Electrospinning of Biocompatible Polymers and Their Potentials in Biomedical Applications

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Abstract Electrospinning has been recognized as a versatile method for the fabrication of continuous ultrafine fibers using electrical forces. Various natural and synthetic polymers have been successfully electrospun into non-woven mats or oriented fibrous bundles with high porosity and large surface areas. Despite the numerous reports on the production of electrospun fibers, these fiber mats did not gain much interest for use in the biomedical field until the past decade. This review summarizes the research and development related to the electrospinning of some common biocompatible polymers as well as an overview of their potential in many biomedical applications such as tissue engineering, wound dressing, carriers for drug delivery or controlled release, and enzyme immobilization.

Keywords Biocompatible polymer · Delivery or release control · Electrospinning · Enzyme immobilization · Tissue engineering scaffold · Wound dressing

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Contents

1 Electrospinning for Biomedical Applications

Over the past couple of decades, one-dimensional nanostructured materials in the form of fibrous structures have received considerable attention because of their unique properties and highly versatile applicability. There are many practical techniques used for preparing such fibrous structures. Among them, electrospinning has been recognized as the simplest technique and can produce continuous ultrafine fibers from diverse materials, including polymers in solution or molten state. The diameters of these fibers range from a few micrometers down to tens of nanometers, a size range that is otherwise difficult to realize using conventional techniques. The smaller size of the individual fibers results in high ratios of surface area to volume or mass. Because of the simple tooling, the size and shape of the individual fibers, including the porosity of the obtained fiber mat, can be easily tuned. In addition, the chemical compositions of the fibrous matrices can be easily manipulated to achieve desired properties and functionalities. These advantages render electrospinning one of the most versatile fiber-fabrication techniques. In biomedical applications, it is popular because of the ease of fabrication of fibers from biocompatible materials, whether naturally derived or synthetic, and the ability to diversify the properties of the fibers through blending and surface functionalization. The ultrafine fibrous scaffolds produced by electrospinning have been demonstrated to be suitable substrates for promoting the adhesion, proliferation, and differentiation of various types of cultured cells [1–4]. As wound dressing materials, a myriad number of therapeutic agents such as drugs, antibiotic agents, proteins, and genes can be facilely incorporated within the electrospun fibrous framework [5–10]. The main purpose of such structures is not to serve as substrate for tissue regeneration, but as platforms in the delivery of the therapeutic agents.

Due to the high surface area to mass/volume ratio of the electrospun fibers, immobilization of enzymes and other biological catalysts can be done with ease [11–14] and the high porosity of the obtained fibrous membrane, with inter-connected porous network, allows the immobilized enzymes and catalysts to

maintain high activities [15]. Despite the wide applicability of electrospinning, the selection of suitable materials is based largely on the requirements of the intended use as well as on the intrinsic properties of the starting materials. Due to the simplicity of the technique, various natural and biocompatible synthetic polymers have been electrospun to satisfy different clinical needs. Some properties of the final fibrous products as submitted by the electrospinning process, such as wettability and mechanical integrity of the obtained fabrics, depend also on the size and morphology of the individual fibers and their organization in the fabrics. Because of the host of biocompatible materials available for selection and the adjustability of the electrospinning setup to achieve the required morphology of the obtained fibers, the physical, physico-chemical, and chemical properties of the electrospun matrices can be easily tailored. This review emphasizes some of the developments in the use of electrospun fibrous matrices (obtained from biocompatible polymers) as substrates for cell and tissue cultures, wound dressings, carriers for drug and gene delivery, and substrates for immobilization of enzymes.

2 Fundamentals of the Electrospinning Process and the Challenges

Electrospinning or electrostatic spinning is an established technique for fiber formation that has recently been rediscovered. It has the ability to fabricate continuous ultrafine fibers with diameters as small as few nanometers in the form of random or aligned non-woven fabrics. The process involves the application of a strong electric field across a conductive capillary, attached to a reservoir containing a polymer solution or melt, and a screen collector. Upon increasing the electric field strength up to a critical value, charges on the surface of a pendant drop destabilize the partially spherical droplet into a conical droplet, which is commonly known as the Taylor cone. Beyond a critical value at which the electric field strength overcomes the surface tension of the 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 causes the jet to become very long and thin. During the course of the trajectory of the jet, the solvent evaporates or solidifies, leaving ultrafine fibers on the collector. For a typical electrospinning setup, the major components are: (1) a polymer reservoir attached to a small capillary tube (e.g., a needle), (2) a high voltage power supply, and (3) a screen collector (Fig. 1).

Although conceptually a simple process, electrospinning involves some significant challenges. A number of parameters can greatly influence the formation and structure of the obtained fibers. In principal, these parameters can be divided into two major categories, namely system and process parameters. By appropriately adjusting all or some of these parameters, fibers with desired morphology can be obtained. To understand the electrospinning process, the different parameters that affect the process are briefly considered.

Fig. 1 Typical
electrospinning setup
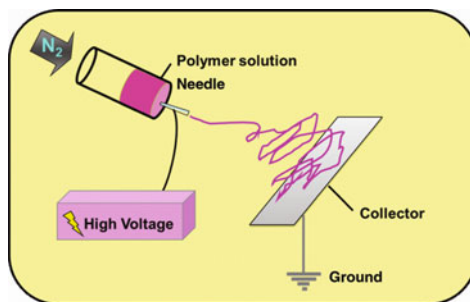
2.1 Process Parameters

Despite the fact that the electrospinning technique is relatively easy to use, there are
a number of process parameters that can greatly affect fiber formation and structure.
Listed in order of relative impact to the electrospinning process, the most important
parameters are applied voltage, polymer flow rate, and capillary–collector distance.
All three parameters can influence the formation of nanofibers with bead-like defects.

2.1.1 Applied Voltage

The fiber diameter can be controlled by the applied voltage, but the results vary
strongly with the polymer system. The strength of the applied electric field controls
the formation of fibers from several micrometers in diameter to tens of nanometers.
Suboptimal field strength can lead to bead defects in the electrospun fibers or even
failure in jet formation. Based on various previous works [16, 17], it is evident that
there is an optimal range of electric field for a certain polymer–solvent system,
because either too weak or too strong a field will lead to the formation of beaded fibers.

2.1.2 Polymer Flow Rate

Polymer flow rate also has an impact on fiber size and can influence fiber porosity
as well as fiber geometry [18–20]. At high flow rates, significant amounts of bead
defects can be observed, largely due to inadequate "drying" of the jet prior to
reaching the collector. Incomplete drying also leads to the formation of ribbon-like
(or flattened) fibers, because the subsequent drying of the core layer results in
collapse of the already-dried skin layer [18, 21].

2.1.3 Capillary–Collector Distance

Playing a much smaller role, the distance between capillary tip and collector can
also influence fiber size. The fiber diameter decreases as the distance from the

Taylor cone to the collector increases [22]. Morphological changes can also occur upon decreasing the distance between the capillary tip and the substrate through the formation of beads or the conglutination of adjacent fiber segments, which can be attributed to inadequate drying of the polymer fiber prior to reaching the collector [20].

2.2 System Parameters

In addition to the process parameters, a number of system parameters play an important role in fiber formation and the obtained structure. System parameters include molecular weight, molecular weight distribution, polymer architecture, and solution properties. Solution properties play a particularly important role. In relation to their impact on the electrospinning process, these factors can be ranked as follows: polymer concentration, solvent volatility, and solution conductivity.

2.2.1 Polymer Concentration

The polymer concentration influences both the viscosity and the surface tension of the solution. The formation of fibers through electrospinning is based on uniaxial stretching of the ejected, charged jet. At concentrations that are too low, the ejected jet does not have enough strength to withstand the electrical forces and hence is broken up into discrete beads [23, 24]. As the concentration increases, the morphology of the obtained products changes into fibers with beads, as more entanglements are formed. Further increase in the concentration finally results in the formation of smooth fibers. However, exceedingly high concentrations disrupt the flow of the solutions because the viscosities are exceedingly high [23, 24]. Within the optimal concentration range of the solutions, uniform fibers can be obtained, and their diameters increase with an increase in the concentration. Depending largely on the size of the fibers, smooth fibers with circular or ribbon-like cross-sections can be obtained. Nonetheless, the range within which morphologies would be observed depends on given polymer–solvent systems.

2.2.2 Solvent Volatility

Choice of solvent determines not only the formation ability of the fibers, but also their surface topographies [18, 21]. The very first criterion for choosing a solvent is based on whether it is a good, marginal, or poor solvent for a polymer (i.e., by comparing the solubility parameters of the solvent and the polymer). Once selected, the resulting polymer solution should not phase-separate during the course of the electrospinning. Because the boiling point of a liquid can be related to its ability to evaporate (i.e., the more relevant parameter is vapor pressure), the boiling point should not be so low that the resulting polymer solution would dry out during the

spinning, and hence cause clogging at the tip of the capillary. If the choosing of a solvent with relatively low boiling point is inevitable, a modifying liquid with relatively high boiling point can be added to adjust the evaporation rate of the resulting solvent mixture. Too high a boiling point would, however, cause other adverse effects, such as the flattening and/or conglutination of fibers or fiber segments on the collector. It should be noted that the choosing of a modifying liquid with relatively low boiling point is often intentional so as to impart certain topographies on the surface of the resulting fibers, as the low-boiling-point liquid would cause local phase-separation of the polymer solution at the liquid–air interface.

2.2.3 Solution Conductivity

Solution conductivity can influence fiber size within 1–2 orders of magnitude [18]. Apart from the solubility parameters and the boiling point of the solvent, the dielectric constant (i.e., the tendency of the molecules to be polarized by an external electric field) is another parameter that needs to be considered. The greater the dielectric constant of a solvent, the greater is the conductivity of the resulting solution. Often, it is necessary to add a modifying liquid with a high dielectric constant, an organic or inorganic salt, or a surfactant to improve the conductivity of the solutions. Solutions with high conductivities have greater charge-carrying capacities than solutions with lower conductivities. Therefore, the fiber jets produced from solutions with high conductivities are subjected to greater tensile forces in an electric field than are fiber jets from solutions with low conductivities. In the presence of a strong electric field, on the other hand, the highly conductive solutions are extremely unstable. This could lead to dramatic bending instabilities, and hence fibers with broad diameter distributions could be obtained [25]. It was suggested that the radius of the fiber jet is inversely related to the cube root of the solution conductivity [26].

The above description of the process suggests that many parameters can influence the morphologies of the resulting electrospun fibers. By appropriately varying all or some of these parameters, fibers with desired morphologies can be obtained.

3 Biocompatible Polymers and Surface Functionalization for Enhancement of Biological and Chemical Activity

The field of biomedical application often requires an interdisciplinary approach that combines the life sciences and medicine with materials science and engineering. For a successful application, the material must be biocompatible, meaning that the material has the ability to perform with an appropriate host response in a specific application. Due to the complicated interaction between materials and biological systems, there is no precise definition or accurate measurement of biocompatibility.

Nevertheless, whether a material is accepted by a living body should be the criterion for evaluating the biocompatibility of materials [27]. The requirements of this response vary from application to application; however, toxicity as well as inflammatory and possibly immune responses should typically be minimized. Initial studies on the biocompatibility of materials relied upon the potential use of bioinert substrates 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 forms no or very little specific interactions with the surrounding environment, including the extracellular fluid or surrounding tissues. The interaction of materials with biological molecules such as proteins, proteoglycan receptors on cell surfaces, and biological molecules normally present in the extracellular matrix (ECM) is largely dependent on the surface chemistry and topography of the materials. Protein adsorption on the material surface is believed to be the initial process when a material comes in contact with a biological environment. These interaction 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 rough surfaces, and random or aligned direction may provide different cell responses both in vitro and in vivo. Therefore, understanding the influence of surface properties is crucial, 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 that can intentionally interact with the biological environment and influence factors such as cell function [28]. These interactions are often accomplished through surface modifications and through functionalization with bioactive molecules such as extracellular proteins (laminin, fibronectin etc.). In addition, the materials must exhibit suitable physical and mechanical properties closely matching the desired requirements, i.e., the modulus of elasticity, strength, structural integrity etc. need to match those of the neighboring tissue. The material selection plays a key role in 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.

Synthetic materials can be divided into biodegradable and non-degradable materials. Biodegradable materials are the more popular choice due to the elimination of the need for a second surgical intervention to remove the implanted scaffold [4, 29–41]. It is imperative that the rate of degradation coincides 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 blend [35, 42] and ratio of amorphous to crystalline segments [18].

In order to more accurately mimic the natural ECM, research has also examined the electrospinning of natural materials such as collagen [43, 44], chitosan [45],

gelatin [46], fibrinogen [47, 48], chitin [49], and hyaluronic acid [50]. 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 consisting of a blend of synthetic and natural materials [50–59].

4 Important Aspects of Biocompatible Polymers in Some Applications

4.1 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" [60]. An important aspect of tissue engineering is the design of polymeric scaffolds with specific mechanical and biological properties similar to native ECM. The ECM is defined as any material that is known broadly as a tissue but is not part of a cell. The main components that make up the ECM are glycoproteins, the most abundant being collagens, proteoglycans, hyaluronic acid, and other molecules depending on the specific tissue, such as fibrin, elastin, fibronectins, laminins, hydroxyapatite (HAp), and even fluids such as serum and bound adhesive motifs [61]. The ECM can also influence cellular function by the physical arrangement of the network of molecules that constitute it [62, 63]. Because fibril arrangements are tissue-specific, it has proven difficult to replicate the physical features of the ECM for regenerative medicine [64]. The complexities of the temporal environment influence phenotypic and other cellular behaviors by providing indirect and direct informational signaling cues [65]. 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 is recreated, the more likely is the success of the tissue engineering scaffold [66–68].

Electrospun fibers mats function as temporary support for cells to regenerate the cellular matrix that has been destroyed by disease, injury, or congenital defects. Although the desired characteristics of a scaffold vary slightly according to the 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 for exchange of nutrients during in vitro or in vivo culture [69, 70]. 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 [69, 71]. In addition, a tissue engineering scaffold should be biodegradable so that a second surgical intervention 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 considered

that involve the type of material, fiber orientation, porosity, surface modification, and type of tissue that will be replaced.

Materials such as natural or synthetic materials as well as polymer blends can provide optimal mechanical and biological properties 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 mat to replace highly oriented tissue such as the medial layer of a native artery of smooth muscle cells [41] or the axons and glial cells in the peripheral nerve system [31]. 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 obtained. Pore size and the density of pores can also play an important role in the 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 two-dimensional (2D) mat or a three-dimensional (3D) 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 the barrier property of electrospun scaffolds, their 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. The small pore size can prevent smooth muscle cell migration into the lumen of the vessel, while still allowing sufficient transport of nutrients and waste removal. However, small pores are not advantageous for all applications. In 3D scaffolds, the cells must be able to infiltrate deep into the scaffold, which require pores of adequate size to allow for cell migration. Recently, researchers have attempted to develop electrospun scaffolds with smaller diameters in order to maximize the surface area. Additionally, it is important to consider the importance of pore size and pore size distribution to allow the use of scaffolds without limiting cell infiltration. Greater enhancement of cellular function can be achieved by attaching bioactive molecules to the surface of the spun scaffolds. Various groups have examined the effects of attaching different bioactive molecules such as RGD peptides [26], gelatin [72], and perlecan (a natural heparan sulfate proteoglycan) [73].

The electrospinning of poly(ε-caprolactone) (PCL) solutions have been reported by Reneker et al. [74]. They varied the PCL solution concentrations between 14 and 18 wt%. The instability in the electrospinning of PCL results in the contact and merging of segments in different loops of the electrospinning jet to form garland-like fibrous structures [74]. Yoshimoto et al. [75] have studied the potential of electrospun PCL fiber mats for use in bone tissue engineering. They found that the

surfaces of the cell–polymer matrixes were covered with mesenchymal stem cell (MSC) multilayers at 4 weeks. Mineralization and type I collagen formation were also observed at 4 weeks [75]. Cardiac nanofibrous meshes 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 α-myosin heavy chain, connexin 43 and cardiac troponin I [76]. In 2004, Li et al. [77] fabricated electrospun PCL fiber mats to support in vitro chondrogenesis of MSCs. Since the level of MSC chondrogenesis in the electrospun fiber mats was enhanced compared to the cell pellet culture, they proposed that these fiber mats are candidate bioactive carriers for MSC transplantation in tissue engineering-based cartilage repair. Fujihara et al. [78] fabricated PCL/CaCO$_3$ composite nanofibers with two different PCL to CaCO$_3$ ratios (PCL:CaCO$_3$ of 75:25 and 25:75 wt%) for use as guided bone regeneration membranes. Erisken et al. [79] prepared functionally graded nanocomposite structures of PCL and β-tricalcium phosphate (β-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 better mimicking of the compositional and structural characteristics of bone tissue, particularly at the bone–cartilage interface [79]. Li et al. [80] studied the ability of the electrospun PCL fiber mats to support and maintain multilineage differentiation of bone marrow-derived human MSCs (hMSCs) in vitro. hMSCs were seeded onto these fiber mats and were induced to differentiate along adipogenic, chondrogenic, or osteogenic lineages by specifically differentiating the specialized cell types. These results indicated that these electrospun PCL fiber mats are promising candidate scaffolds for cell-based, multiphasic tissue engineering [80]. A hybrid process incorporating direct polymer melt deposition (DPMD) and an electrospinning process was developed to fabricate a highly functionalized 3D scaffold with an open porous network, a controllable shape, and a biocompatible nanofibrous inner architecture [81]. 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 ECM-like tissue engineering scaffold. The results showed that these scaffolds supported cell adhesion and proliferation [81]. Yang et al. [82] developed a facile and efficient process to provide the electrospun PCL scaffold with a bone-like calcium phosphate coating while maintaining its fibrous and porous structure. The biomimetic method including the plasma surface treatment was efficient at mineralizing the electrospun PCL scaffolds with a layer of bone-like apatite. It was concluded that these scaffolds can serve as potential materials for use in bone tissue engineering.

Li et al. [83] fabricated silk fibroin fiber scaffolds containing bone morphogenetic protein 2 (BMP-2) and/or nanoparticles of hydroxyapatite (nHAp) by electrospinning. These scaffolds were used in vitro to study bone formation from 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 [83].

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. A chitosan/PCL nanofibrous scaffold was prepared by a single-step electrospinning technique. The presence of chitosan in the scaffold enhanced the hydrophilicity, bioactivity, and protein adsorption on the scaffold. The cytocompatibility of the chitosan/PCL scaffold was examined using human osteoscarcoma cells (MG63) and was found to be nontoxic and support the attachment and proliferation of various cell lines such as mouse embryo fibroblasts (NIH3T3), murine aneuploid fibro sarcoma (L929), and MG63 cells. These studies demonstrate that a chitosan/PCL nanofibrous scaffold would be useful for bone and skin tissue engineering [84].

Bhattarai et al. [85] reported chitosan/poly(ethylene oxide) (PEO) nanofibrous scaffolds that promoted the attachment of human osteoblasts and chondrocytes and maintained characteristic cell morphology and viability throughout the period of study. This nanofibrous matrix is of particular interest in tissue engineering for controlled drug release and tissue remodeling. Similarly, Subramanian et al. [86] prepared chitosan/PEO nanofibers for cartilage tissue engineering. Chitosan/PEO nanofibers were biocompatible to chondrocytes. Cells attached to the chitosan/PEO nanofiber mats, and the results indicated that the electrospun chitosan/PEO mats could be used for cartilage tissue repair. Mo et al. [87] also reported that smooth muscle cells attached to the electrospun chitosan/collagen nanofibers used for tissue engineering applications. Biocomposite nanofibrous scaffolds were prepared by using chitosan/poly(vinyl alcohol) (PVA) and N-carboxyethyl chitosan (N-CECS)/PVA for tissue engineering applications [88]. The cell attachment of the prepared biocomposite nanofibers was studied using mouse fibroblast (L929) cells. The L929 cell culture revealed the attachment and growth of mouse fibroblasts on the surface of biocomposite scaffolds. Similarly, the potential use of the N-CECS/PVA electrospun fiber mats as scaffolding materials for skin regeneration was evaluated in vitro using L929 [89]. Indirect cytotoxicity assessment of the fiber mats indicated that the N-CECS/PVA electrospun mat was nontoxic to the L929 cells. Cell culture results showed that fibrous mats were good at promoting cell attachment and proliferation. This electrospun matrix could be used as potential wound dressing for skin regeneration.

Biomimetic nanocomposite nanofibers of HAp/chitosan were prepared by combining an in situ co-precipitation synthesis approach with an electrospinning process [90]. The incorporation of HAp nanoparticles into chitosan nanofibrous scaffolds induced bone-forming ability as compared to pure chitosan scaffolds due to the excellent osteoconductivity of HAp. Moreover, a carboxymethyl chitin (CMC)/PVA fibrous scaffold was successfully prepared by electrospinning for use as a scaffold for tissue engineering [91]. The results showed that the CMC/PVA fibrous scaffold supported cell attachment and proliferation. Ren et al. [92] fabricated gelatin/siloxane fibrous scaffolds by sol–gel processing and electrospinning to support the growth of hMSCs for bone tissue engineering. Randomly

oriented and aligned poly(lactic-*co*-glycolic acid) (PLGA) and PLGA/gelatin biocomposite scaffolds were successfully prepared by electrospinning for use in bone tissue engineering [93]. The results showed that the elongation of the osteoblast on the aligned nanofibrous scaffolds was parallel to the fiber arrangement, and that the cell number was similar to that of randomly oriented scaffolds. The aligned nanofibrous scaffolds thus provide a beneficial approach for bone regeneration.

A bilayered tubular scaffold composed of a stiff and oriented poly(lactic acid) (PLA) outer fibrous layer and a randomly oriented PCL fibrous inner layer (PLA/PCL) was fabricated through sequential multilayering electrospinning [94]. The rotation speed of the collector was used to control the level of fiber orientation of each layer. The PLA/PCL bilayered scaffolds proved to support the attachment, spread, and growth of mouse fibroblasts and human myofibroblasts. Therefore, these scaffolds could be considered as a candidate scaffolds for blood vessel tissue engineering [94]. Lee et al. [95] fabricated vascular scaffolds composed of PCL and collagen by electrospinning. The results showed that the PCL/collagen composite scaffolds are biocompatible, possess biomechanical properties that resist a high degree of pressurized flow for up to 4 weeks, and provide a favorable environment that supports the growth of vascular cells. Since silk fibroin has unique mechanical properties and good biocompatibility, it is used in biomedical applications. Sato et al. [96] prepared a small diameter graft made of 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 showed that the electrospun silk fibroin graft with the silk sponge is attractive for cardiovascular applications. In addition, silk fibroin/collagen tubular scaffolds were successfully prepared by electrospinning by Zhou et al. [97] for use in vascular tissue engineering.

An 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. [98]. They studied cell proliferation and the morphology of cell–matrix interaction with the electrospun nanofibrous matrix. The results showed that this scaffold supported cell attachment and proliferation and thus was a candidate scaffold for skin tissue engineering. In 2006, Pan et al. [99] studied the interaction between dermal fibroblasts and an electrospun dextran/PLGA scaffold using cell viability, proliferation, attachment, migration, ECM deposition, cytoskeleton organization, and 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 [99]. Noh et al. [49] compared the cellular response of the electrospun chitin nanofibers (Chi-N) and commercial chitin microfibers (Chi-M) for potential use in wound healing or tissue engineering applications. Good cell attachment and spreading of all cell types was observed on Chi-N in comparison to Chi-M. The Chi-N treated with type I collagen also promoted the cellular response. These results indicated that Chi-N scaffold was useful for wound healing and regeneration of oral mucosa and skin [49]. Electrospun hexanoyl chitosan scaffolds were prepared by Neamnark et al. [100]. The authors investigated the potential of these scaffolds

for skin tissue engineering in terms of attachment and proliferation of human keratinocytes (HaCaT cells) and human foreskin fibroblasts (HFF). The results showed that these scaffolds supported cell attachment and proliferation of both types of cells, especially HaCaT. Electrospun PLGA fiber matrices of varying fiber diameters were studied for use in skin tissue engineering [101]. The results indicated that the electrospun PLGA fiber matrixes with a diameter range of 350–1,100 nm had a higher proliferation rate than the fiber matrices below and beyond this fiber diameter range. Lowery et al. [102] studied the effect of fiber diameter and pore size on cellular proliferation in tissue engineering scaffolds composed of electrospun 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 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 [102].

4.2 Controlled Drug and Gene Delivery

Controlled drug or gene delivery and tissue engineering are closely related areas. Sometimes, release of therapeutic agent can be combined with implantable scaffolds to increase the efficiency of tissue regeneration. Electrospun fibers provide the advantage of increased drug release as compared to cast films due to the high surface area to volume ratio [103]. Due to the great flexibility in material selection, several biocompatible polymeric materials, either biodegradable or non-biodegradable, have been electrospun to produce the fiber matrices for drug or bioactive agent delivery [18]. A number of therapeutic agents including biological active agents (i.e., anticancer drugs, anti-inflammatory drugs, antibiotics, and proteins), herbal extracts, and genes (i.e., DNA) can be loaded and delivered to the desired targets. When selecting a material to be used as a drug delivery device, a number of requirements must be considered. First, the material should protect the drugs from decomposition in the blood stream and, second, it should undergo biodegradation, which eliminates the need for explantation. Third, it should allow controlled release of the therapeutic agent over a time period with a constant release rate and continue as long as necessary for the treatment. It should also ensure that only the drug is released in the targeted tissue.

Materials that undergo biodegradation are generally more popular; however, these materials add an extra level of complexity to drug release as compared to non-degradable materials. Release of the therapeutic molecules can be controlled via diffusion alone or via both diffusion and material degradation. For non-degradable materials, the release profile tends to be primarily controlled by diffusion. In biodegradable systems, the drug may be released by diffusion or by degradation, which can lead to overdosing and local drug concentrations reaching toxic levels. Thus, special care must be taken to tailor both the release rate and the degradation rate if a degradable material is to be used [18].

The drug release profile is dependent on the dispersion of drug in the polymer matrix. Conventionally, the drugs are dissolved or dispersed in the polymer solution before being electrospun into fiber mats, which results in the presence of drug on the fiber surface as well as being encapsulated inside. This can lead to burst release in the initial stages. An alternative method of encapsulating the therapeutic agents inside polymeric nanofibers is by coaxial electrospinning, an advanced electrospinning technique for fabricating core–shell structures [18]. This advanced technique has the advantages of high loading efficiency and controllable release behavior over conventional methods of encapsulating bioactive agents. In coaxial electrospinning, the burst release effect can be suppressed but not eliminated completely. In order to overcome the burst release effect, an extra, protective coating on the drug reservoir of the electrospun fibers has been used. Recently, two-stream electrospinning has been developed to fabricate fibrous composite sheets wherein one component stream provides antibiotic release while the other provides mechanical properties for the desired application.

Zeng et al. [104, 105] used electrospun poly(L-lactic acid) (PLLA) fiber mats as drug carriers. In 2003 [104], the authors studied the influence of surfactants and drugs on the diameter and uniformity of electrospun PLLA fibers. Various types of surfactants, including anionic and cationic surfactants, were used to improve the size distribution of the PLLA fiber diameters. Moreover, various types of drugs including rifampin (a drug for tuberculosis), paclitaxel (PTX, an anticancer drug), and doxorubicin hydrochloride (Dox, an anticancer drug) were also investigated. The results showed that the drugs were encapsulated inside the fibers, and that drug release in the presence of proteinase K followed nearly zero-order kinetics due to the degradation of PLLA fibers. In 2005, Zeng et al. [105] studied the influence of solubility and compatibility of drugs in the drug–polymer system. They also used PLLA fiber mats as carriers to incorporate various types of drugs including the anticancer drugs PTX and Dox. The results showed that there was good compatibility of PTX and Dox with PLLA, whereas Dox was found on the surface of PLLA fibers, resulting in the burst release. Therefore, the compatibility of the drugs in the drug–polymer system is an important factor for controlled release in a drug delivery system. The electrospun amphiphilic PEG-PLLA diblock copolymer fiber mats containing Dox were successfully prepared using water-in-oil emulsion electrospinning [106]. The results showed that the sustained release of Dox was observed for emulsion-electrospun fiber mats in comparison with the suspension-electrospun fiber mats. Both hydrophobic and hydrophilic drugs, PTX and Dox, were also successfully loaded in PEG-PLLA fiber mats by emulsion-electrospinning [107]. The solubility and distribution of both drugs in the drug–polymer system were affected by the release behaviors. Due to the more hydrophilic nature of Dox compared with PTX, Dox was easier to diffuse into buffer solution, leading to a higher release rate. Moreover, the release rate of PTX was accelerated by Dox's release from the same drug-loaded fiber mats, but the release rate of Dox was not affected by PTX. Moreover, PTX-loaded PLGA materials in the form of microfibers, discs, and sheets were prepared by electrospinning [108].

Jiang et al. [5] developed ibuprofen-loaded electrospun PEG-*g*-chitosan with PLGA for controlled drug delivery applications. The presence of PEG-*g*-chitosan significantly moderated the burst release rate of ibuprofen from the electrospun PLGA membranes. Moreover, in this study, ibuprofen was conjugated to the side chains of PEG-*g*-chitosan for prolonged release of more than 2 weeks. These results indicated that chitosan nanofibers could be useful for controlled drug delivery applications.

Kenawy et al. [109] studied the release characteristics of electrospun PVA fiber mats made from partially and fully hydrolyzed PVA containing ketoprofen at a concentration of 5 wt%. Moreover, they studied the stabilization of these fiber mats by treatment with methanol for 1 and 24 h. The authors found that the burst release of ketoprofen was eliminated by the treatment with methanol. Moreover, the higher temperature of the medium showed higher release rates. The degree of hydrolysis of PVA also affected the release rate of ketoprofen. The incorporation of non-steroidal anti-inflammatory drugs (NSAIDs) into electrospun cellulose acetate (CA) fiber mats was investigated by Tungprapa et al. [110]. Four different types of NSAIDs with varying water solubility (naproxen, indomethacin, ibuprofen, and sulindac) at a fixed concentration of 20 wt% based on the weight of CA were used to investigate the release characteristics in acetate buffer solution (pH 5.5) at 37°C. The ability of the drug to release from the polymer matrix depends on many factors such as solubility of the drug in polymer matrix, the solubility of drug in the medium, swelling and solubility of the polymer matrix in the medium, and the diffusion of the drug from the polymer matrix to the medium [110]. Electrospun poly(D,L-lactide) (PDLLA) fiber mats incorporating paracetamol at different loadings (2, 5, and 8 wt%) were fabricated by Cui et al. [111]. The authors studied the drug release characteristics and degradation of fiber mats as a function of fiber characteristics. The effect of different diameters and drug contents on the drug release characteristics and degradation of fiber mats was also studied. The results demonstrated that the release characteristics of paracetamol from these fiber mats showed an initial burst release or steady release phase, followed by a plateau or gradual release. The authors concluded that the drug release behavior from fiber mats can be controlled by fiber size and drug content. Peng et al. [112] also used paracetamol as a model drug. They prepared electrospun PEG-*co*-PDLLA fiber mats with different amounts of PEG (0–20 wt%) and containing paracetamol at a fixed concentration of 5 wt%. The results showed that the drug release rate increased with decreasing PEG content. Moreover, the drug burst release behavior was mainly related to the drug–polymer compatibility, and the sustained release phase depended on polymer degradation. Metoxicam-loaded PVA fiber mats were prepared by electrospinning [113]. The amount of metoxicam loaded in PVA solution was 2.5, 5, 10, and 20 wt% based on the weight of PVA. Comparisons were made against films. The results showed that all the metoxicam-loaded PVA fiber mats exhibited a higher amount of metoxicam permeation than the corresponding films. This could be due to the highly porous structure of the fiber mats that contributed to higher swelling in an aqueous medium.

Kenawy et al. [114] used electrospun fiber mats prepared either from PLA, poly (ethylene-*co*-vinyl acetate) (PEVA), or from a 50:50 blend of the two as carriers to

deliver tetracycline hydrochloride. The release characteristics from the electrospun fiber mats were compared to a commercially available drug delivery system (Actisite) as well as to the films. The electrospinning process was used to fabricate biodegradable amorphous PDLA and semi-crystalline PLLA fiber mats for biomedical applications [115]. The antibiotic drug (mefoxin) was incorporated in these fiber mats to study the release behaviors. The results showed that the burst release of mefoxin was observed within the first 3 h, which is an ideal drug release profile for the prevention of post-operation-induced adhesion, because most infections occur within the first few hours after surgery. The highly water-insoluble drugs itraconazole and ketanserin were incorporated into electrospun segment polyurethane (PU) fiber mats for use in topical drug administration and wound healing [116]. The results showed that both itraconazole and ketanserin were released from the water-insoluble PU fiber mats into an aqueous solution. Moreover, ketanserin was released more rapidly from the PU fiber mats within the first 4 h than itraconazole. The cellulose acetate (CA) fiber mats were used as carriers to deliver the model vitamins, all-*trans* retinoic acid (vitamin A acid) and α-tocopherol (vitamin E) [117]. The release characteristics of these vitamin-loaded CA fiber mats and films were investigated by immersion in acetate buffer solution containing either 0.5 vol% Tween 80 or 0.5 vol% Tween 80 and 10 vol% methanol. The results showed that the vitamin-loaded CA fiber mats exhibited a gradual release over the time periods, whereas the vitamin-loaded CA films exhibited a burst release of the vitamins. PLGA fiber mats were also used as carriers to incorporate all-*trans* retinoic acid [118]. Comparisons were made against the PLGA films. The results showed that the release rate of the vitamin from the PLGA fiber mats exhibited a sustained controlled release and that the PLGA fiber mats preserved their fibrous structure for 4 months.

The encapsulation of human β-nerve growth factor (NGF), which was stabilized in the carrier protein bovine serum albumin (BSA), in a copolymer of ε-caprolactone and ethyl ethylene phosphate (PCLEEP) was achieved by electrospinning [39]. A sustained release of NGF from PCLEEP fiber mats was obtained for up to 3 months. PC12 neurite outgrowth assay also confirmed that the bioactivity of NGF-loaded PCLEEP fiber mats was retained throughout the period of sustained release. Therefore, these fiber mats had the efficiency for use in peripheral nerve regeneration applications. Luong-Van et al. [119] prepared heparin-loaded PCL fiber mats by electrospinning. Heparin is used to prevent vascular smooth muscle cell (VSMC) proliferation, which can lead to graft occlusion and failure, and was loaded at concentrations of 0.5 and 0.05 wt% in PCL solutions. Two model proteins, BSA and lysozyme, were incorporated into core–shell fibers with PCL as shell and protein-containing PEG as core by a coaxial electrospinning method. The obtained diameter of both shell and core was increased with increasing feed rate. The release behavior of protein from the fiber mats were carried out in 0.05 M phosphate buffer solution (pH 7.4) at 37°C. A slight burst release was observed on the first day followed by a relatively steady release. Moreover, the release rate could be controlled by varying the feed rate of the PEG/protein solution: higher feed rate resulted in higher protein release. In addition, Yan et al.

[120] investigated the core–shell structure of fiber mats prepared by coaxial electrospinning from poly(L-lactide-*co*-caprolactone) solution as shell and phosphate buffer saline (pH 7.4) solution containing BSA or/and NGF as core for use in nerve tissue engineering applications. Lu et al. [121] prepared composited fiber mats composed of cationized gelatin (CG)-coated PCL by coaxial electrospinning. CG was used as the shell material for fabricating core–shell fiber mats. They investigated the adsorption behaviors of fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (FITC-BSA) or FITC-heparin onto the fiber mats. Moreover, vascular endothelial growth factor (VEGF) was impregnated into fiber mats through specific interactions with adsorbed heparin in the outer CG layer. Maretschek et al. [122] also prepared cytochrome C (a hydrophilic model protein)-loaded fiber mats based on PLLA by emulsion-electrospinning. Moreover, Yang et al. [123] prepared lysozyme encapsulated within the core–shell structured fibers by emulsion electrospinning. The release of lysozyme loaded in core–shell poly(DL-lactic acid) (PDLLA)/methyl cellulose (MC) fiber mats was carried out in phosphate buffer solution (pH 7.4) containing 0.02% sodium azide as a bacteriostatic agent. The release kinetics for all the fiber mats could be shown to take place in two stages: an initial fast release followed by a constant linear release. An increase of lysozyme loading caused a higher amount of lysozyme release. Thus, the core–shell structured fibers could reduce the initial burst release, sustain the release period dependent on the protein loading, and protect the structural integrity and bioactivity of encapsulated lysozyme during incubation in medium.

4.3 Wound Dressing

Wound healing or wound repair is a native process of regenerating dermal and epidermal tissues in which the skin repairs itself after injury. In normal skin, the epidermis and dermis exist in steady state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, a set of complex biochemical actions occur immediately to repair the damage. The classic model of wound healing is divided into inflammatory, proliferative, and remodeling phases and epithelialization. Normally, the body cannot heal thick burns or deep ulcers because there is no source of cells remaining for regeneration, except from the wound edges. As a result, complete re-epithelialization takes a long time and is complicated, with scarring of the base. Dressings for wound healing function to protect the wound, exude extra body fluids from the wound area, decontaminate the exogenous microorganisms, improve the appearance, and sometimes accelerate the healing process. For these functions, a wound dressing material should provide a physical barrier to a wound, but be permeable to moisture and oxygen. For a full-thickness dermal injury, the adhesion and integration of an artificial dermal layer consisting of a 3D tissue scaffold with well-cultured dermal fibroblasts will considerably assist the re-epithelialization [124].

Recently, an electrospun membrane has been reported to be a good candidate for wound dressing applications because of its unique properties. These include a highly porous membrane structure and well-interconnected pores that are small enough to protect the wound from bacterial penetration via aerosol particle capturing mechanisms [125]. High surface area and high porosity are essential for the exchange of liquids and gases with the environment. They also provide local release of drugs onto the skin. In addition, the electrospinning process provides a simple way to incorporate drugs into the nanofibers for medical treatment and antibacterial purposes, which can then be released into the healing wound in a homogeneous and controlled manner. Usually, the electrospun membrane can also be used for the treatment of wounds or burns of human skin. These kinds of wounds heal particularly fast and without complications if they are covered by a thin web of fiber mats that can let wounds heal by encouraging the formation of normal skin growth and eliminate the formation of scar tissue, which would occur in a traditional treatment [124].

Min et al. [126] fabricated electrospun silk fibroin fiber mats by electrospinning to study the cytocompatibility and cell behavior on these fiber mats. The results showed that these fiber mats supported the attachment and spreading of normal human keratinocytes and fibroblasts. Moreover, electrospun silk fibroin fiber mats containing epidermal growth factor (EGF) to promote the wound healing processes were prepared by electrospinning [127]. The preservation of the structure of these fiber mats during the healing period and the biocompatibility of these fiber mats indicated that these fiber mats are the new type of materials for medical applications, especially for patients suffering from chronic wounds. The collagen nanofibrous matrix produced by electrospinning was investigated for use in wound dressing [128]. The collagen nanofibrous matrix showed good tensile strength, even in aqueous solution. The electrospun collagen nanofibers coated with type I collagen or laminin were found to promote cell adhesion and spreading of normal human keratinocytes. These results indicated that these materials might be good candidates for biomedical applications such as wound dressing and tissue engineering. Gu et al. [129] fabricated electrospun PLLA and gelatin/PLLA fiber mats. The porous structured electrospun gelatin/PLLA fiber mat showed controlled evaporative water loss, promoted fluid drainage ability, and exhibited excellent biocompatibility. Therefore, this fiber mat has a potential for wound dressing application. Electrospun PLGA/collagen fiber mats were prepared by electrospinning [130]. The cytocompatibility and cellular responses to these fiber mats, cell and material interactions, and open wound healing in rats were studied. The results showed that these fiber mats were active in responses in human fibroblasts and were very effective as wound-healing accelerators in early-stage wound healing. These results indicated that the electrospun PLGA/collagen fiber mat might be a good candidate as a wound dressing material [130].

Quaternized chitosan (QCh) derivatives have a high activity against bacteria and thus are potential candidates for wound dressing applications. Electrospun QCh fiber mats were successfully prepared by electrospinning QCh solutions mixed with PVA [131]. The electrospun QCh/PVA fiber mats were stabilized against dissolution in aqueous environment using photomediated crosslinking.

The photo-crosslinked electrospun QCh/PVA fiber mats showed good bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* and thus could serve as potential candidates for wound dressing applications. To achieve continuous defect-free fibers from QCh derivatives, electrospinning of mixed solutions of QCh with poly(vinyl pyrrolidone) (PVP) was carried out [132]. The photo-crosslinked QCh/PVP fibers were irradiated to be water-stable. These fibers showed high antibacterial activity against *S. aureus* and *E. coli* and thus might be used in wound dressing applications. Chen et al. [133–135] developed a composite nanofibrous membrane of chitosan/collagen for wound healing applications. The composite nanofibrous membrane showed enhanced wound healing, and induced cell migration and proliferation. Animal studies also demonstrated that the nanofibrous membrane was better than gauze and commercial collagen sponge for wound healing.

Son et al. [136] prepared electrospun CA fiber mats from a CA solution containing 0.5 wt% of $AgNO_3$. The Ag^+ ions and Ag clusters diffused and aggregated on the surface of the CA fibers during UV irradiation. The Ag nanoparticles with an average size of 21 nm exhibited strong antimicrobial activity. Moreover, electrospun PLLA fiber mats containing nanosilver particles were prepared by electrospinning [137]. These fiber mats had strong antibacterial activities against *S. aureus* and *E. coli* and thus might be used in wound dressings or anti-adhesion membranes. Rujitananroj et al. [138] fabricated electrospun gelatin fiber mats containing 2.5 wt% $AgNO_3$. They studied the potential of these fiber mats as wound dressing materials and found that these fiber mats had greatest antibacterial activity against *Pseudomonas aeroginosa*, followed by *S. aureus*, *E. coli*, and methicillin-resistant *S. aureus*. Electrospun PVA fiber mats containing 1 wt% $AgNO_3$ were prepared and stabilized by heat treatment at 150°C for 10 min [139]. The cytotoxicity of the Ag ions and/or nanoparticles on normal human epidermal keratinocytes (NHEKs) and fibroblasts (NHEFs) was evaluated. The results showed that both Ag ions and Ag nanoparticles had similar cytotoxicity to the NHEK and NHEF cells. Moreover, the NHEFs appeared to be more sensitive to Ag ions or particles than NHEKs. The NHEK cells were more sensitive to the nitrate ions than NHEFs. Therefore, an antimicrobial Ag-containing matrix should be used to minimize the damage to epidermal cells [139]. In addition, electrospun chitosan/gelatin fiber mats containing silver nanoparticles were prepared by electrospinning [140] for use in wound dressing applications. Hang et al. [141] prepared PVA/chitosan fiber mats containing 1 wt% silver nanoparticles by electrospinning. They found that the addition of $AgNO_3$ to the polymer blends improved the electrospinnability of the blends. Moreover, the silver nanoparticles in the polymer blends showed antibacterial activity and acted as a nucleating agent during cold crystallization.

Burn healing is one of the most important problems in modern surgery due to the high percentage of burns among other traumas, and the high lethality and disability after the treatment of burns of high surface area. The problem of covering large burnt surfaces is still a challenge. Recently, a chitosan-based electrospun nanofibrous material was proposed as a new material for burn dressing [142]. Chitosan

nanofiber mats were created and tested as wound dressings for IIIa and IIIb degree burns. The results showed that chitosan nanofiber dressings provide effective absorption of exudate, ventilation of the wound, protection from infection, and stimulation of the process of skin tissue regeneration. Degradation of these materials prevents mechanical damage of the wound during removal. In another work, composite nanofibrous membranes of chitosan and silk fibroin were fabricated by electrospinning [143]. In this study, the antibacterial activities against *E. coli* (Gram negative) and *S. aureus* (Gram positive) were evaluated using turbidity measurements and the results suggested that the antibacterial effect of composite nanofibers varied with the type of bacteria. Furthermore, the biocompatibility of murine fibroblasts on the prepared nanofibrous membranes was investigated by hematoxylin and eosin (H&E) staining and MTT assays in vitro, and the membranes were found to promote cell attachment and proliferation. These results suggested that chitosan/silk fibroin composite nanofibrous membrane could be a promising candidate for wound healing. Shalumon et al. developed sodium alginate (SA)/PVA fibrous mats using an electrospinning technique. ZnO nanoparticles were further prepared, characterized, and introduced (at different concentrations) into SA/PVA fibrous mats through electrospinning to obtain SA/PVA/ZnO composite nanofibers. The prepared composite nanofibers were characterized. Cytotoxicity studies indicated the less toxic nature of composite SA/PVA fibers with low ZnO concentrations. The cell adhesion potential of these mats was further proved by studies with L929 cells for different time intervals. The SA/PVA/ZnO mats showed antibacterial activity against two different bacteria strains, *S. aureus* and *E. coli*, due to presence of ZnO nanoparticles. Hence, these fibrous mats could be ideal biomaterials for wound dressing applications at an optimal concentration of ZnO [144].

4.4   Catalysts, Enzyme Carriers, and Biosensors

Enzymes, which are green catalysts with a high degree of specificity, have received much attention in the fields of fine chemistry, pharmaceutical synthesis, food processing, biosensor fabrication, bioremediation, and protein digestion in proteomic analysis due to several advantages over conventional inorganic catalysts. These advantages include stereo- and regioselectivity, fewer side reactions, and mild reaction conditions [126]. However, some drawbacks limit their use at large industrial scales. Because enzymes are proteins, any changes in reaction conditions could lead to the deformation of their structure and loss of activity. Additionally, the difficultly in removal and recycling of the enzymes after reaction increases the cost of the processes [15]. Enzyme immobilization is an effective way to use enzymatic reactions at the industrial scale. This process is believed to retain the stability of the enzyme. In addition, the immobilized enzyme can be reused throughout several reactions and is easily separated from the product, which decreases the cost problem and also reduces contamination of the product [15].

However, a high loading efficiency of enzyme is a crucial problem in enzyme immobilization due to the loss of enzyme activity after immobilization. Increasing the amount of enzyme immobilized onto the substrate can compensate for the loss of activity [15]. The performance of an immobilized enzyme strongly depends on the properties of the substrate, which usually depend on the material type, composition and structure. Various forms of substrate have been used in enzyme immobilization, including beads, particles, hydrogels, membranes, films, and fibers. However, some of the support materials have disadvantages that are difficult to overcome. For example, mesoporous material usually entraps enzyme molecules on the inner surface, which limits the diffusion and results in lower enzyme activity [12]. Nanoparticles and nanotubes remarkably decrease the mass transfer limitation, whereas their dispersion and recycling are more difficult. By contrast, fiber mats obtained from electrospinning have a great potential to overcome these problems and are promising supports for enzyme immobilization because of the simple and versatile method of fabricating ultrafine fibers. These fibers have an extremely large surface area that provides an enormous number of active sites, thus enhancing the capability to bind more enzymes. Moreover, the interconnected pores of fiber mats provide effective interactions between the reactant and enzyme, which is valuable for continuous-flow chemical reactions or biological, processes [11].

The enzymes can be grafted onto the electrospun fiber surface via surface attachment and encapsulation [15]. Surface attachment refers to physical adsorption or covalent attachment of enzymes on the as-spun supports. However, physical adsorption on a hydrophobic support is often limited because of the poor wettability of the support surface by the enzymes. To improve the performance of the immobilized enzymes, surface modification is crucial for providing reactive groups on the fiber surface. The encapsulation of enzymes in the nanofibers can be achieved by direct co-electrospinning of enzymes with other components. However, the encapsulation approach has several disadvantages, resulting in the loss of enzyme during measurement and storage due to the residual enzyme molecules residing on the surface. Also, the enzyme molecules are confined inside the nonporous fibers, inhibiting the accessibility of the substrate to the enzyme [15].

The immobilization of cellulose in electrospun PVA fiber mats was prepared by electrospinning [14]. These fiber mats were crosslinked by glutaraldehyde vapor, and the catalytic efficiency for biotransformations was studied. The activity of immobilized cellulose in the electrospun PVA fiber mats after crosslinking was over 65% of that of the free enzyme, and fiber mats were superior to casting films for use in immobilization of cellulose. Similarly nanofibrous chitosan/PVA membrane was fabricated for enzyme immobilization [145]. This chitosan/PVA nanofibrous membrane was used as a support for lipase immobilization, with the advantages of high enzyme loading up to 63.6 mg/g and activity retention of 49.8%. The stabilities of the immobilized lipase towards pH, temperature, reuse, and storage were enhanced. These results imply that the chitosan nanofibrous membrane (with excellent biocompatibility) is a potential support for enzyme immobilization and can be used for biosensor applications. Ye et al. [146] fabricated electrospun poly(acrylonitrile-*co*-maleic acid) fiber mats tethered with two natural

biomacromolecules, chitosan and gelatin, to achieve dual-layer biomimetic supports for lipase immobilization. Lipase from *Candida rugosa* was then immobilized on these dual-layer biomimetic supports using glutaraldehyde. After immobilization, the pH, thermal, and reuse stabilities of the immobilized enzyme can be enhanced. Therefore, these immobilized fiber mats could be potential supports in enzyme immobilization technology for industrial applications [59].

Electrospun polysulfone fiber mats containing poly(*N*-vinyl-2-pyrrolidone) (PVP) and PEG as additives were prepared to immobilize *C. rugosa* by physical adsorption [147]. The results showed that the electrospun polysulfone fiber mats were potential supports in enzyme immobilization technology for industrial applications. Blending biopolymers into the fiber mats was a feasible method of improving enzyme activity, whereas the amount of bound enzyme decreased little. Moreover, electrospun polyacrylonitrile (PAN) fiber mats were immobilized with lipase from *C. rugosa* by an amidation reaction [148]. The enzyme molecules are covalently bound to the fiber mats and form aggregates on the fiber surface, which also became more hydrophilic and robust after enzyme immobilization. After enzyme immobilization, the storage stability was improved over that of free enzyme. Lipase enzyme from *C. rugosa* has been successfully immobilized in electrospun PVA fiber mats by electrospinning [149]. The enzyme loading in these fiber mats reached as high as 50 wt%. The lipase-loaded electrospun PVA fiber mats exhibited superior activity to the crude enzyme following exposure to elevated temperatures and humidity.

The immobilization of lipase on electrospun poly(acrylonitrile-*co*-2-hydroxyethyl methacrylate) (PANCHEMA) fiber mats was also achieved [150]. Epoxy-activated PANCHEMA fiber mats seemed to be an almost-ideal system for enzyme immobilization and could preserve relatively high activity of immobilized enzyme. The stabilities of the immobilized lipase were also improved. It was concluded that the lipase-immobilized PANCHEMA fiber mat bioreactor possessing high enzyme loading and catalytic efficiency might have great potential as a biocatalyst for a wide range of reactions including hydrolysis, alcoholysis, aminolysis, and transesterification [150]. To achieve a biofriendly microenvironment for enzyme immobilization, collagen or protein hydrolysate from egg skin was tethered on electrospun poly(acrylonitrile-*co*-acrylic acid) (PANCAA) fiber mats [151]. Lipase from *C. rugosa* was then immobilized on the protein-modified fiber mats by covalent binding using glutaraldehyde as coupling agent, and on the nascent PANCAA fiber mats using EDC/NHS as coupling agent. The enhancement of both the activity retention and stabilities of the immobilized lipase could be found on egg skin hydrolysate-modified and collagen-modified PANCAA fiber mats compared with those on the nascent PANCAA fiber mats. The results indicate that the immobilization of biomacromolecules onto PANCAA fiber mats could provide a biofriendly microenvironment for further tethering of enzymes [64].

Li et al. [152] fabricated electrospun PAN fiber mats immobilized with *C. rugosa* lipase by amidation. Enzyme molecules were covalently bound to the fiber mats and formed small protein aggregates. The immobilized lipase on the electrospun PAN fiber mats showed a good biocatalystic activity for soybean oil

hydrolysis. Moreover, the reusability of immobilized lipase on the electrospun PAN fiber mats was very high. These results implied that the immobilized fiber mats had good potential for industrial applications [152]. *Pseudomonas nitroreducens* LY was immobilized in the electrospun PVA fiber mats by electrospinning [153]. Moreover, theanine was synthesized by the immobilized *P. nitroreducens* LY in fiber mats with a yield of 10.75 g/L. These results indicate that these immobilized fiber mats could be useful in microbial cell immobilizing technology. Stoilova et al. 154] functionalized electrospun styrene-maleic anhydride copolymers by modification with two types of spacers – a polymer with a flexible hydrophilic polyether chain (Jeffamine ED) and a rigid low molecular weight spacer (*p*-phenylenediamine). Acetylcholinesterase was then immobilized onto the modified fiber mats using glutaraldehyde as a binding agent. The results showed that the immobilized acetylcholinesterase had higher thermal and storage stability than the free enzyme.

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