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Control of Stem Cell Fate and Function by Polymer Nanofibers

Deepti Rana[1], Maria Leena[2,†], Manasseh Nithyananth[3], Renu Pasricha[4], Geetha Manivasagam[5], and Murugan Ramalingam[1,6,*]

[1] *Centre for Stem Cell Research (CSCR), A unit of Institute for Stem Cell Biology and Regenerative Medicine-Bengaluru, Christian Medical College Campus, Vellore 632002, India*
[2] *Department of Nanoscience and Technology, Karunya University, Coimbatore 641114, India*
[3] *Department of Orthopedics, Christian Medical College, Hospital Campus, Vellore 632004, India*
[4] *National Centre for Biological Sciences Tata Institute of Fundamental Research, Bangalore 560065, India*
[5] *Centre for Biomaterials Science and Technology, VIT University, SMBS, Vellore 632014, India*
[6] *WPI-Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan*

Stem cells are considered as an integral part of tissue engineering and regenerative medicine. Cellular functions of stem cells, which are responsible for tissue organization, can be controlled and regulated by providing an appropriate microenvironment, which mimics native stem cell niche. Nanotechnology is a powerful tool for engineering cellular microenvironment in the form of scaffolds. The scaffolds that have nanoscale features, for example, nanofiber, are considered as an effective substratum for tissue regenerative applications because they structurally mimic the native extracellular matrix (ECM). Electrospinning is a technique which produces polymer nanofiber scaffolds with controlled size and orientation of the fibrous structure. These polymer nanofibers can be used to control stem cell fate and function, in particular cell adhesion, proliferation and differentiation, during tissue engineering. In this article, we focus on recent developments and research trends in polymer nanofibrous scaffolds and their impact in controlling and regulating stem cell fate and function.

Keywords: Nanofibers, Stem Cells, Scaffolds, Cell-Material Interaction, Tissue Engineering.

1. INTRODUCTION

Tissue engineering aims to repair and regenerate the damaged tissues and organs, which fails to heal by themselves.[1,2] There are three key factors to be considered for the success of tissue regeneration: (i) the cells that create tissue/organ, (ii) the scaffold that provides structural support to cells, and (iii) cell-matrix (scaffold) interactions that direct the tissue growth.[3] Although the specialized adult cells remain an important source for tissue engineering, the use of stem cells has recently been recognized as a promising alternative to specialized cells owing to their enormous potential to differentiate into spectrum of tissues with adequate functions.[4] Stem cells, by definition, are immature or undifferentiated cells that are able to renew by themselves and differentiate into more specialized, tissue- or organ-specific cells.[5] This ability allows them to act as a good repair system for the defective tissues or organs in our body.

Stem cells require unique microenvironment to control and regulate their growth in three-dimension (3D) and probe their physiological functions during tissue organization. The cellular functions of stem cells can be modulated by various external cues/stimuli, such as mechanical stress, matrix elasticity/stiffness/orientation, soluble biochemical factors or matrix-mediated signals, presented by the microenvironment where the cells reside and grow. For example, it has been reported that in an artificially designed nanofibrous scaffold, the matrix fiber alignment or orientation controls the growth and functions of neural stem cells where the directional growth of neuronal axons is specifically controlled by the fiber orientation.[6] In another phenomenal study, mesenchymal stem cells (MSCs) are shown to specify lineage commitment and exhibit phenotypes with extreme sensitivity to matrix elasticity of the culture substrate.[7] In native tissues, stem cell

*Author to whom correspondence should be addressed.
†Short Term Project Internship Student of CSCR, India.

niche provides a complex array of physiochemical cues in a temporal and spatially defined manner, instructing the cells to attach, proliferate, migrate and differentiate. Thus creating a biomimetic microenvironment that supports the physiological growth and function of stem cells is of great importance for the success of tissue engineering.

A rapid advance in nanotechnology has led to the realization of engineering biomimetic stem cell microenvironment.[8] Creation of a nano-featured environment is believed to be one of the appropriate conditions for the proper growth of cells and subsequent tissues. This is because cells in the human body live in a complex mixture of pores, ridges, and fibers of ECM in a nano-featured environment.[9] Currently, there are several methods available for creating nano-featured scaffolds, which include electrospinning,[6] self-assembly[10] and phase separation.[11] Among them, electrospinning is considered a simple and versatile method in designing polymer nanofibrous scaffolds with controllable fiber size and orientation. Electrospun polymer nanofibers have unique functional properties such as high surface area, high aspect ratio, high porosity, small pore size, and low density.[6] These features are essential for the better cell adhesion, which is a significant issue in the initial stage of tissue engineering because cell migration, proliferation, and differentiation functions typically depend on it.[12, 13] In this article, we therefore focus on recent developments and research trends in polymer nanofibrous scaffolds and their impact in controlling and regulating stem cell fate and function.

2. STEM CELLS AND THEIR MICROENVIRONEMT

Stem cells hold promise for expanding list of therapeutic uses. Stem cells retain the exclusive properties of self-renewal and multi-lineage differentiation potential (potency) and in addition, they can contribute to the healing and tissue regeneration through their trophic and inflammatory effects. Depending on the potency of the cells, stem cells can broadly be categorized as multipotent (undifferentiated adult stem cells that can develop into more than one cell type, for example, cord blood stem cells) and pluripotent (primary undifferentiated cells that can develop the whole organism, for example, embryonic stem cells) in nature. Induced pluripotent stem cells (iPSCs) are, a new entry among the listed conventional types of stem cells. The iPSCs are, a type of pluripotent stem cells that can be generated by the reprogramming of the somatic adult cells (not the stem cells) to attain the pluripotent state. Regardless of the cell type, to direct cells to differentiate into specific lineage requires a right microenvironment.

For designing of an ideal microenvironment for stem cell culture one needs through understanding of structure and function of stem cell's native niche or microenvironment. Microenvironment contains both cellular and acellular components to provide complex array of physical and biochemical signals to the residing stem cells in a temporal and spatial fashion. In terms of biology, stem cell niche could be referred as the native microenvironment of the residing stem cells which could interact with them in order to regulate and manipulate their cellular fate and functions. For tissue development, various microenvironmental factors act on the resident stem cells to alter their gene expression, and induce proliferation or differentiation. It has been found that after a tissue injury, the surrounding microenvironment produces active signals for the stem cells to stimulate either self-renewal or differentiation to form new tissues. The major factors that control the stem cell characteristic within its microenvironment are stem cell-stem cell interactions, stem cell-neighbouring differentiated cells interactions, stem cell-adhesion molecules interaction, various ECM components, oxygen tension, growth factors, cytokines and the physiological conditions of the environment (such as pH, ionic strength and metabolites). ECM being the most important component of the native stem cell microenvironment, consists of hydrated and crosslinked networks of ECM proteins along with sugars and cytokines.[14] Stem cells and their microenvironment maintain a dynamic behaviour to induce the development and reciprocal maintenance of the tissues.

In the view of tissue engineering and regenerative therapies, stem cell microenvironments are of great interest for controlling the cellular fate and also to replicate the *in vivo* microenvironmental conditions *in vitro*. Through this way, stem cell proliferation and differentiation could be controlled in laboratory conditions (i.e., culture flasks/plates) and would result into sufficient amount of the proper cell type prior to their transplantation. These observations make the studies related to mimicking the native stem cell microenvironment as an alternative way for regenerating or repairing the damaged tissue, mainly for tissue engineering and regenerative medicine applications. Native microenvironment provides numerous biochemical, biomechanical, structural and topographical cues at micro and nano-scales to manage different signalling pathways of the residing stem cells.[15]

With the advancements in engineering techniques, nanomaterials have been used to provide these biochemical (growth factors, cytokines, enzymes and small cell-permeable molecules) and biophysical (surface topography, 3D geometry, matrix elasticity, substrate stiffness and external forces) cues to manipulate and regulate the cellular functions of the stem cells.[16] Nanomaterials could also provide the nanoscale topography of the collagen fibrils (50–500 nm) and its fibrous matrix structure to facilitate better stem cell attachment and proliferation. Since stem cells actively sense their microenvironment and react to the properties of their surroundings, polymeric nanofibrous scaffolds due to their tunable and nanoscale fibrous structure could provide a 3D microenvironment similar to the

native stem cell niche. Therefore, polymeric nanofibrous scaffolds could prove as a promising nanoscale substrate for mimicking the native stem cell microenvironment and optimizing their application into the clinic.

3. POLYMER NANOFIBER SCAFFOLDS

The scaffold, by definition, is a temporary supporting structure for growing cells and tissues. It is also called synthetic ECM. Nanofibers are defined as fibers with diameters on the order of a few hundreds of nanometres. ECM contains nanofibrous proteins that provide biological and chemical functions as well as physical support for cells to grow into specific tissues. In order to mimic such fibrous structures for cell culture and tissue engineering, nanofibers are developed using natural or synthetic materials. Polymers nanofibers are one of the most studied classes of materials as scaffold for tissue engineering, meniscus, cartilage, ligament and control of stem cell fate and function, owing to their structural and functional properties that can mimic the microenvironment of native cells/tissues.

A distinct advantage of nanofiber scaffolds is that they can be tailored to resemble the native ECM. Moreover, the orientation of fiber formation (i.e., alignment) can be optimized during fabrication to match the functional properties of the tissue to be engineered with these nanofibers as scaffolds. The nanofibers are also suitable for the surface modification of bioactive agents, where the biomolecules can either be immobilized or be adsorbed to enhance certain cellular functions, such as guiding neural stem cell (NSCs) elongation and their neurite outgrowth. The main challenge in utilizing electrospun nanofibers as tissue engineering scaffolds is cell seeding, which can be overcome by sacrificial biopolymer or cryospinning by creating pores of desired size within the electrospun matrix.[17]

Nanofibers can be manufactured by several techniques, including self-assembly, phase separation and electrospinning. Among them, Electrospinning is a simple, cost-effective, and versatile technique that essentially employs electrostatic forces to produce polymer fibers. The basic configuration of electrospinning consists of three major components (See Fig. 1): (i) a spinneret (ii) a fiber collector and (iii) a high-voltage power system. The spinneret is directly connected to a syringe, which acts as a reservoir for the polymer solution to be electrospun. This polymer solution can be fed through the spinneret with the help of a syringe pump at a steady and controllable feed rate. The fiber-collecting device is positioned right below the spinneret, with an appropriate gap (usually a few centimetres). A high-voltage/low-current power system is required for the conversion of polymer solution to a charged polymer jet. The electric voltage (usually up to 30 kV) is applied across the spinneret and the grounded metallic counter electrode (fiber collector) to facilitate the charged jet to eject from the spinneret tip toward the surface of the fiber collector.

The nanofiber scaffolds prepared by electrospinning have interconnected pores and high porosity in combination with large surface areas. Other characteristics, including ease of processing and tailorable size, shape, and mechanical properties of the fibers make electrospinning suitable for stem cells and tissue engineering applications.[18–20] This versatile process has the potential to produce 2D and 3D nanofiber assemblies that can provide various physical cues like random orientation; alignment;[21] layered nanofiber;[22] porous fiber;[23] core/shell structures;[24] multilayer structures, hollow structure; multichannel microtubes;[25] yarn;[26] nano/micro fiber composite;[27] patterned nanofiber;[28] 3D nanofibrous structure;[29] and Nanofiber Hierarchical Structures.[30] The following section discusses about the stem cells response to these polymeric nanofibrous scaffolds, associated interactions and the underlying mechanisms involved.

4. STEM CELL RESPONSE TO POLYMER NANOFIBERS

Stem cells have an ability to respond to the topographical features of the microenvironment and maintain a dynamic relationship with it.[31] For mimicking the natural stem cell microenvironment, electrospun polymeric nanofibers have proved to be the most promising candidate. Owing to its controlled nanoscale fiber dimensions, high porosity and large surface to volume ratios, polymeric nanofibers have been used widely to harmonize with stem cells to support cellular functions as well as to delivery various biomolecules/growth factors.[32, 33] Fibrous structure with nanoscale geometry of nanofibers mimics the collagen fibrils and make the cultured stem cells to sense these synthetic substrates as its natural ECM.[34] Topographic specificity and material specific properties have made nanofibers as the most relevant choice for cell substrate. In a recent study, Mirzaei et al. 2015, have studied the differentiation potential of the random and aligned electrospun carbon nanofibers (CNFs) cultured with human endometrial stem cells (hEnSCs) for neural differentiation.[35] The results revealed topographical sensitive behaviour of stem cells. In proliferative conditions, lower proliferation of hEnSCs was observed on aligned CNFs whereas under neural inductive conditions significant upregulation in neuronal markers was observed. Notably, slight upregulation of the oligodendrocyte-specific markers was observed on the hEnSCs cultured on random CNFs. Phenotypically, differentiated cells on aligned CNFs were found to extend along the main axis but stretched multidirectional on random CNFs. Except the topographical cues, material specific properties such as electrical conductivity and biocompatibility could also be attributed to its regenerative potential for neuronal lineage.[35] Similar to topographical cues, mechanical stress or matrix stiffness of the substrate have also been shown to manipulate differentiation potential of the stem cells. For instance, Sridhar et al. reported

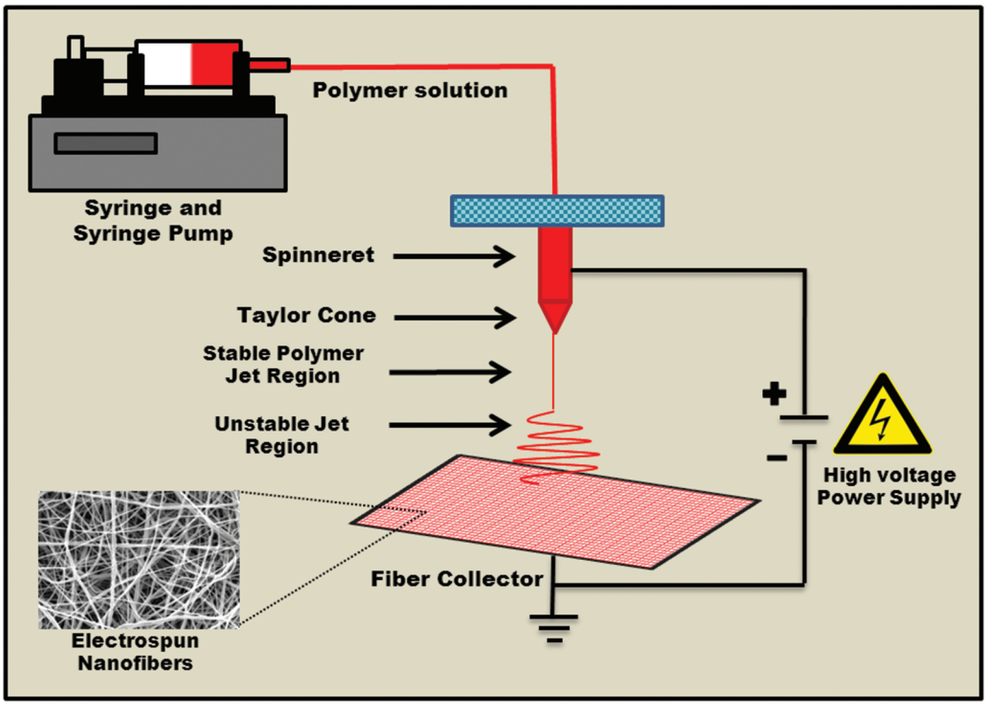

Figure 1. Schematic illustration of the electrospinning setup and process conditions.

that scaffolds with similar mechanical strength to the native myocardium could support MSCs to produce contractile proteins and achieve typical cardiac phenotype.[36] Gold nanoparticle (16 nm) loaded poly(ε-caprolactone) (PCL) nanofibers with mechanical strength of 2.56 MPa, were found to highly resonate the phenotype and cardiac marker expression in the differentiated MSCs.[36]

Other biophysical cues which could influence stem cell fate and functions are either passive mechanical inputs (provided from the base properties of the materials like fiber alignment, surface roughness and fiber elasticity) or active mechanical inputs (from extrinsic applied mechanical deformations). Nanofiber orientation can control the anisotropic mechanical properties of the scaffold, for example, non-aligned scaffolds shows isotropic tensile modulus of 2.1 ± 0.4 MPa whereas aligned scaffolds shows anisotropic modulus of 11.6 ± 3.1 MPa, suggesting that fiber alignment has a profound effect on the mechanical properties of scaffolds.[37] It has been reported that anisotropic nanofibers could control the human MSCs actin filament organization and cellular alignment, therefore could regulate the cellular behaviour.[37] Subsequently, fiber alignment was found to be strongly influenced by the morphology and distribution of stem cells. T*ussah* silk fibroin (TSF) aligned nanofibers with smaller diameter (400 nm) improved MSCs migratory speed in comparison with random large diameter (800 and 1200 nm) nanofibers.[38] Similarly, PLLA nanofibers cultured with MSCs showed topography dependent induction of various lineage-specific differentiation such as tenogenic, chondrogenic and osteogenic *in vitro* and *in vivo*. MSCs on the aligned substrate exhibited tenocyte-like morphology and enhanced tenogenic differentiation whereas randomly-oriented fiber scaffolds displayed enhanced osteogenic differentiation.[39] Elasticity, surface roughness and stiffness are other passive mechanical cues. Influence of elasticity on differentiation of MSCs was clearly demonstrated by Kuo[40] and wingate.[41] Polyurethane nanofibers which exhibit higher elasticity than PCL-nanofiber, supported osteogenic and chondrogenic induction potential of MSCs as compared to PCL fibers.[40] In order to study the role of compressive elasticity on MSCs differentiation towards endothelial cells, Wingate et al. developed 3D polyethylene glycol dimethacrylate nanofiber hydrogel matrix using electrospinning and photopolymerization techniques with elastic modulus ranged from 2 to 15 kPa (similar to the native intima basement membrane and media layer).[34] It was found that matrix elasticity could guide the cells to express different vascular-specific phenotypes with high differentiation efficiency. Almost 95% of MSCs seeded onto 3-D matrices with 3 kPa elasticity showed Flk-1 endothelial markers within 24 h, whereas only 20% of MSCs seeded on matrices with >8 kPa elasticity demonstrated Flk-1 marker. In contrast, $\sim$80% of MSC seeded on 3-D matrices with >8 kPa elasticity showed smooth muscle α-actin marker within 24 h, while less than 10% of MSC seeded on 3-D matrices with <5 kPa elasticity showed α-actin markers. This demonstrate that the local

elasticity of the substrate could control the MSC differentiation into either endothelial or smooth muscle-like cells.[41]

Other than the biophysical cues, these polymeric nanofibers and their composites could also be used to provide biochemical cues to the stem cells. Interestingly, biomimetic composite nanofibrous membrane of gelatin/β-tricalcium phosphate (TCP) have been shown to enhance osteogenic differentiation of bone marrow derived MSCs *in vivo* by activating calcium sensing receptor signalling.[42] Continuous release of calcium ions into the medium through the composite nanofibrous scaffold (gelatin/TCP) induced new bone regeneration with increased level of calcium-sensing receptor as a calcium signalling molecule in comparison with pure gelatin based nanofibers. With increase in TCP content within the nanofibrous scaffolds, more bone formation could be achieved *in vitro* as well as *in vivo*.[42, 43]

In addition to provide microenvironmental cues, polymeric nanofibrous scaffold could mimic the natural ECM and can be used as an alternative for natural ECM systems or feeder cells. For instance, mouse ESCs (mESCs) cultured on polymethylglutarimide (PGMI–a synthetic thermoplastic polymer) nanofibrous scaffolds without any feeder cells supplement, were able to self-renew and retain their pluripotency i.e., could be differentiated into all three germ layers.[44] Furthermore, gelatin nanofibrous substrates cultured with human pluripotent stem cells (hPSCs) under feeder- and serum-free conditions showed its suitability for long-term expansion of hPSCs and maintenance of stemness for over more than 20 passages without any abnormality. Through this way, nanofibrous scaffolds could be used to avoid enzymatic disassociation and mechanic cutting during passaging of these stem cells.[45] Interestingly, polyethersulfone (PES)-nanofiber based *ex-vivo* stem cell expansion technology in combination with subsequent re-expansion methods have been showed to support the expansion of human umbilical cord blood stem cells to upto 5 million fold yields within 24 days of the initial seeding. The re-expanded stem cells preserved their phenotype, biological functionality and multi-potential differential capabilities *in vitro*, such as, endothelial and smooth muscle lineages.[46] Altogether these findings further suggest that nanofiber-based *ex-vivo* expansion technology can generate sufficient numbers of biologically functional stem cells for potential clinical applications.[46]

Surface chemistry of the polymeric nanofibrous scaffold could also significantly affect the colony formation rate of the human iPSCs by controlling the colony edge

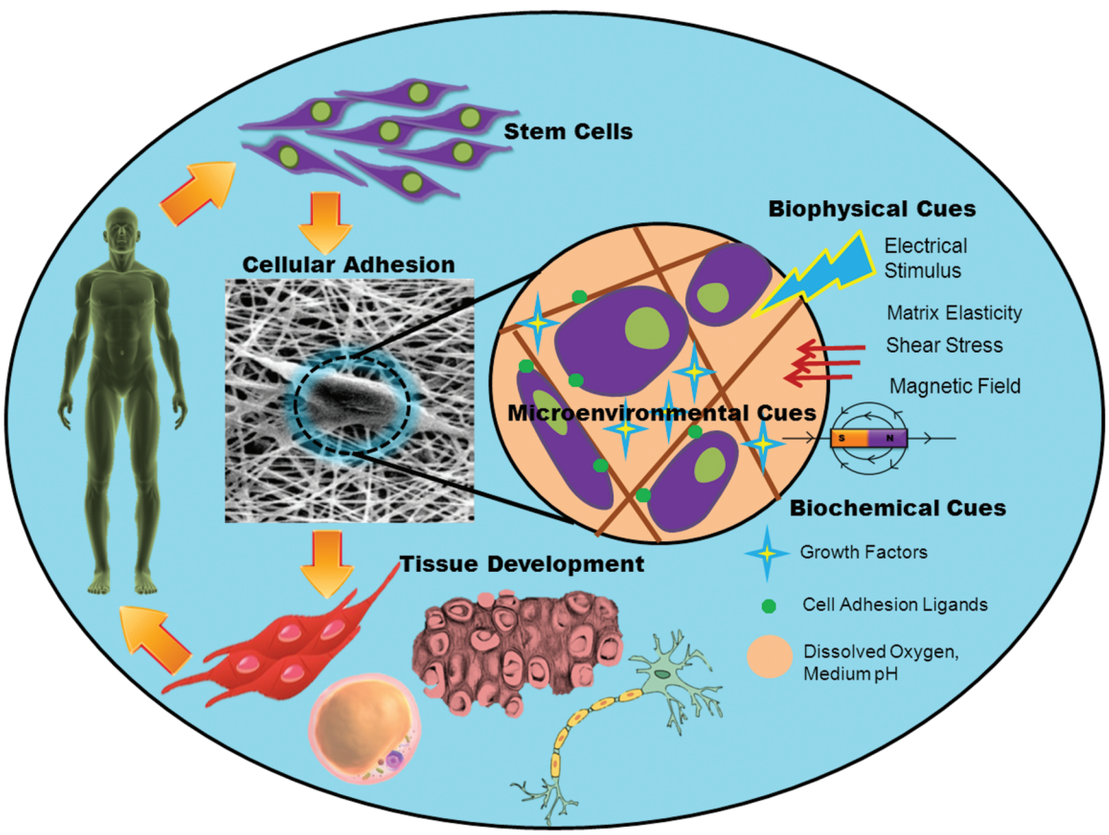

Figure 2. Schematic representation of stem cell response to the microenvironmental cues (such as biophysical and biochemical) provided by nanofibrous scaffolds. Biophysical cues include electrical stimulation, matrix elasticity, shear stress and magnetic field induction whereas biochemical cues relate to growth factors, signaling biomolecules, oxygen tension and matrix pH.

propagation. Moreover, nanofibrous substrates with surface chemistry controlled uniformly by collagen conjugation, the stiffness of the substrates were found to be inversely related to the sphericity (a degree of 3D colony morphology). The difference in the sphericity subsequently could affect the spontaneous differentiation of iPSCs during long-term culture. Therefore, electrospun substrates could be used to modulate the iPSCs self-renewal and lineage commitment by controlling the colony morphology. All these findings conclusively indicate nanofibrous substrates could effectively control the stem cell fate and functions by providing appropriate biophysical and biochemical cues as shown in Figure 2.

5. CONCLUDING REMARKS

Polymer nanofibers mimic the structural organization of native tissue microenvironment and thus these fibers have the ability to control the fate and function of stem cells, and other cells, primarily in the context of tissue engineering applications. Experimental studies discussed in this article elucidate some of the recent advancement and trend in nanofiber biomaterials for stem cells-based tissue engineering. The studies show that recent research in electrospinning techniques are focused on modulating properties of nanofiber scaffolds in terms of providing appropriate biochemical cues, biophysical cues, mechanical cues, and controlling delivery of biomolecules. Another main focus of recent studies is towards exploring the stem cell interaction with nanofiber biomaterials, in terms of signalling mechanism, cell migration etc., which are very essential to control stem cell behaviour and thus development of engineered tissues and organs. Collaborative efforts made in recent years between stem cell biologists and materials scientists give details on critical interaction between stem cells and nanofiber biomaterial, taking the stem-cell based tissue engineering research in directions of clinical relevance. Ongoing developments suggest that stem cell based tissue engineering with nanofiber biomaterial will outshine in the development of more patient specific tissues and organs.

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