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Review

Development and biomedical applications of nanofibrous scaffolds

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ABSTRACT

Nanofibrous (NF) scaffolds have been exploited in various fields because of the fascinating advantages of nanofibers. NF scaffolds have ultrathin fiber diameter and large surface-volume ratio. Among various methods for NF scaffold fabrication, electrospinning is one of the most preferred ones because it is simple, scalable and versatile in fabrication of nanofibers with a variety of polymers, additives and structures based on its applications. Electrospun NF scaffolds can be prepared from synthetic and natural polymers. Especially, electrospinning generates fibers that are similar to the fibrous structures of extracellular matrix. Therefore, NF scaffolds have great potential for applications in various biomedical fields, including three-dimensional cell and tissue culture, regeneration of tissues, drug delivery to cells and in screening devices. In this paper, current techniques related to fabrication of NF scaffolds and biomedical applications of these scaffolds will be reviewed.

KEYWORDS: nanofiber, scaffold, electrospinning, tissue regeneration, three-dimensional culture

INTRODUCTION

Many techniques have been developed to construct engineered human tissues at the laboratory level. Among many developed cell or tissue-scaffold constructs, nanofibrous (NF) scaffolds can mimic the structure and morphology of the extracellular matrix (ECM) [1]. Therefore, tissue-specific biochemical and structural features can be reproduced in NF scaffolds through optimal positioning of functionalized nanofibers. Natural biomaterials used for nanofibers are nontoxic, biodegradable, biocompatible and cost effective. Human and animal cells can be cultured into complex three-dimensional (3D) structures by using appropriate scaffolds. Limitations in cell infiltration and migration in NF scaffolds can be overcome by alignment of nanofibers. NF scaffolds are able to provide not only mechanical support for the cells but also the chemical cues for organization, growth, differentiation and migration of cells. This article focuses on the electrospinning methods for fabrication and biophysical modification of NF scaffolds and introduces the biochemical and medical applications of electrospun NF scaffolds.

1. Nanofibers

Elongated, slender and threaded structures are referred to as fibers. Structures having dimensions in nanometer range are called nanostructures, while nanotechnology is the study of the science and technology of structures having at least one dimension in nanometer range [2]. Nanofiber is one of the typical nanostructures, especially one-dimensional (1D), with its diameter up to several hundred nanometers. The small diameter

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and large surface to volume ratio of nanofibers lead to their wide range of applications in many fields including tissue engineering, drug delivery and nanoelectronics [2]. In general, native ECM is composed of nanofibers that offer structural integrity to tissues [3]. In this regard, NF structures mimicking ECM structures could be one of the most viable candidates for biomaterials in bioengineering applications [4].

Nanofibers are primarily developed using several methods, such as self-assembly, drawing, template synthesis and electrospinning [2]. Self-assembly is a process in which individual pre-existing components organize themselves into desired patterns and structures through weak and non-covalent forces, such as H-bonding and electrostatic interactions [5]. It yields radially arranged fibers with diameters less than 100 nm and lengths up to few micrometers. This process can offer novel properties and functionalities [6, 7]; however, it is a complex, long and an extremely elaborate technique with low productivity [8]. Drawing is a process capable of producing individual long nanofibers of viscoelastic material by micromanipulation-based stretching [2]. This process requires minimum equipment and produces fibers with diameter in the range of 2 to 100 nm; however it is a discontinuous process and highly susceptible to viscosity change induced by solvent evaporation [2]. Template synthesis is an extrusion process using a nanoporous membrane. Nanofibers of a wide variety of materials such as electronically conductive polymers, metals, semiconductors and carbons can be fabricated by this process [9]. In this case, the fiber diameter is dependent on the template pore size, and it varies from nano to submicron dimensions [10].

2. Fabrication of NF scaffolds using electrospinning

The term electrospinning, derived from electrostatic spinning, was used relatively recently (around 1994). However the fundamental idea of this process dates back to more than 60 years. During 1934 to 1940, Formhals obtained a series of patents, describing an experimental setup for production of polymer filaments using an electrostatic force [11-13]. Since 1980s and especially in the recent years, the electrospinning process has regained more attention and the number of publications in this field has been increasing exponentially in the past few years.

Electrospinning process consists of four stages, which are the formation of Taylor cone, the ejection of a stable straight jet, the evolution to the unstable whipping jet and the deposition on a collector, in order. A conventional electrospinning setup consists of a syringe pump, a conductive tip/nozzle, a high voltage supplier and a grounded collector. A droplet of a polymer solution is held at the nozzle tip by its own surface tension. Then, the interactions of the electrical charges in the droplet with the external electric field result in the formation of the well-known Taylor cone [14]. When the Taylor cone is subjected to a strong electric field with an appropriate field gradient, the droplet becomes unstable, and a single fluid jet is stretched out from the apex of the Taylor cone. After traveling linearly for a specific distance, the ejected liquid jet usually becomes unstable with respect to the jet propagation [15-17] before deposition on a collector. Electrospun nanofibers are influenced by various process parameters such as flow rate, electric voltage, tip-to-collector distance (TCD), size and shape of nozzle and collector, solvent and solution concentration as well as the polymer itself [2]. In addition, environmental factors like temperature, humidity and atmospheric pressure must be carefully considered [3].

3. NF scaffolds

A scaffold plays a pivotal role in tissue regeneration, which is drawing attention as one of the most promising therapeutic approaches to lost or damaged tissues [18, 19]. The design and development of scaffolds that mimic both the structure and function of the native ECM is considered as one of the most important tasks in the field [19] because the scaffold is expected to elicit specific cellular functions and to direct cell-cell interactions when it serves as matrices for tissue regeneration and cell transplantation [20]. One of the most crucial determining factors of the scaffold in cell viability is the polymer itself [21], because it has its own chemical and physical properties, such as surface roughness, degradation,

Table 1. Electrospun NF scaffolds of various polymers.

Target	Polymer(s)	Reference
Skin	Polycaprolactone (PCL)-Collagen	22
	Type I collagen	23
	Gelatin-Hyaluronic acid	24
	Collagen, Silk fibroin	25
	PCL-Polyethylene oxide	26
	Heparin-Poly(L-Lactic acid) (PLLA)	27
	Chitin	28
	Chitosan	29
	Poly(ethylene-co-vinyl alcohol)-Ag particles	30
Cartilage and bone	PCL	31
	PCL-Nanoclay	32
	PCL-Gelatin	33
	PCL-Poly(methyl methacrylate) (PMMA)	34
	Poly(lactic-co-glycolic acid) (PLGA)-Gelatin	35
	PLGA-Hydroxyapatite (HA)-Multi-walled carbon nanotubes	36
	PLLA-HA	37
	PCL-Poly(vinyl alcohol) (PVA)-Chitosan	38
	PLGA-HA	39
Nerve	Silk	40
	Collagen-PLGA	41
	Poly(anhydride-ester)	42
	PLLA	43
	PCL-Collagen	44
	PCL-Gelatin	45
Vascular	Polyurethane	46
	Polyurethane-Degrapol	47
	PCL	48
	Polydioxanone, Elastin, Collagen	49
	PGA (Polyglycolic acid)-Gelatin	50
	Gelatin-Heparin	51
	Polyurethane-Gelatin	52

strength, stiffness and permeability, which can be extended to NF scaffolds. Therefore, various polymers have been used in the fabrication of NF scaffolds using electrospinning to introduce their own material properties. Some of the polymers, composites and blends used in tissue regeneration as NF scaffolds are listed in Table 1 with their target tissues.

NF structures can be classified into three types based on the aspect of dimension: 1D, 2D and 3D structures. Here, we considered a single fiber as a 1D fibrous structure, which is invaluable to the applications such as nanocapacitor, nanocircuit and carbon nanotubes (CNT)-based nanowires [53]. Bioengineering predominantly requires 2D or 3D electrospun structures because of their geometrical similarity to ECM. Therefore, 2D and 3D NF scaffolds are mainly investigated here. A 2D NF scaffold can be defined as a structure with mat or membrane geometry fabricated by electrospinning. On the other hand, a 3D scaffold should occupy certain extent of the 3D space. However, the scaffold dimensions should be

considered carefully because it is relevant not only to geometry but also to cell behavior.

In general, NF structures fabricated using conventional electrospinning has a strong limitation in cell migration depth [54] because of its inherent small pore size. Therefore, a NF scaffold fabricated using conventional electrospinning cannot provide the environment favorable for cell migration even though it has a complete 3D geometry. In this aspect, regular pores and their interconnectivity (especially along the direction towards the scaffold center) must be considered as the most important factor in 3D NF scaffolds. As mentioned above, typical 2D scaffolds can be fabricated using conventional electrospinning, and thus, it is restrictively possible to adjust pore size and interconnectivity among pores by means of change in fiber diameter and density, or by introduction of holes using additional post-processing. However, it is difficult to extend the same structural changes to 3D NF scaffolds. Hence there is a strong need for 3D NF scaffolds with regular pores and interconnectivity among its outer and inner pores.

4. Advanced NF scaffolds

During the past decade there have been numerous improvements in fabrication of nanofibers, which are useful to various bioengineering and tissue engineering applications. Improvements in NF structure can be arranged as improvement in nanofiber morphology, improvement in nanofiber deposition and extension to 3D structures.

Shape and surface morphology of nanofibers can significantly influence cell behaviors, such as attachment and proliferation of cells on nanofibers. Therefore, variation in shape and surface morphology can lead to significant improvement of cell viability on the nanofibers. Bognitzki *et al.* presented that poly-L-lactide (PLLA) porous nanofibers could be fabricated by the consideration of solvent evaporation, which leads to phase separation into polymer-rich and polymer-deficient phases. Polymer-deficient sites on nanofiber surface are especially shown to form pores [55]. Moreover, flattened or ribbon-like nanofibers can be fabricated via the different approaches to solvent evaporation [56, 57]. Composite polymer solution has been electrospun through conventional electrospinning to attain material-coated fibrous structures for various applications. Some examples of composite fibrous structures include carbon nanotubes-poly(methyl methacrylate) (PMMA) composite fibers [53], hydroxyapatite-coated PCL for bone tissue engineering applications [58] and silver-polydopamine coatings on poly(vinyl alcohol) (PVA) fibers [59]. One of the dramatic improvements in nanofiber morphology resulted from introduction of coaxial dual nozzles, which were utilized in electrospinning two polymer materials. As a result, core-shell structured nanofibers could be attained [60, 61]. Moreover, the concept of the usage of multiple channels is extended to the fabrication of multichannel nanofibers [62]. This structure serves as a highly efficient method for delivering bioactive agents like drugs [63], genes and growth factors [64], as well as for cell encapsulation [65].

A variety of nanofiber deposition shapes can be made by the modification of collectors. There are several approaches to the modification of collectors, such as drum collector, pre-patterned collector and direct-writing, which can align nanofibers or control pore geometry and spatial distribution [66-70]. Aligned nanofibers can be fabricated using rotary drum or parallel conducting collectors [66, 67] and are widely used in various applications because aligned fibrous mats achieved the guidance and migration of cells in the desired direction in addition to adhesion, proliferation and differentiation of cells on the fibrous matrix [68]. In due course, various modified collectors were used to produce continuous aligned nanofibers. Sundaray *et al.* fabricated perpendicularly oriented and aligned nanofibers with the help of a rotary drum and grounded pinpoint electrode [69]. Another group used two parallel-positioned permanent magnets to produce aligned fibers [70].

Patterning nanofibers using a pre-patterned grounded collector is another attractive approach. Zhang and Chang demonstrated that patterned woven collector of metal wires could be useful in patterning nanofibers [71]. Moreover, they fabricated woven nanofibers using switched perpendicularly arranged parallel electrodes [71]. Cho *et al.* replicated complex patterns with multilayered nanofiber patterns [72]. In addition, photolithography using a pre-patterned mask was successfully introduced in patterning NF membranes or mats [73].

Direct-writing can be an effective approach to fabricate nanofibers with quality control of nanofiber deposition. Sun *et al.* demonstrated that electrospinning under short TCD and fast collector scanning could allow precise deposition of straight single nanofibers [74]. However, the process is more valuable in 1D NF structures with nanoelectronic applications, as mentioned above. On the other hand, direct-writing based on melt-electrospinning was developed and simple NF line patterns with a width of about 500 μm were fabricated under the condition of larger TCD and low speed 2D motion of a plate collector [75]. Recently, Lee *et al.* proposed a direct-write electrospinning (DWES) setup, which had both functionalities of focusing nanofibers and scanning collector, to fabricate various NF patterns [76]. They also employed a layer-by-layer approach to control pattern thickness without pattern width change. Consequently, it has been shown that useful patterned NF mats with various mesh size and thickness can be fabricated [76].

5. 3D NF scaffolds

The approaches to improve nanofiber deposition were successfully extended to building 3D NF structures. Pre-patterned collector based electro-spinning could be applied to the fabrication of 3D NF scaffolds by designing a template of the desired structure followed by deposition. Zhang and Chang enhanced their concept of using pre-patterned collector [71] to apply to the fabrication of 3D NF tubular structures [77]. They presented wonderful 3D tubular NF structures with fiber alignments using a cylindrical collector with equally spaced circular protrusions. Brown *et al.* presented that direct-writing based on melt-electrospinning could be directly used in building 3D fibrous structures without any post-processing [78].

Recent advances include the combination of electrospinning with other techniques to produce 3D NF structures. Yan *et al.* presented a well-defined 3D honeycomb-patterned NF structure fabricated using self-assembly of electrospun nanofibers [79]. In addition, electrospinning could be combined with 3D printing process to build 3D hybrid structures consisting of nano- and micro-fibers [80]. Moreover, 3D NF scaffolds, which were built by stacking the patterned NF mats fabricated using DWES [76], were successfully applied in tissue regeneration [81]. These 3D NF scaffolds had regular pores and higher interconnectivity among pores along the vertical direction. Moreover, their 3D NF scaffolds were combined with hydrogel encapsulating cells mediated by water layers to introduce a soft-tough mechanical property like that of human connective tissues [82].

6. Functional NF scaffolds

The behavior and fate of cells in ECM can be controlled by their microenvironment, including adhesion, secreted growth factors and cytokines. Cells attach strongly to nanofibers through improved focal adhesion of cells to the nanofibers and the physical entrapment of cells in the nanofibrillar network [83]. Nanofibers can be functionalized through the incorporation of bioactive materials during the spinning or through surface modifications after spinning. Addition of collagen, heparin and cationized gelatin to the surface of NFs was shown to increase cell infiltration [27, 84, 85]. Interestingly, immobilized heparin in nanofibers improved cell infiltration into the scaffolds, leading to prevention of blood clot formation around scaffold implanted in wound healing animal models [27]. Many types of bioactive agents, including growth factors, nucleic acids and integrin-binding ligands have been incorporated into polymer scaffolds [86, 87]. It was also demonstrated that cardiomyocytes cultured on fibronectin-coated chitosan surfaces exhibited a typical elongated shape with improved cell adhesion when compared to cells cultured on chitosan surface [88].

7. Cell culture in 3D NF scaffolds

Two-dimensional *in vitro* culture systems have different conditions from the *in vivo* niche. Therefore, various 3D cell culture systems to preserve structural and functional *in vivo* mimic complexity have been developed. The expression levels of heat shock protein (HSP)-70, a marker of cellular stress in astroglial cells, was shown to be significantly decreased in bioactive 3D culture when compared with a standard 2D culture system, and is similar to that of cells *in vivo* [89]. This result suggests that 3D culture systems provide a less stressful and *in vivo*-like culture condition.

Nanofibers form self-sustaining 3D scaffolds, but the desired infiltration of some kinds of cells into the inner regions of NF scaffold was not successful because of a lower porosity due to a denser packing of the fibers and the inherent planar structure of the meshes [90]. Recently, initial surface proliferation and subsequent depth penetration of cells could be observed with scaffolds electrospun from PCL [91]. A scaffold made of electrospun nanofibers has a high porosity which can directly affect the infiltration of cells [90].

NF scaffolds can be made of multiple fiber layers. Thin nanofiber meshes were constructed by electrospinning of a mixed solution of PCL and collagen and they were layer-by-layer stacked with cells to fabricate a 3D cell-scaffold construct [92, 93]. It has been demonstrated that in the multilayered 3D scaffold, human bone marrow-derived mesenchymal stem cells and human dermal fibroblasts continuously proliferated and deposited new extracellular matrix [92]. Moreover, subcutaneous implantation of the cultured construct containing stem cells into nude mice was demonstrated to be integrated with the surrounding tissues and neovascularization [92]. Nanofiber orientation in 3D scaffolds may affect cellular behavior [94]. When 3D cell/nanofiber constructs with pre-osteoblasts embedded among nanofiber layers were built via layer-by-layer assembly, cell polarization and osteogenesis were induced [95]. These results suggest that nanofiber alignment in 3D scaffold favors osteogenic differentiation of pre-osteoblasts and induce cellular elongation of embedded cells. The proliferation rate of cells in 3D scaffolds may also be influenced by the limited nutrient supply due to early formation of dense cell layers at the periphery of a scaffold, which leads to nutrient deprivation inside the scaffold [96]. These problems can be overcome by perfusion of media to NF scaffolds with the help of microfluidic devices.

Traditional 3D NF scaffolds have been constructed by “top-down” approach, in which cells are seeded onto a scaffold with biocompatible and biodegradable properties. Currently, bottom-up methods can be used to assemble microscale building blocks (e.g. microscale hydrogels) for 3D structures [97-101]. Bottom-up approaches will provide several advantages, including spatiotemporal control of 3D complex constructs with desired geometry, superior diffusion properties of microgels and fabrication of vascular-like structures [98].

8. Cell growth and migration in NF scaffold

Terminal differentiation of stem cells in NF scaffolds depends on physical and biochemical interactions with nanofibers as well as growth factors and cytokines. Recently, Zhong *et al.* developed multi-angle fluid flow stimuli in a microscale-platform integrated with aligned nanofibers to mimic the fibro-cartilage microenvironment [102]. Using this device, mesenchymal stem cells could be differentiated into fibro-chondrogenic phenotypes.

In vitro assays commonly used to study cell migration have some limitations to predict cell migration *in vivo*. Electrospun nanofibers have been used to study cell migration but have relatively small pore size for cell migration, because cell migration in NF scaffolds seems to be dependent on fiber diameter and pore sizes. Thus, none or a few cellular infiltrations occur inside the NF scaffold. Moreover, there are controversies on the mobility on and inside the NF scaffold. Some groups demonstrated that nanofibers were able to accelerate cell migration because they mimic the ECM, whereas in other reports they were seen to decrease cell migration due to increased adhesion of cells to nanofibers by increased expression of vinculin [83]. Rho *et al.* have shown that the mobility of keratinocytes on NF scaffolds was improved by the adsorption of ECM proteins to the surface of electrospun nanofibers [23].

Recently, alignment of electrospun nanofibers was shown to allow rapid cell migration and extension in NF scaffold compared to random NF scaffold [43, 44, 103, 104]. It has been suggested that the aligned nanofibers might provide greater spacing and change the spatial organization of pores between the fibers for more efficient cell migration [27]. Glioma cells seeded on aligned fibers maintained an elongated morphology and displayed decisive motion compared to cells seeded on random fibers [104]. Cell migration in aligned nanofibers may be due to fiber alignment-induced morphological alterations via focal adhesion formation [95, 105]. Moreover, migration of cancer

cells in aligned NF scaffold showed characteristic molecular features of 3D migration because glioma cells cultured on aligned NF scaffolds were more sensitive to myosin II inhibition and were less affected by stress fiber disruption than cultured cells on rigid surfaces [106]. Another group also demonstrated that aligned NF scaffolds enhanced epidermal skin cell migration across dermal wound when compared to a control group without scaffold [27]. Moreover, aligned NF promoted the infiltration of endothelial cells into the scaffolds.

9. Tissue regeneration

The cells in tissue regeneration should be attached to or cultured within some supporting structure or matrix that has been designed to facilitate the regeneration of tissue [1]. Cells can be seeded on biodegradable scaffolds made from natural biopolymer [107]. In addition, synthetic polymers such as PLGA, PCL, PLA (Polylactic acid) and PGA have been approved by the Federal Drug Administration (FDA) as biocompatible and biodegradable materials for clinical use. It has been demonstrated that coaxial electrospun NF scaffolds made from synthetic or natural polymers did not elicit immunological response *in vivo* [108]. In addition, functionalized nanofibers include growth factors, enzymes and DNAs to control development and differentiation of seeded cells. Therefore, nanofibers have been applied to many areas of tissue engineering such as skin, kidney, bone, cartilage, tendon/ligament, neural and cardiovascular tissues [97, 109].

For example, NF scaffolds have been used for nerve repair. Electrospun nanofibers provide contact guidance for directed neurite extension, leading to longer axonal protrusion to bridge the severe nerve defect [43, 110]. It was demonstrated that silk fibroin nanofibers with small diameters are more favorable to the development and maturation of neutrons cultured in neurobasal medium containing brain-derived neurotropic factor (BDNF), than larger diameter silk fibroin scaffolds [111]. Moreover, axially aligned fibers were able to improve peripheral nerve regeneration compared with random or circumferentially aligned fibers [104, 112, 113].

10. Delivery of biomaterials

As we described earlier, NFs can be functionalized by electrospinning. Functionalized NFs contain bioactive molecules (cytokines, proteins, drugs etc.) and deliver them to the cells [114]. The drug release profile can be controlled by the modulation of scaffold morphology, porosity and composition [1]. Chemically immobilized nerve growth factor (NGF) on aligned and random electrospun scaffolds produced from poly(ethylene glycol) and poly(e-caprolactone) (PEG-PCL) co-polymers enhanced neurite elongation and alignment of mesenchymal stem cells (MSCs) compared to non-biofunctionalized scaffolds [115]. Sahoo *et al.* demonstrated increased cell proliferation in fibroblast growth factor-2 (FGF-2) functionalized poly(lactide-co-glycolide) (PLGA) NF scaffolds [116]. However, electrospun nanofibers have the problem of burst release of drugs or proteins to a certain extent and the release of drugs in nanofibers cannot be controlled. It was demonstrated that the protein or biomaterials encapsulated in the core of nanofibers by coaxial electrospinning released slowly and steadily, while proteins in the blended nanofibers released in a burst manner [117, 118].

CONCLUSION

Nanofibers can be applied in various biomedical fields. Electrospunned NF scaffolds provide an ECM-mimic structure for cell attachment, spreading and infiltration through defined nanofiber diameter, alignment, density and functionalization. Cells cultured in the bioactive 3D NF scaffolds resemble cells *in vivo* when compared to cells cultured in 2D plastic surfaces. Alignment of nanofiber in scaffolds could induce dynamic morphological changes and 3D cell migration. In the near future, co-culture of different cell types will be possible in NF scaffolds by means of bioactively coated nanofibers.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interest.

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