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Review Article

Electrospinning of Nanofibers for Tissue Engineering Applications

Haifeng Liu,[1] Xili Ding,[1] Gang Zhou,[1] Ping Li,[1] Xing Wei,[2] and Yubo Fan[1]

[1] *Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, School of Biological Science and Medical Engineering, Beihang University, Xue Yuan Road No. 37, Haidian District, Beijing 100191, China*
[2] *Orthopaedic Department, First Affiliated Hospital, PLA General Hospital, Beijing 100048, China*

Correspondence should be addressed to Haifeng Liu; haifengliu@buaa.edu.cn and Yubo Fan; yubofan@buaa.edu.cn

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Electrospinning is a method in which materials in solution are formed into nano- and micro-sized continuous fibers. Recent interest in this technique stems from both the topical nature of nanoscale material fabrication and the considerable potential for use of these nanoscale fibres in a range of applications including, amongst others, a range of biomedical applications processes such as drug delivery and the use of scaffolds to provide a framework for tissue regeneration in both soft and hard tissue applications systems. The objectives of this review are to describe the theory behind the technique, examine the effect of changing the process parameters on fiber morphology, and discuss the application and impact of electrospinning on the fields of vascular, neural, bone, cartilage, and tendon/ligament tissue engineering.

1. Introduction

Electrospinning, which is an ultrafine fiber manufacturing technology, was coined in 1990s from the earlier used term of "electrostatic spinning." It has now attracted increasing attention in both the academic and industrial communities [1–4]. Electrospinning is capable of fabricating fibers with nanometer range diameters, which yields very high specific surface areas, up to one to two orders of magnitude higher than current microfibers produced from conventional melting and dry/wet spinning methods. Therefore, electrospun nanofibers are very useful for developing a variety of products or structures whose functions are dependent on surface area.

Among those potential applications, one of the most promising uses is for developing nanofibrous cellular scaffolds for tissue engineering. The underlying rationale of using nanofibers for scaffolding is based on the biomimetic principle that electrospun nanofibers can mimic the physical structure of the native extracellular matrix (ECM). This is because, from the biological viewpoint, almost all of the tissues and organs, such as bone, nerve, blood vessel, ligament, tendon, and cartilage, are synthesized and hierarchically organized into fibrous form (structure) with fiber dimensions down to nanometer scale [5–9]. Nanofibrous scaffold can therefore provide environmental or physical cues to cells and promote cell growth and function well towards the synthesis of genuine extracellular matrices over time [10]. To date, electrospun fibers have been applied towards a broad range of regenerative medicine applications. Electrospinning has emerged as a new scaffold fabrication method.

2. The Electrospinning Process

Electrospinning is a method in which materials in solution are formed into nano- and micro-sized continuous fibers. The elements required for electrospinning include a polymer source, a high-voltage supply, and a collector (as shown in Figure 1) [11]. What makes electrospinning unique from other methods of spinning (i.e., dry spinning, melt spinning, etc.) is the electrostatic force stretching the solubilized polymer as it falls and solvent starts to evaporate out [12]. Through several different collection methods, this process yields nonwoven, nanoporous materials. The basis of electrospinning is derived from a large change in electric potential. The material in solution is forced through an electrified orifice with voltage applied, usually between 5 and 30 kV. This charges the

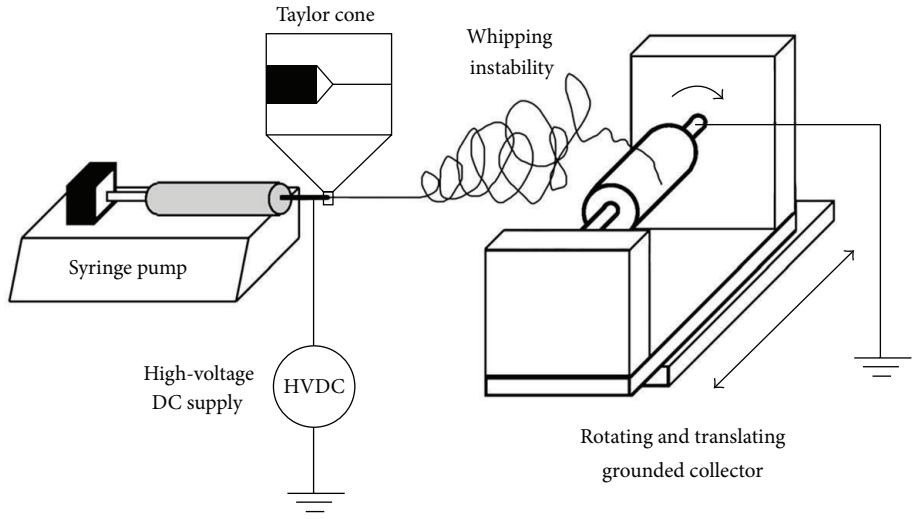

Figure 1: Schematic of a typical electrospinning system. A polymer solution is forced through a needle using a syringe pump. The needle is connected to a high-voltage DC supply, which injects charge of a certain polarity into the polymer solution. If the electrostatic force created by the repulsion of similar charges is sufficient to overcome the surface tension of the polymer solution, the Taylor cone is formed and a fiber jet is emitted from its apex. While the fiber jet is traveling toward the grounded collector it undergoes a chaotic whipping instability. The fiber jet is then deposited on the collector, which can be rotating and translating as depicted here [11].

solution inducing what is known as a Taylor cone or envelope cone located at the tip of the needle. The Taylor cone is the foundation for the jet of material that whips down toward a collection area. This motion is driven by bending instabilities in the jet as well as effects of evaporation and solidification of the solvent [13].

An equally charged auxiliary electrode at the site of the needle is also present to propel the jet downward. This electrode, normally a disk, inverted cone, or cylinder, provides an extra "electrostatic push" to both focus on the jet and overcome the surface tension of the solution [14, 15]. This supplementary electrode also plays a role in reducing the instability of the initial jet leaving the apex of the Taylor cone, resulting in the stable, visible region of solution immediately under the needle. As the jet moves toward the collector plate, it is elongated by electrostatic interactions between charges nearby segments of the same jet. Meanwhile, the solvent evaporates, and finally the jet solidifies into a fiber [16]. If the surface tension of the solution exiting the jet is strong enough, the effect will be a reduction in the surface area of the jet [17].

The collection area is either grounded or supplied with a negative charge to further attract the solution. Alignment of the fibers has been attempted using several different configurations such as an array of counter electrodes, disc collection, placement of knife-edge electrodes, and rotating wire drums [18, 19]. Significant alignment fibers have been achieved with a combination of an inverted cone supplementary electrode and a motorized dual-axis stage collection system [20]. Other methods such as multiple jets and two-fluid electrospinning have also been attempted to maximize desirable properties of the scaffold [21].

Due to the nano- and microscale of the process, many variables affect both the fiber formation and the consistency of the final product. It has been shown that humidity plays a large role in electrospinnability [22]. A lower humidity (less than 35%) is ideal for spinning. Humidility higher than 35% will make the jet difficult to spin continuously. Other external variables that affect the process include gravity, temperature, air density, and air velocity [23]. It is very important for electrospinning to be performed in a closed and regulated environment. Properties of the solution that have a large effect on the process include concentration, viscosity, conductivity, surface tension, and homogeneity [24, 25].

3. Polymers for Electrospinning

Since electrospinning began, many polymers have been successfully electrospun for different applications. Electrospinning technology can be used to generate nanofibrous scaffolds made of synthetic polymers as well as native matrix such as collagen, gelatin, chitosan, silk, and elastin [26–34]. Synthetic polymer scaffolds such as those composed of lactic or glycolic acids are biocompatible and biodegradable, have configurable mechanical properties, and can be easily modified to incorporate proteins and peptides [35, 36]. PLA and PGA have been approved by the Food and Drug Administration (FDA) as suture material and use in drug delivery. Recently, copolymer and polymer mixtures have been found to be advantageous over homopolymers and can be incorporated to vary the mechanical properties and degradation time of nanofibrous scaffold [37–40].

4. Nanofibers for Tissue Engineering Applications

Electrospun fibrous scaffolds possess an extremely high surface-to-volume ratio, tunable porosity, and malleability to conform over a wide variety of sizes and shapes, which have found wide applications in biomedical fields. The scaffolds

contain nanofibers with microscale interconnected pores, loosing three-dimensional assemble, resembling the topographic features of ECM, and resulting in suitable substrates for tissue engineering [41]. Being an emerging field applied to tissue engineering, electrospinning has yet to make a significant impact on *in vivo* applications. Although many different polymeric scaffolds and cell types have been studied, the bulk of the research thus far is restricted to preliminary, qualitative analyses of the cytocompatibility of the electromaterials in terms of cell adhesion, proliferation, and changes in cell morphology. However, increasing attention is being paid to the more quantitative evaluation of the changes in cellular functions as a result of the topographical cues provided by the nanofibrous scaffolds. By varying the previously discussed processing and solution parameters the fiber orientation (aligned versus random) and porosity/pore size (cell infiltration) of the electrospun scaffold can be controlled and optimized for each individual application. After fabrication the surface of the scaffold can be modified with a high density of bioactive molecules due to the relatively high scaffold surface area. Due to the flexibility in material selection as well as the ability to control the scaffold properties, electrospun scaffolds have been employed in a number of different tissue applications including vascular, neural, bone, cartilage, and tendon/ligament.

4.1. Vascular Tissue Engineering

4.1.1. Smooth Muscle Cells. Xu et al. [42] demonstrated the potential of poly(L-lactide-co-s-caprolactone) [P(LLA-CL)] (75 : 25) electrospun fibrous scaffolds as a material for the engineering of vascular grafts. After 7 days of culture, the human coronary artery smooth muscle cells (SMCs) integrated well into the scaffold, reaching close to 90% confluence. The SMCs also maintained their phenotypes as evidenced by being stained positively for x-actin and myosin. Similarly, using human coronary artery smooth muscle cells, Venugopal et al. [43] compared the biocompatibility of electrospun scaffolds made of collagen type I and poly(caprolactone) in terms of cell proliferation, cell adhesion, and cell growth rate assays after 3 days of *in vitro* cell culture. They concluded that while all scaffolds promoted cell-matrix and cell-cell interactions and preserved the phenotypic morphologies of SMCs, PCL scaffolds with collagen type I coating were the preferred choice. In such combination, the PCL provides the desired mechanical characteristics and collagen provide the cytocompatibility.

In another study, Stankus et al. [44] evaluated the feasibility of integrating high density of vascular smooth muscle cells directly into poly(ester urethane)urea (PEUU) fibrous scaffolds during the electrospinning process, in order to obtain a well-integrated, three-dimensional distribution of cells throughout the fibrous scaffold. Two orthogonally positioned separate supplies of polymer and cells were used during the electrospinning process, and despite exposure to a large electric field, cells electrosprayed from cell culture media were found to be more than 90% viable after the fabrication process. Cellular constructs of thickness between 300 to 500 pm were obtained and cells proliferated under 7 days of transmural perfusion culture, compared to static culture, where cell number remained unchanged. Cellular morphological analyses also revealed healthier-looking cells uniformly located throughout the scaffold under perfusion culture as opposed to static culture.

4.1.2. Endothelial Cells. The potential of electrospun poly(L-lactic acid) (PLLA) fibrous scaffolds in supporting the growth of human vascular endothelial cells (ECs) was evaluated by Xu et al. [45]. Electrospun scaffolds comprising fibers with diameters of 235 ± 71 nm and 3500 ± 854 nm, respectively, were studied in parallel with tissue culture polystyrene (TCPS) and PLLA solvent-cast film as controls. Immunostaining for cell adhesion protein, CD31, revealed that ECs appeared to adhere less well to the electrospun scaffolds, maintaining a rounded morphology compared to the typical cobblestone appearance of ECs when seeded onto flat surfaces. Cell proliferation also appeared to be better on flat surfaces compared to the fibrous scaffolds. However, no significant differences in cell behavior were observed in cells cultured on the micro- and nanofibrous scaffolds.

In a separate study, the same group demonstrated good interaction and integration of human coronary artery ECs with poly(L-lactide-co-E-caprolactone) [P(LLA-CL)] (75 : 25) scaffolds [42]. The ECs reached close to 75% confluence after 7 days of culture. Immunostaining for CD31 and CD62E in ECs demonstrated that the cells maintained their phenotypes. The study demonstrated the potential of P(LLA-CL) fibrous scaffolds as a material for vascular grafts. However, since the study was done in the absence of a flat surface control, it was inconclusive as to whether the structure of the nanofibers, hence surface roughness, affected cell attachment and proliferation compared to a smooth surface.

Kwon et al. [46] studied the effects of fiber diameters on the adhesion, proliferation, and morphology of human umbilical vein endothelial cells (HUVECs). Electrospun micro- and nano-fibrous scaffolds of a copolymer of L-lactide and ε-caprolactone, PLA-CL 50/50, were obtained by varying the processing parameters. The authors observed that cell adhesion and proliferation were better on the small-diameter fiber meshes (0.3 and 1.2 μm diameter fibers). The cells also spread and elongated along the small-diameter meshes. In contrast, cells seeded on the 7 pm diameter fibrous mesh showed reduced cell adhesion, restricted cell spreading, and no signs of proliferation. Together with the observations made by Xu et al., it appears that a threshold fiber diameter may exist between 3.5 and 7 pm to significantly affect the adhesion and morphology of the cultured ECs.

Kwon and Matsuda [47] combined the copolymer poly(L-lactide-co-s-caprolactone) (PLCL 50 : 50) with collagen type I. However, instead of serving as a coating, collagen was blended into PLCL and electrospun into a composite fibrous mesh. The advantage is perhaps the ease of introducing collagen in the single-step process of electrospinning. HUVECs were used to evaluate the potential of the composite mesh as a tissue scaffold by observing the cell adhesion and proliferation for up to 5 days of culture on the mesh. PLCL scaffolds coated with fibronectin and blended with small

amounts of collagen (5 and 10 wt%) were found to increase cell attachment, spreading, and proliferation compared to plain PLCL meshes. Scaffold shrinkage due to large amounts of collagen present (30 and 50 wt%) appeared to offset the advantages of blending collagen by resulting in a much lower number of cells on the scaffolds after 5 days of culture.

A functional human endothelium on the lumen of the graft was successfully tissue engineered by Zhang et al. [48] on a highly porous composite electrospun scaffold composing of PCL, PGC, elastin, and gelatin. Fluorescent microscopy showed that human aortic endothelial cells (HAECs) adhered to and then spread on the scaffold upon seeding, verifying that HAECs favored the biomechanics and biochemistry of the scaffold. This tissue-engineered endothelium formed tight junctions between adjacent HAECs, secreted PGI2 molecules, a vital antithrombotic and vasodilatation factor, and successfully prevented human platelet adherence and aggregation, suggesting that it is functionally comparable to its native counterparts.

4.1.3. Vascular Grafts. Electrospinning can be employed to fabricate small-diameter tubular scaffolds, providing a nanofibrous network structure to mimic the natural arterial ECM. Zhang et al. [49] developed a tissue-engineered construct that mimicked the structure of blood vessels using tubular electrospun silk fibroin scaffolds with suitable mechanical properties. Human coronary artery smooth muscle cells (HCASMCs) and human aortic endothelial cells (HAECs) were sequentially seeded onto the luminal surface of the tubular scaffolds and cultivated under physiological pulsatile flow. The results demonstrated that TEVGs under dynamic flow conditions had better outcome than static culture controls in terms of cell proliferation and alignment, ECM production, and cell phenotype based on transcript and protein level assessments. The metabolic activity of HCASMCs present in the TEGs indicated the advantage of dynamic flow over static culture in effective nutrient and oxygen distribution to the cells.

One of the major downfalls of tissue-engineered small-diameter vascular grafts is the inability to obtain a confluent endothelium on the lumenal surface. Loosely attached endothelial cells (ECs) are easily separated from the vessel wall when exposed to the *in vivo* vascular system. Thus any denuded areas on the lumenal surface of vascular grafts may lead to thrombus formation via platelet deposition and activation. If the denuded areas could express anticoagulant activity until the endothelial cell lining is fully achieved, it may greatly improve the chances of successful vascular reconstruction. Therefore, Liu et al. [50–52] fabricated sulfated silk fibroin nanofibrous scaffolds by electrospinning and assessed the anticoagulant activity and cytocompatibility of the scaffolds *in vitro* in order to improve the antithrombogenicity and get some insights into its potential use for vascular tissue engineering (Figure 2). Sulfated silk fibroin was prepared by reaction with chlorosulfonic acid in pyridine and then was developed to form a nanofibrous scaffold by electrospinning technique. It was found that the anticoagulant activity of sulfated silk fibroin nanofibrous scaffolds was significantly enhanced compared with silk fibroin nanofibrous scaffolds. Vascular cells, including ECs and SMCs, demonstrated strong attachment to sulfated silk fibroin nanofibrous scaffolds and proliferated well with higher expression of some phenotype-related marker genes and proteins (Figure 3).

With the use of electrospun PLA fibrous meshes and collagen type I fibers, Stitzel et al. [53] fabricated a prototypic vascular graft. The authors demonstrated that the vascular graft prototype was able to support the growth of human aortic SMCs, with confluent layers of SMCs being observed in the luminal and external surfaces of the vascular graft after 10 days of culture. The SMCs were also observed to align and organize in the presence of collagen I fibers, as opposed to cells that were seeded on grafts without collagen I fibers. The authors attributed the cell alignment to the stress exerted by the collagen fibers, which may mimic the stress of a closed section of an artery, thereby aligning the cells. The work was further expanded with the fabrication of a prototypic vascular graft composed of a mixture of collagen type I, elastin, and poly(D,L-lactide-co-glycolide) (PLGA) electrospun nanofibrous scaffold [54], in attempt to more closely mimic the mechanical properties and material composition of a blood vessel. Bovine SMCs were used to assess the *in vitro* biocompatibility of the material by seeding the cells in wells along with the electrospun scaffolds and assessing cell viability and proliferation up to 7 days, instead of directly seeding the cells on the electrospun scaffolds. Nonetheless, cell attachment was also evaluated by coculturing bovine endothelial cells and SMCs on the inner and outer surfaces of the electrospun prototypic vascular graft, respectively. Confluent layers of cells were observed after 3-4 days of culture, demonstrating the biocompatibility of the material.

4.2. Neural Tissue Engineering. Nerve tissue engineering is one of the most promising methods to restore nerve systems in human health care. Scaffold design has pivotal role in nerve tissue engineering. Interest in employing electrospinning for scaffold fabrication is mainly due to the mechanical, biological, and kinetic properties of the scaffold being easily manipulated by altering the polymer composition and processing parameters [55]. The orientation of nanofibers is one of the important features of a perfect tissue scaffold, because the fiber orientation greatly influences cell growth and related functions in cells such as nerve and smooth muscle cells [56–59]. Yang et al. [60] evaluated the effects of fiber alignment and fiber diameter on the morphology and proliferation of the neuronal stem cells (NSC). The scaffolds included random nanofibrous mesh (average diameter = 700 nm), aligned nanofibrous mesh (average diameter = 300 nm), random microfibrous mesh (average diameter = 3.5 μm), and aligned microfibrous mesh (average diameter = 1.5 μm). The neuronal stem cells attached well onto all fibrous scaffolds, with extensive neurite-like outgrowth. They elongated and aligned in the direction of the aligned fibers, but adopted a random morphology on random fibrous scaffolds, thus demonstrating the contact guidance provided by the structure of the aligned fibers. Cells were also observed qualitatively to elongate more on nanofibers compared to microfibers, regardless of fiber orientation. While this study

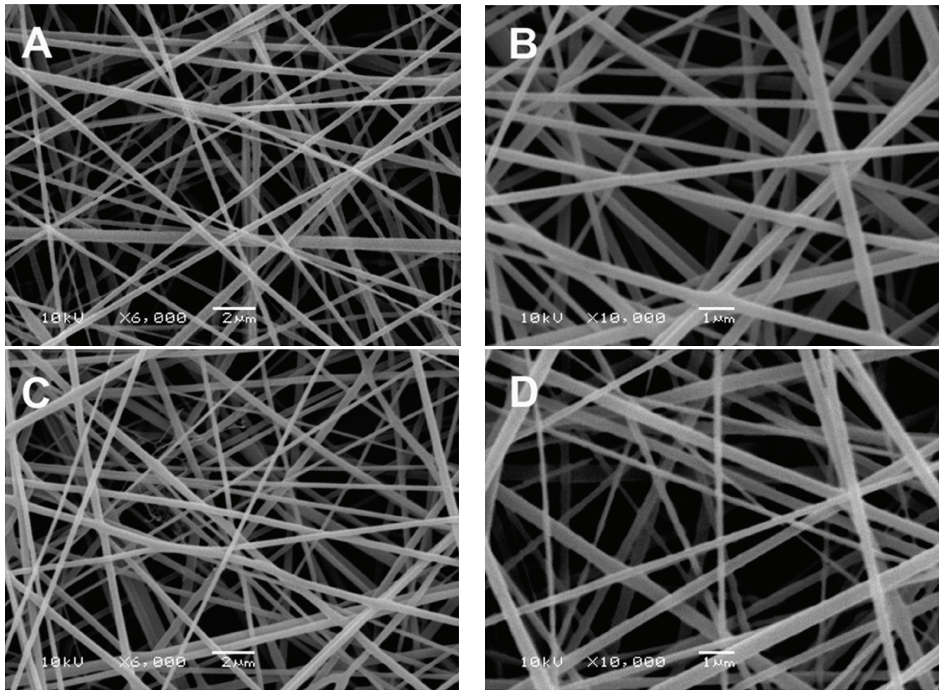

Figure 2: SEM images of silk (A, B) and S-silk (C, D) scaffolds showing similar surface morphology. (A, C) Scale bars = 2 μm; (B, D) scale bars = 1 μm [50].

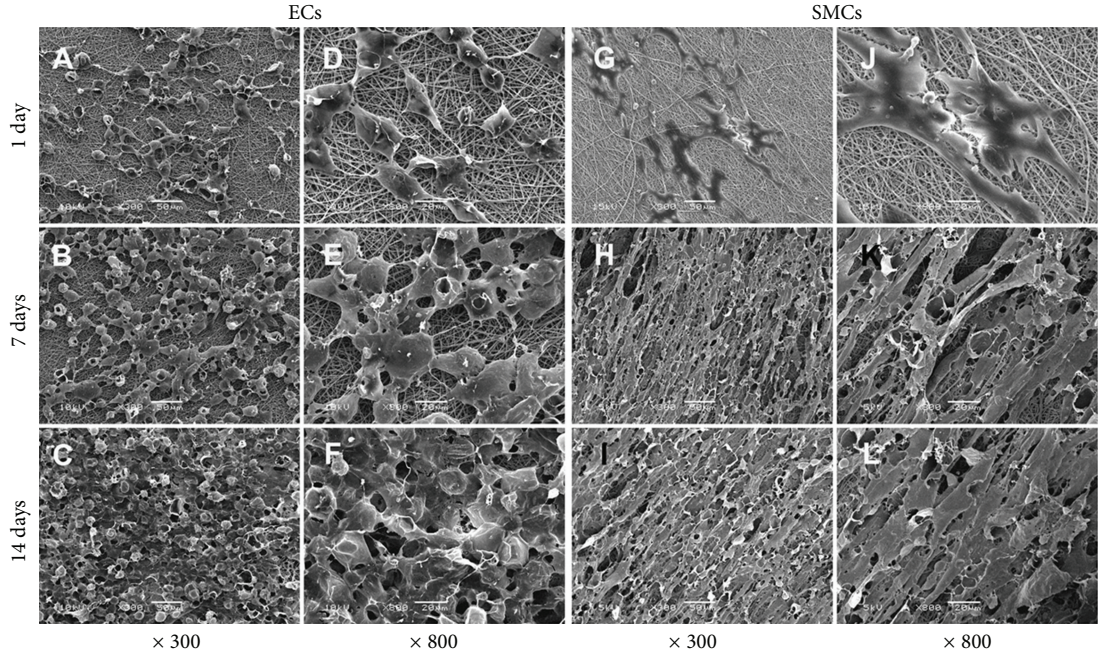

Figure 3: SEM photomicrographs showing adherence and proliferation of ECs and SMCs cultured on S-silk scaffolds for 1, 7, and 14 days. (A–C, G–I) Scale bars = 50 μm; (D–F, J–L) scale bars = 20 μm [50].

suggested the intriguing effect of nanoscale features on NSC neurite outgrowth, electrospinning will always produce fibers with size disparity in diameter and imperfect alignment.

Bone marrow mesenchymal stem cells (BMSCs) capable of differentiating into neuronal cells on engineered nanofibrous scaffolds have great potential for bionanomaterial-cell transplantation therapy of neurodegenerative diseases and injuries of the nervous system. Prabhakaran et al. [61] investigated the potential of human bone-marrow-derived mesenchymal stem cells (MSCs) for neuronal differentiation *in vitro* on poly(l-lactic acid)-co-poly-(3-caprolactone)/collagen (PLCL/Coll) nanofibrous scaffolds. PLCL and PLCL/Coll nanofibrous scaffolds were fabricated by electrospinning process, and their chemical and mechanical characterizations were carried out using SEM, contact angle, FTIR, and tensile instrument. PLCL/Coll nanofibrous

scaffolds were suitable substrates for the neuronal differentiation of MSCs in the presence of neuronal inducing factors. Grown on PLCL/Coll nanofibrous scaffolds, the differentiated MSCs showed multipolar elongations along with neurofilament protein and nestin expressions, typical of neuronal cells.

4.3. Bone Tissue Engineering. Fibrous nanocomposites of hydroxyapatite (HA) and biodegradable polymers capable of compositionally and structurally emulating the basic building blocks of those naturally mineralized collagen nanofibers would possess great potential for engineering functional native bone-like substitutes [62]. The composite fibers were usually fabricated by blend electrospinning of HA and biodegradable polymers [63]. Cui et al. applied electrospun nanofibers as the reaction confinement for composite fabrication. Poly(DL-lactide) (PDLLA) ultrafine fibers with calcium nitrate entrapment were prepared by electrospinning and then incubated in phosphate solution to form *in situ* calcium phosphate on the polymer matrix. The formation of nanostructured HA and good dispersion of HA particles on the electrospun fibers were observed [64]. Moreover, electrospun PDLLA nanofibers were surface modified with gelatin grafts to control the nucleation and growth of HA in simulated body fluid (SBF). The average size of HA can be modulated by changing the concentration of gelatin on the surface of electrospun fibers, and the quantitative evaluation with kinetic models was established for HA growth rate and crystal size [65].

Silk fibroin fiber scaffolds containing human recombinant bone morphogenetic protein 2 (BMP-2) and/or nanoparticles of hydroxyapatite (nHAP) prepared via electrospinning were used for *in vitro* bone formation from human bone-marrow-derived mesenchymal stem cells (hMSCs) [66]. BMP-2 survived the aqueous-based electrospinning process in bioactive form. hMSCs were cultured for up to 31 days under static conditions in osteogenic media on the scaffolds. Electrospun silk fibroin-based scaffolds supported hMSC growth and differentiation toward osteogenic outcomes. The scaffolds with the coprocessed BMP-2 supported higher calcium deposition and enhanced transcript levels of bone-specific markers than in the controls, indicating that these nanofibrous electrospun silk scaffolds were an efficient delivery system for BMP-2. X-ray diffraction (XRD) analysis revealed that the apatite formed on the silk fibroin/BMP-2 scaffolds had higher crystallinity than that on the silk fibroin scaffold controls. In addition, nHAP particles were incorporated into the electrospun fibrous scaffolds during processing and improved bone formation. The coexistence of BMP-2 and nHAP in the electrospun silk fibroin fibers resulted in the highest calcium deposition and upregulation of BMP-2 transcript levels when compared with the other systems.

Zhang et al. [67] reported a novel biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan (HAp/CTS) prepared by combining an *in situ* coprecipitation synthesis approach with an electrospinning process. A model HAp/CTS nanocomposite with the HAp mass ratio of 30 wt% was synthesized through the co-precipitation method so as to attain homogenous dispersion of the spindle-shaped HAp nanoparticles (ca. 100×30 nm) within the chitosan matrix. By using a small amount (10 wt%) of ultrahigh molecular weight poly(ethylene oxide) (UHMWPEO) as a fiber-forming facilitating additive, continuous HAp/CTS nanofibers with diameters of 214 ± 25 nm had been produced successfully and the HAp nanoparticles with some aggregations were incorporated into the electrospun nanofibers. Biological *in vitro* cell culture with human fetal osteoblast (hFOB) cells for up to 15 days demonstrated that the incorporation of HAp nanoparticles into chitosan nanofibrous scaffolds led to significant bone formation oriented outcomes compared to that of the pure electrospun CTS scaffolds.

4.4. Cartilage Tissue Engineering. While most of the studies on the use of electrospun fibrous scaffolds for orthopedic implant applications revolve around the evaluation of the cytocompatibility of the scaffolds, the study by Li et al. [68] illustrated the potential of electrospun fibrous scaffolds for chondrogenic differentiation. Electrospun scaffolds, as shown by Li et al., may have distinct advantages over other 3D scaffolds that are currently used for chondrogenic differentiation. Firstly, unlike other scaffolds such as hydrogels, chondrocytes cultured on electrospun scaffolds may not require the addition of growth factors for cartilage tissue development, and secondly nanofibrous scaffolds have superior mechanical integrity as opposed to hydrogels. Using a random nonwoven mesh of PCL nanofibers (average diameter = 700 nm), the functionality of the 3-dimensional structure of electrospun nanofibrous scaffolds in controlling chondrogenic differentiation in fetal bovine chondrocytes (FBCs) was evaluated. The dedifferentiated chondrocytes were able to redifferentiate without the addition of growth factors after 21 days of cell culture, suggesting that the electrospun fibers may be able to stimulate the seeded cells to release the endogenous growth factors necessary for chondrocytic differentiation. Cells seeded on the fibers proliferated in the presence of serum. However, differentiation of the cells was encouraged in serum-free medium. This suggested that the PCL nanofibrous scaffolds can support both cellular proliferation and differentiation. Cartilage-associated genes in FBCs, such as collagen type II, collagen IX, aggrecan, and COMP, were all upregulated on PCL fibrous scaffolds compared to cells seeded on TCPS. Changes in cellular functions were also assessed by Alcian Blue staining, revealing a higher amount of sulfated matrix production in the FBCs seeded on electrospun scaffolds.

More recently, in order to engineer cartilage with precise three-dimensional (3D) structures by applying electrospun fibrous membranes of gelatin/polycaprolactone (GT/PCL), Xue et al. prepared the electrospun GT/PCL membranes into rounded shape and then seeded chondrocytes in the sandwich model [69]. After *in vitro* and *in vivo* cultivation, the newly formed cartilage-like tissues were harvested. Macroscopic observations and histological analysis confirmed that the engineering of cartilage using the electrospun GT/PCL membranes was feasible. An ear-shaped cartilage was then constructed in the sandwich model, with the help of an ear-shaped titanium alloy mold. After 2 weeks of culture *in vitro* and 6 weeks of subcutaneous incubation *in vivo*, the

ear-shaped cartilage largely maintained its original shape, with a shape similarity up to 91.41% of the titanium mold. In addition, the engineered cartilage showed good elasticity and impressive mechanical strength. These results demonstrated that the engineering of 3D cartilage in a sandwich model using electrospun fibrous membranes was a facile and effective approach.

4.5. Tendon/Ligament Tissue Engineering. Reconstruction of ACL is one of the major concerns, and tissue engineering strategies for its repair face the challenge of fabricating ideal biomaterial scaffolds. Scaffolds provide a structural and logistic template for cell attachment and tissue development and should biodegrade in parallel with the accumulation of new tissue components. Electrospinning has recently received attention as a possible polymer processing technique is for the fabrication of scaffolds to be used in tendon and ligament tissue engineering. Due to the relatively high tensile strength of tendon and ligament, the deposition of a collagenous connective tissue matrix is crucial for successful tissue reconstruction. Ouyang et al. examined a knitted PLGA scaffold for applications in tendon regeneration in adult female New Zealand White rabbits with 10 mm gap defects of the Achilles tendon [70]. They observed that the regenerated tendons contained collagen type I and type III fibers as early as 2 weeks postimplantation. Additionally, at 12 weeks postimplantation both the tensile stiffness and modulus of the regenerated tendon were 50% more than those of normal tendon, with even better results achieved for scaffolds seeded with bone marrow stromal cells. However, knitted scaffolds required gel systems, such as fibrin or collagen gel, for cell seeding and were found to be unsuitable for ligament reconstruction in the knee joint, because the cell-gel composite dissociated from the scaffold during motion. Gel systems are also likely to encounter nutrient transmission problems, and cells seeded in a three-dimensional (3D) gel are observed to proliferate more near the surface than in the center of the gel. In order to solve this problem, Sahoo et al. electrospun PLGA nanofibers onto a knitted PLGA scaffold in order to provide a large area for cell attachment, thereby removing the need for a gel system for cell seeding [71]. Porcine bone marrow stromal cell attachment, proliferation, and extracellular matrix synthesis were examined on the electrospun/knit composite scaffold compared to a knit PLGA scaffold in which cells were immobilized using a fibrin gel. The results showed that cell proliferation and cellular activity were both increased in the electrospun/knit composite scaffold, while the cell attachment was comparable between the two scaffolds. Additionally, Lee et al. found that human ligament fibroblasts synthesize significantly larger amounts of collagen when they are seeded on aligned nanofibers compared to randomly oriented nanofibers [72]. Thus, electrospun nanofibrous scaffolds can be used not only to improve cell attachment but also to increase cellular activity such as extracellular matrix generation in tissue engineering scaffolds for tendon/ligament repair.

Biomaterial scaffolds with gradients in architecture and mechanical and chemical properties have the potential to improve the osseointegration of ligament grafts by recapitulating phenotypic gradients that exist at the natural ligament-bone (L-B) interface. In order to regenerate the L-B interface, Samavedi et al. investigated the potential of two scaffolds with mineral gradients in promoting a spatial gradient of osteoblastic differentiation (as shown in Figure 4) [73]. The first graded scaffold was fabricated by coelectrospinning two polymer solutions (one doped with nanohydroxyapatite particles) from offset spinnerets, while the second was created by immersing the first scaffold in a 5 × simulated body fluid. Rat bone marrow stromal cells, cultured in the presence of osteogenic supplements, were found to be metabolically active on all regions of both scaffolds after 1 and 7 days of culture. Gene expression of bone morphogenic protein-2 and osteopontin was elevated on mineral-containing regions compared to regions without mineral, while the expression of alkaline phosphatase mRNA revealed the opposite trend. Finally, the presence of osteopontin and bone sialoprotein confirmed osteoblastic phenotypic maturation by day 28. This study indicates that coelectrospun scaffolds with gradients in mineral content can guide the formation of phenotypic gradients and may thus promote the regeneration of the L-B interface.

5. Controlled Delivery Carriers

The emergence of coaxial electrospinning has allowed the development of many new designs of functional nanotechnological materials. Co-axial electrospinning is a simple and rapid technique to produce micro/nanotubes [74, 75] drug- or protein-embedded nanofibers [76–78] and hybrid core-shell nanofibrous materials [79, 80]. The greatest advantage of co-axial electrospinning is its versatility in the type (hydrophobic or hydrophilic) and size (ranging from 100 nm to 300 μm) of fibers it can produce. Monoaxial electrospun fibers have been reported to be able to incorporate and release antibiotics, drugs, and proteins in a sustained manner [81–83]. However, the distribution and release of drugs from the fibers are poorly controlled. Moreover, growth factors and cytokines embedded in polymer matrixes also suffer from significant decrease in bioactivity [84]. As delivery system for tissue engineering, coaxial electrospun fibers offer better drug stability, more complete drug encapsulation, and tighter control of release kinetics compared to monoaxial fibers. Co-axial electrospinning circumvents technical limitations of monoaxial electrospinning by its core-shell design, allowing cytokines and growth factors to be dissolved in aqueous solution for encapsulation. Encapsulated lysozyme and platelet-derived growth factor-bb released from core-shell nanofibers have maintained high bioactivity over a period of 1 month [85]. The core-shell design also allows better control over the release kinetics of the drug of interest due to an increased number of variable parameters. Changes in the shell and core material properties via variation in molecular weight, polymer type, and addition of porogen can fine-tune the release profile [86].

In another study, VEGF-loaded core/shell fibrous membranes were prepared by coaxial electrospinning with dextran (DEX) as the core component and poly(lactide-co-glycolide) (PLGA) as the shell polymer, respectively [87, 88]. The

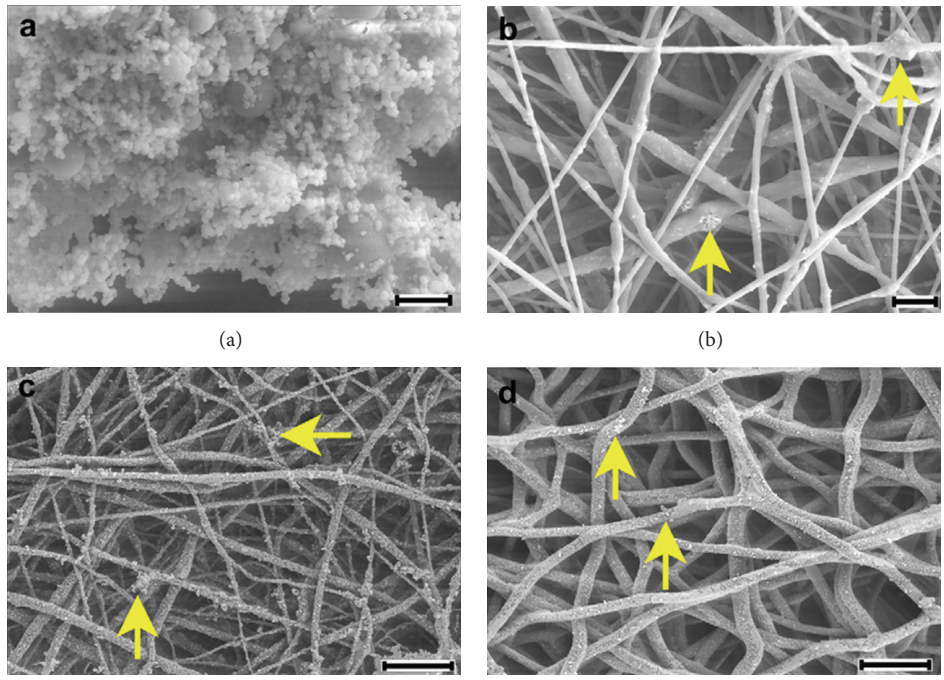

Figure 4: SEM micrographs of (a) nHAP particles used to fabricate ES scaffold (scale bar: 600 nm), (b) nHAP-PCL(ES) region, with arrows showing incorporated nHAP partially exposed on the surface of fibers (scale bar: μm), (c) nHAP-PCL(SBF) region, with arrows showing mineral crystallites (grown from 5 × SBF) decorating fibers (scale bar: 10 μm), and (d) PUR(SBF) region, with arrows showing mineral crystallites (grown from 5 × SBF) decorating fibers (scale bar: 10 μm) [73].

electrospun DEX/PLGA fibers were observed by scanning electron microscopy, transmission electron microscopy, and confocal microscopy to identify the core/shell fiber structure and the protein distribution. The results of tensile tests showed that the DEX/PLGA membranes possessed lower tensile strength and higher Young's modulus than the PLGA one. The release profiles demonstrated that vascular endothelial growth factor (VEGF) release was sustained for more than 28 days. Studies on cell viability and spreading demonstrated that the DEX(VEGF)/PLGA membranes positively promoted cell proliferation and cell-membrane interaction, which further testified that the processed VEGF remained bioactive.

6. Conclusions

Electrospinning has gained popularity with the tissue engineering community as a potential means of producing scaffolds. This fabrication technology provides the ability to control biomaterial composition, fiber diameter, fiber alignment, geometry, and drug/protein incorporation into a scaffold. Nanoscaled fibers fabricated by electrospinning are able to improve the cellular interactions of a wide variety of cell types; moreover, the cells are able to maintain their phenotypic and functional characteristics on nanofibrous scaffolds. In addition, a growing body of evidence demonstrates that the topography of nanofibrous scaffolds plays an important role in controlling cell adhesion, proliferation, and differentiation. Therefore, electrospun nanofibrous scaffolds can serve as a tool for studying the topographical aspects of cellular interactions that would lead to improved tissue formation. Furthermore, these electrospun scaffolds can be functionalized by adding biochemical and mechanical cues to enhance cellular interactions for tissue engineering applications.

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