaffold with a high degree of anisotropy.

Depending on the system parameters and the process parameters a number of different pore sizes can be modulated. Pore size and the density of pores can also play an important role in the ability of migration and filtration of cells and macromolecules which can be used to control transport of nutrients and waste products. The pore size of an electrospun scaffold will essentially dictate whether it is viewed as a 2-dimensional mat or a 3-dimensional scaffold by cells depending on the application either might be desirable. Initially, small pore size was seen as a hindrance in many situations; however, it can actually serve as an advantage in applications where cell infiltration is unwanted such as skin and the endothelium. Due to their barrier property of electrospun scaffolds, the application in tissue engineered vascular grafts has grown significantly. An electrospun scaffold can provide superior endothelial cell attachment due to the large fraction of surface available for interacting with cells and its small pore can prevent smooth muscle cell

migration into the lumen of the vessel, while still allowing sufficient transport for nutrients and waste removal. However, small pores are not advantageous for all applications. In more 3-dimensional scaffolds the cells must be able to infiltrate deep into the scaffold, which requires pores of adequate size to allow for cell migration. Recently, the current researches attempt to develop electrospun scaffolds with smaller diameters in order to maximize surface area. Additionally, it is important to consider the importance of pore size and pore size distribution that allow for the use of scaffolds without limiting cell infiltration. The grater enhancement of cellular function can be achieved by attaching bioactive molecules to the surface of the as spun scaffolds. Various groups have examined the effects of attaching different bioactive molecules such as RGD peptides (Baumgarten *et al.*, 1971), gelatin (Ma *et al.*, 2005) and perlecan which is a natural heparan sulfate proteoglycan (Reneker *et al.*, 2002) etc.

The electrospinning of PCL solutions have been reported by Reneker et al. (Reneker *et al.*, 2002). They varied the PCL solution concentrations ranging between 14 and 18 wt.%. The instability in the elctrospinning of PCL results in the contact and merging of segments in different loops of the electrospinning jet to form garland-like fibrous structures. Yoshimoto et al. (Yoshimoto *et al.*, 2003) have been studied the potential of electrospun PCL fiber mats for using in bone tissue engineering. They found that the surfaces of the cell-polymer matrixes were covered with mesenchymal stem cells (MSCs) multilayers at 4 weeks. Mineralization and type I collagen were also observed at 4 weeks. The cardiac nanofibrous meshes (CNM) have been successfully prepared by electrospinning of PCL solutions in a 1:1 mixture of chloroform and methanol. They found that the cardiomyocytes attached well on the PCL meshes and expressed cardiac-specific proteins such as β-myosin heavy chain, connexin43 and cardiac troponin I (Shin *et al.*, 2004). In 2004, Li et al. (Li *et al.*, 2005) fabricated the electrospun PCL fiber mats to support in vitro chondrogenesis of MSCs. Since the level of MSC chondrogenesis in the electrospun fibe mats is enhanced compared to the cell pellet culture. They proposed that these fiber mats are the candidate bioactive carriers for MSC transplantation in tissue engineering based cartilage repair. Fujihara et al. (Fujihara *et al.*, 2005) fabricated polycaprolactone (PCL)/$CaCO_3$ composite nano-fibers with two different PCL to

$CaCO_3$ ratio (PCL:$CaCO_3$ = 75:25 wt% and 25:75 wt%) for use as the guided bone regeneration membranes. Erisken et al. (Erisken *et al.*, 2008) have been prepared functionally graded nanocomposite structure of PCL and β-tricalcium phosphate (TCP) by using a hybrid twin-screw extrusion electrospinning process. They found that the ability to incorporate the β-TCP nanoparticles into PCL nanofibers enabled the better mimicking of the compositional and structural characteristics of bone tissue particularly at the bone-cartilage interface (Erisken *et al.*, 2008). Li et al. (Li *et al.*, 2005) studied the ability of the electrospun PCL fiber mats to support and maintain multilineage differentiation of bone marrow-derived human mesenchymal stem cells (hMSCs) in vitro. hMSCs were seed onto these fiber mats and were induced to differentiate along adipogenic, chondrogenic, or osteogenic lineages by culturing in specific differentiated into the specified cell types. These results made the electrospun PCL fiber mats as a promising candidate scaffold for cell-based, multiphasic tissue engineering (Li *et al.*, 2005). A hybrid process incorporating direct polymer melt deposition (DPMD) and an electrospinning process was developed to fabricate a highly functionalized three-dimensional (3D) scaffolds with an open porous network, a controllable shape and a biocompatible nanofibrous inner architecture (Part *et al.*, 2008). Each microfibrous layer of the scaffold was built using the DPMD process with computer-aided design modeling data. Between the layers of the 3D structure, PCL/collagen nanofiber matrices were deposited via an electrospinning process. Chondrocytes were seeded and cultured for 10 days to evaluate the potential of these scaffolds for use as an extracellular matrix-like tissue engineering scaffold. The results showed that these scaffolds supported for cell adhesion and proliferation (Part *et al.*, 2008). Yang et al. (Yang *et al.*, 2008) developed a facile and efficient process to provide the electrospun PCL scaffold with a bone-like calcium phosphate (CaP) coating while maintaining its fibrous and porous structure. The biomimetic method including the plasma surface treatment was efficient to mineralize the electrospun PCL scaffolds with a layer of bone-like apatite. It was concluded that these scaffolds were the potential materials for use in bone tissue engineering.

Li et al. (Li *et al.*, 2006) fabricated silk fibroin fiber scaffolds containing bone morphogenetic protein 2 (BMP-2) and/or nanoparticles of hydroxyapatite

(nHAP) by electrospinning. These scaffolds were used for in vitro to study the bone formation from human bone marrow-derived mesenchymal stem cells (hMSCs). The results showed that the incorporation of BMP-2 and/or nHAP into silk fibroin scaffolds enhanced bone formation significantly and thus suggested that these scaffolds were potential candidates for bone tissue engineering. Chitosan has been considered as one of the most attractive natural polymers for bone tissue engineering due to its biocompatibility, biodegradability and excellent mechanical properties. Therefore, a biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan (HAp/CTS) was prepared by combining an in situ co-precipitation synthesis approach with an electrospinning process (Zhang *et al.*, 2008). The incorporation of HAp nanoparticles into chitosan nanofibrous scaffolds induced the bone forming ability as compared to pure chitosan scaffolds due to the excellent osteoconductivity of HAp. Moreover, a carboxymethyl chitin (CMC)/poly(vinyl alcohol) (PVA) fibrous scaffold was successfully prepared by electrospinning to use as a scaffold for tissue engineering (Shalumon *et al.*, 2009). The results showed that the CMC/PVA fibrous scaffold supported cell attachment and proliferation. Ren et al. (Ren *et al.*, 2010 fabricated gelatin/siloxane fibrous scaffolds by a sol-gel processing and electrospinning process to support the growth of bone marrow derived mesenchymal stem cells (BMSCs) for bone tissue engineering. Randomly-oriented and aligned poly(lactic-co-glycolic acid) (PLGA) and PLGA/gelatin biocomposite scoffolds were successfully prepared by electrospining for use in bone tissue engineering (Meng *et al.*, 2010. The results showed that the elongation of the osteoblast on the aligned nanofibrous scaffolds was parallel to the fiber arrangement and the cell number was similar to that of randomly-oriented scaffolds. The aligned nanofibrous scaffolds thus provide a beneficial approach for bone regeneration.

A bi-layered tubular scaffold composed of a stiff and oriented poly(lactic acid) (PLA) outside fibrous layer and a randomly oriented PCL fibrous inner layer (PLA/PCL) was fabricated a sequential multilayering electrospinning (ME) (Vaz *et al.*, 2005). The rotation speed of the collector was used to control the level of fiber orientation of each layer. The PLA/PCL bi-layered scaffolds were proved to support the attachment, spread and growth of mouse fibroblasts and human myofibroblasts. Therefore these scaffolds could be considered as a candidate scaffold for blood

vessel tissue engineering. Lee et al. (Lee *et al.*, 2008) fabricated the vascular scaffolds composed of PCL and collagen by electrospinning. The resulted showed that the PCL/collagen composite scaffolds are biocompatible, possess biomechanical properties that resist high degree of pressurized flow over long term up to 4 weeks, and provide a favorable environment that supports the growth of vascular cells. Since silk fibroin had unique mechanical property and good biocompatibility. Thus it was used in biomedical applications. Sato et al. (Sato *et al.*, 2010) prepared a small diameter graft made of a silk fibroin by electrospinning. Moreover, the electrospun silk fibroin graft was coated with a silk sponge in order to improve mechanical strength and water permeation. These results made the electrospun silk fibroin graft with the silk sponge was attractive for cardiovascular applications. In addition, the silk fibroin/collagen tubular scaffolds were successfully prepared by electrospinning by Zhou et al. (Zhou *et al.*, 2010) for use in vascular tissue engineering.

The electrospun nanofibrous scaffold of the novel poly(p-dioxanone-co-L-lactide)-block-poly(ethylene glycol) (PPDO/PLLA-b-PEG) copolymer was prepared by Bhattarai et al. (Bhattarai *et al.*, 2004). They studied cell proliferation, morphology of cell-matrix interaction with the electrospun nanofibrous matrix. The results showed that this scaffold supported to cell attachment and proliferation and thus was a candidate scaffold for skin tissue engineering. In 2006, Pan et al. (Pan *et al.*, 2006) studied the interaction between dermal fibroblasts and the electrospun dextran/poly lactide-co-glycolide (PLGA) scaffold such as viability, proliferation, attachment, migration, extracellular matrix deposition, cytoskeleton organization and the functional gene expression. The results showed that cells interacted favorably with the scaffold. The collagen gel assay results showed that gel contraction was enhanced by the presence of the scaffold. Therefore, the dextran/PLGA scaffold can be used in enhancing the healing of chronic or trauma wounds (Pan *et al.*, 2006). Noh et al. (Noh *et al.*, 2006) compared the cellular response to the electrospun chitin nanofibers (Chi-N) and commercial chitin microfibers (Chi-M) for potential use in wound dressing or tissue engineering applications. They found that high cell attachment and spreading of all cells tested were observed on Chi-N in comparison to Chi-M. The Chi-N treated with type I collagen also promoted the cellular response. These results made Chi-N scaffold was useful for wound healing and regeneration of

oral mucosa and skin. The electrospun haxanoyl chitosan scaffolds were prepared by Neamnark et al. (Neamnark *et al.*, 2008). They investigated the potential of these scaffolds for skin tissue engineering in terms of the attachment and the proliferation of human keratinocytes (HaCaT) and human foreskin fibroblasts (HFF). The cell cultured results showed that these scaffolds supported the cell attachment and the proliferation of both types of cells, especially for HaCaT. The electrospun poly(lactic acid-co-glycolic acid) (PLGA) fiber matrices of varying fiber diameters were studied to use in skin tissue engineering (Kumbar *et al.*, 2009). The results indicated the electrospun PLGA fiber matrixes with the diameter range of 350-1100 nm had the higher proliferation rate than the fiber matrices below and beyond this fiber diameter range. Lowery et al. (Lowery *et al.*, 2010) studied the effect of fiber diameter and pore size on cellular proliferation in tissue engineering scaffolds composed of electrospun poly(ε-caprolactone) (PCL). The results showed that the cells proliferated at a faster rate on scaffolds with peak pore diameters greater than 6 μm. Moreover, the cells bridged pores on the surface of a fiber mats with 6.5 μm pores, but extended along single fibers in a mat with more than 20 μm pores, showing a pore diameter effect on cell conformation.

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