The Nanofiber Matrix as an Artificial Stem Cell Niche

Hai-Quan Mao, Shawn H. Lim, Shuming Zhang, Gregory Christopherson, Korey Kam and Stephen Fischer

Abstract Nanofibrous matrices serve as artificial substrates to partially mimic the key physical and structural characteristics of the stem cell niche. Three methods for the preparation of nanofibrous matrices, including electrospinning, solution phase separation and self-assembly are reviewed and contrasted. Their effect on stem cell adhesion, survival, migration, proliferation and differentiation are discussed. In particular the applications of these nanofibrous matrices for the expansion, differentiation and delivery of stem/progenitor cells are highlighted in this chapter. These matrices provide great opportunities to modulate cellular behavior and tissue regeneration at the nanometer scale.

1 The Stem Cell Niche

The term "stem cell niche" was coined in 1978 by Schofield, who proposed the notion that stem cells reside in vivo within specialized compartments, or niches, which maintain these cells in an immature and proliferative phenotype [1]. The

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stem cell niche constitutes the natural microenvironment that surrounds stem cells, and encompasses both cellular and acellular components. The niche is believed to provide a complex array of physical and biochemical signals to stem cells in a temporal and spatial fashion; by integrating both local and systemic cues, the niche actively engages these cells and guides their proliferation, migration, and fate specification [2–5]. Consequently, the niche is both an anatomical and functional entity, and it plays a vital role in regulating how stem cells participate in tissue regeneration and repair [6, 7].

Important lessons can be learned by examining the structure–function relationships of various niche components, and recent studies have provided greater insight into how these niche components act in concert to regulate stem cell behavior [6–9]. For instance, there is mounting evidence demonstrating the importance of cell adhesion molecules in providing selective anchorage to various types of stem cells through both cell–cell and cell–extracellular matrix (ECM) interactions [2, 5, 10, 11]. Such adhesion-based interactions are likely cell-type dependent and are vital to stem cell survival and activity (e.g. phenotype maintenance, migration and proliferation). In addition to providing anchorage, another important function of niche ECM components is the local regulation of "instructional" signaling cues by manipulating the concentration and presentation pattern of signaling molecules (Fig. 1) [12]. In other words, the niche appears to provide the context by which these biochemical cues act on stem cells to orchestrate their biological functions. Thus, these signaling molecules have to be viewed as parts of a multifaceted spatial and temporal network regulated by the ECM and niche cells [13].

In light of the vital role that the stem cell niche appears to play in vivo, the niche concept has been increasingly recognized as a guiding principle for designing systems for the ex vivo manipulation of stem cells and stem cell-based therapies. Engineered artificial matrix systems with a well-controlled presentation of cellular,

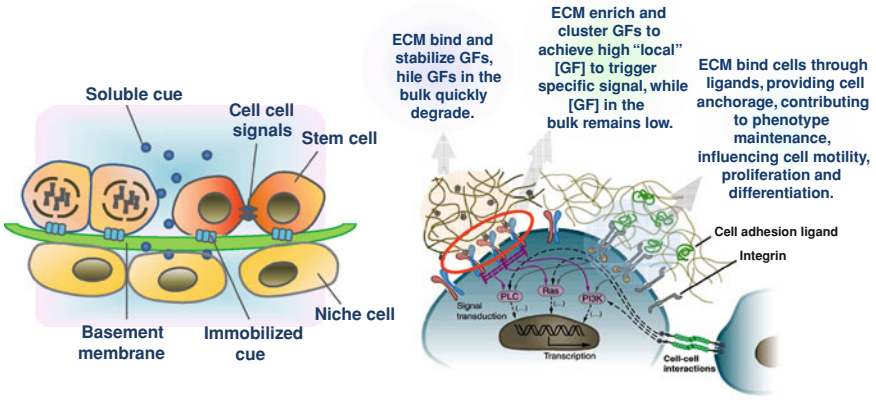

Fig. 1 The concept of the stem cell niche, and role of the extracellular matrix in regulating stem cell survival and signaling. *ECM* extracellular matrix, *GF* growth factor; [*GF*] growth factor concentration. Matrix topography may facilitate these processes. Adapted from [14]

molecular, and physical cues offer a systematic approach to correlate effects of niche components on stem cell behavior. Such systems will not only help to further identify the key niche components and their functions, but also provide feasible matrices for ex vivo stem cell expansion and controlled differentiation. Certainly, the level of complexity of native stem cell niches makes replicating these a great challenge.

2 Nanoscale Topography in the Extracellular Matrix

It has long been recognized that the physical properties of a cell culture substrate, including its topographical, mechanical, and electrical properties, significantly impact cell adhesion, survival, migration, proliferation, and differentiation in vitro. In many cases, the cell type-specific behaviors observed in response to these physical stimuli in vitro can be correlated in some fashion to the native environment in which the cells reside in vivo. For example, recent studies have demonstrated that stem cells can be differentiated towards tissue-specific lineages by matching the mechanical stiffness of a substrate to that of the desired tissue [15, 16]. Likely, the physical properties of stem cell niches play an important role in regulating stem cell behavior in vivo. A major challenge in engineering artificial stem cell niches is to properly identify the relevant physical features of native niches, which are likely different for each stem cell type.

A fairly obvious physical feature of many stem cell niches is the presence of nanoscale topography. For example, ample evidence has shown that the in vivo ECM (e.g. basal lamina) possesses ubiquitous nanoscale fibrous morphology (Fig. 2) [17–21]. Additionally, in-depth microscopic analysis of tissues where stem cells reside has revealed topographical features with both micro- and nanoscale fibrous structure [22–25]. Despite the existence of ECM fibrous features associated with the various niches, the most substantial evidence of the role of topography in regulating stem cell fate has been derived in vitro. A number of studies have shown that various cell types respond to topographical features of the substrate upon which they are cultured [21, 26]. Various model substrates with controlled micro- and nanoscale surface features including islands, pillars, gratings, and fibers have been used to investigate the impact of surface topography on stem cell behavior. For instance, when cultured on parallel grooves, cells elongated and aligned in the direction of the grooves; and this morphological change was facilitated by reorganization of the cytoskeleton structure [27–30]. This reorganization affects cell phenotype and function by triggering cytoskeleton-linked intracellular signaling pathways, for example, the reduction in smooth muscle cell proliferation when cultured on 350 nm-wide gratings [21]. Using the mammalian visual system as a model, Ellis-Behnke et al. have shown that a nanofiber scaffold provided a permissive environment to promote axon regeneration and brain repair, resulting in functional recovery of vision [31]. These results, while highlighting the significance of nano-topographical factors as a general signaling cue, provide impetus for further mechanistic investigation into their influence on stem cells.

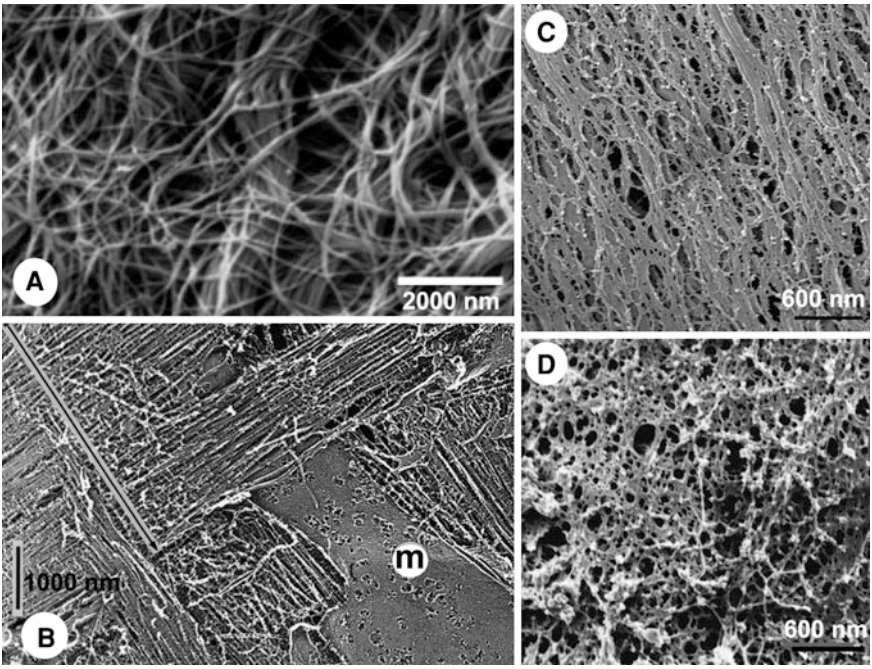

Fig. 2 Examples of extracellular matrices and associated nanotopography. **a** The marginal layer of pars distalis facing the cavum hypophysis in goat; **b** replicas of the developing embryonic corneal stroma at 18 days, and areas with collagen fibrils that are arranged in lamellar structure that criss-cross in a quasi-perpendicular manner. **c** Ultrastructural basement membrane architecture of carotid basement membrane, and **d** inferior vena cava basement membrane. Adapted from **a** [18]; **b** [32]; **c** and **d** [24]

3 Methods to Generate Nanofibrous Matrices

Several methods have been developed to investigate the role of nanoscale topography in regulating stem cell behavior. We will highlight three common techniques, which have been used to generate nanofibrous structures both for ex vivo stem cell culture and in vivo stem cell delivery.

3.1 Electrospinning

Electrospinning is the most widely used method for producing nanofiber matrices, and is prized for its high versatility and capacity to generate nanofibers from a variety of polymer solutions or melts. The diameter of electrospun fibers ranges from tens of nanometers to a few microns [33]. A standard electrospinning setup includes a spinneret (a single or an array of blunt needles or glass pipettes), a

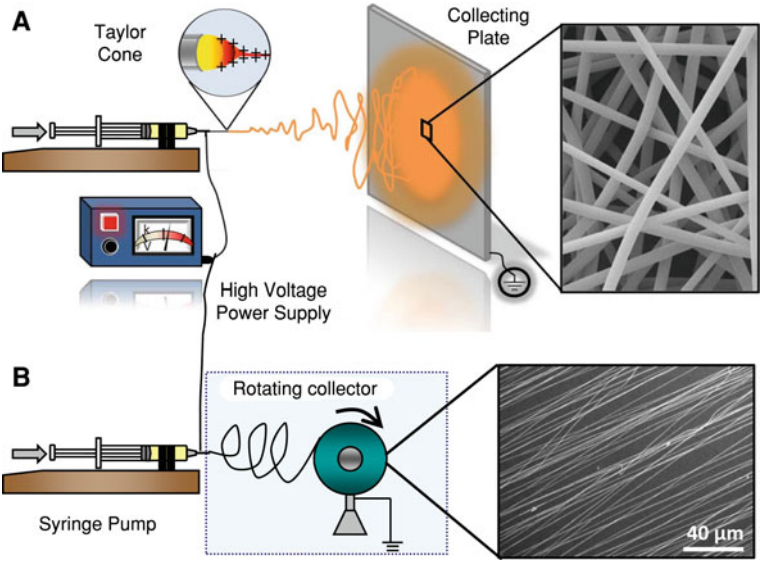

Fig. 3 Electrospinning setup and collection. **a** A typical Electrospinning setup includes a spinneret, a syringe pump, and a collecting plate. Polymer solution or melt is stretched into a thin fiber under applied high voltage, whipping in a random manner before depositing on grounded collecting plate. **b** When a rotating wheel or frame is used for collection, electrospun fibers can be aligned

syringe pump, and a collector plate (Fig. 3a). When the polymer solution or melt is extruded, a high electrostatic potential (usually 5–50 kV) is applied between the spinneret and grounded collector plate, inducing charge accumulation on the surface of the fluid. With increasing electrostatic repulsion, the surface of the fluid at the needle opening forms a conical shape zone (i.e. the Taylor cone) [34]. When the electrostatic repulsion overcomes the intrinsic surface tension of the polymer solution or melt, a charged jet of fluid is ejected from the Taylor cone, whipping in a random orientation before accumulating on the grounded collecting plate. The small diameter of electrospun fibers is mainly achieved by the stretching and acceleration of the viscous jet before fibers land on the collecting plate. Solvent evaporation and polymer melt solidification can occur before or after the fiber collection, depending on the parameters and electrospinning set-up. Due to the bending instability associated with a spinning jet, electrospun fibers usually deposit on the collecting surface as randomly oriented nonwoven mats. However, when these fibers are collected onto rotating wheels or frames, they can form a mesh of aligned nanofibers (Fig. 3b) [35, 36]. Flexibility in controlling the nanofiber spatial orientation is a distinct advantage of electrospinning method.

Although relatively straightforward in design, the electrospinning process itself is rather complicated. Many materials properties (such as solubility, glass-transition temperature, melting point, crystallization velocity, molecular weight, molecular weight distribution, entanglement density, solvent vapor pressure, and pH) and processing parameters (such as polymer concentration, electrical

conductivity, surface tension, feed rate, electrode separation and geometry, temperature, and relative humidity) can affect fiber diameter and arrangement of collected nanofibers. Systematic optimization is often required to achieve control over nanofiber morphology, diameter, composition, secondary structures and spatial arrangement. In such a process, the polymer material and solvent combination is generally the first consideration. After selecting an appropriate solvent, parameters such as solution feeding rate, solution concentration, electrical conductivity and inclusion of surfactant can be modulated to control the diameter of produced nanofibers. Generally speaking, lower flow rate and concentration, addition of surfactant [37] and higher electrical conductivity lead to a reduction of fiber diameter [38]. For spatial organization of nanofibers, aligned fibers can usually be obtained by utilizing a rotating collector [36] or creating a quadratic arrangement of four electrodes to force a cross deposition of nanofibers [39]. A shorter distance between spinneret and collection plate (e.g. centimeter or millimeter range) can also achieve a higher degree of alignment [40, 41].

To expand the scope of possible application for electrospun fibers, a variety of improvements have been made to increase the diversity of materials and introduce surface functionality. Polymer blends have been used to prepare electrospun fibers with nanoscale morphologies [42] or a core-sheath structure [43], and natural biomaterials have been electrospun into fibers. The most frequently used natural materials include collagen [44], chitosan [45], gelatin [46] and laminin [47]. Carrier polymers can also be used to facilitate the electrospinning of materials that otherwise cannot be processed alone, and composite or protein nanofibers.

Electrospun fibers have been extensively used as tissue engineering scaffolds and as culture platforms for various types of stem cells. To facilitate cell adhesion, fiber surfaces have been modified using different techniques, including physical adsorption or covalent conjugation of cell adhesion proteins, plasma treatment to introduce surface charge, and grafting polymerization to generate surface functional groups, etc. [48]. For example, electrospun fibers have been coated with positively charged poly-L-ornithine (PLO) and laminin to promote the adhesion of neural stem cells [49]. As one example of chemical functional group grafting, Chua et al. have introduced polyacrylic acid chains to the surface of polyethersulfone nanofibers by UV-initiated surface grafting polymerization, and subsequently converted the carboxylic groups to amino groups to achieve high amine density on fiber surface. These amine-grafted nanofibers have promoted the phenotype maintenance of hematopoietic stem cells during expansion culture [50–52]. Beyond these efforts, electrospun nanofibers have also been covalently conjugated with cell-specific bioactive ligands to enhance cell adhesion, proliferation, and differentiation [53].

3.2 Self-assembly

The formation of functional nanofibers via self-assembly is also an area of growing interest. The term “self-assembly” is used to describe the process in which a

disordered system of pre-existing components forms an organized structure or pattern as a consequence of specific, local interactions among the components themselves, without external direction [54, 55]. Using this approach, scientists have developed a family of self-organized nanostructures such as zero-dimensional objects, spherical micelles, cylindrical micelles, ribbons, two-dimensional sheets, and three-dimensional networks [56]. The strategies employed in controlling geometries of self-assembled structures usually require careful design of both molecular shape and molecular interactions [57].

One example of a self-assembled nanofiber matrix is prepared from a de novo-designed water-soluble amphiphilic peptide, consisting of a hydrophobic alkyl tail connected to a short peptide sequence (Fig. 4a) [58–60]. Upon addition of counter ions or pH titration, these molecules can self-assemble into networks of well-defined nanofibers. This process is triggered by charge screening using counter ions and facilitated by the collapse of the alkyl groups from aqueous solution as well as hydrogen bond formation between adjacent peptide residues.

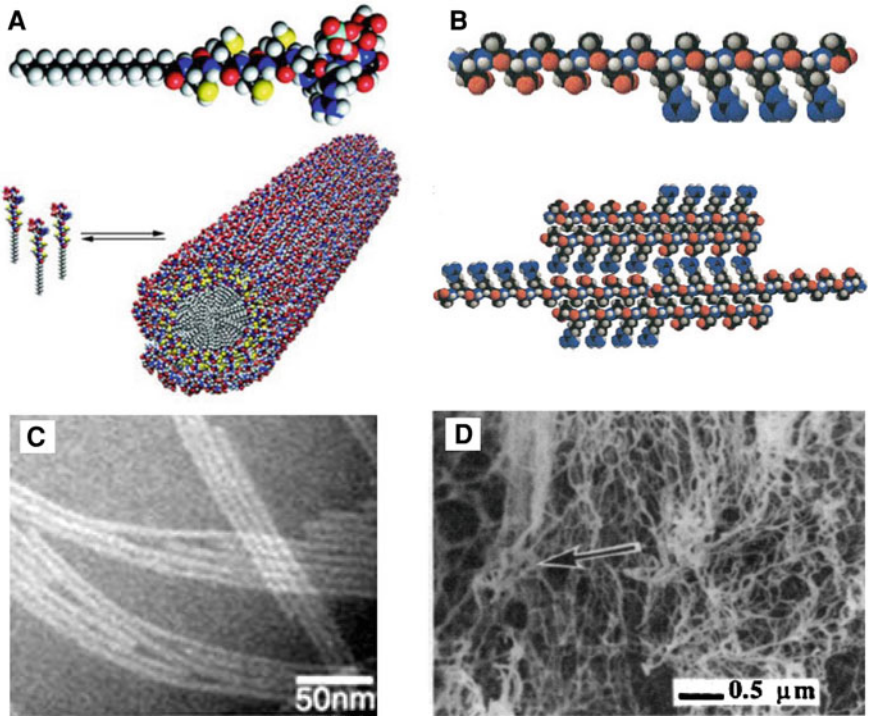

Fig. 4 Self-assembly illustration of **a** a peptide amphiphile [peptide sequence: $C_{15}H_{31}CO$-CCCCGGGS(P)RGD, a transmission electron microscopic image of the assembled fiber is shown in **c**] and **b** a β-sheet forming peptide (DADADADARARARARA, a scanning electron microscopic image of the assembled fibers is shown in **d**). Schematic of the molecules are shown, which further self-assemble together to form a cylindrical micelle. Adapted from [58] and [65]

This nanofiber formation can be initiated at physiological pH and ionic strength, making it possible for cell encapsulation and in vivo applications [61, 62].

Another type of well-studied nanofiber matrix is prepared from peptides that self-assemble into β-sheets in physiological medium (Fig. 4b) [63–65]. The amino acid sequence of these peptides consists of alternating hydrophobic and hydrophilic residues, with the hydrophilic residues carrying alternating positive and negative charges. Upon addition of physiological medium, these molecules self-assemble into networks of well-defined nanofibers, resulting in a hydrogel with about 99% water content [64]. The mechanical moduli of these peptide hydrogels approach that of a hydrogel prepared from type I collagen. Since self-assembly is mediated by intermolecular forces, the hydrogels can be easily deformed and re-assembled under shear, allowing them to be delivered in vivo through injection (with or without cells) to promote tissue regeneration.

In both of these peptide nanofiber systems, ECM-derived short cell adhesion peptides can be incorporated into the sequences of self-assembling peptides to enable cell binding and migration through the matrix. For example, a laminin-derived peptide sequence, isoleucine-lysine-valine-alanine-valine (IKVAV), included in a peptide amphiphile can be decorated onto self-assembling nanofiber surfaces. This IKVAV-conjugated nanofiber matrix enhanced cell adhesion and differentiation of neural progenitor cells [61]. While linking functional short peptide sequences to self-assembling peptides has been successful, the incorporation of larger bioactive proteins is challenging. An alternative approach is to covalently tether biotin to the nanofiber surface, and subsequently immobilize growth factors to biotin moieties using the biotin–streptavidin linkage. Using this approach, Davis et al. have achieved local and sustained release of insulin-like growth factor 1, known to increase cardiac stem cell growth [66].

It is generally difficult to control the alignment of these self-assembled nanofibers due to a lack of long-range order. Recent developments by Zhang et al. have shown that through a thermal induction process, long range alignment of some peptide-based self-assembled nanofibers can be achieved [67]. It remains to be demonstrated whether these aligned nanofibers can be produced in larger scale for stem cell cultures and/or therapeutic delivery.

3.3 Solution Phase Separation

Polymer solutions can be thermally induced to undergo phase separation. Under appropriate conditions, the separated polymer-rich phase can form a nanofibrous structure [68, 69]. This fiber formation occurs through the spinodal liquid–liquid phase separation (Fig. 5) and a consequential crystallization of the polymer-rich phase. When temperature decreases to below the spinodal transition such as that shown in Fig. 5a, the liquid–liquid phase separation spontaneously occurs through a spinodal-decomposition mechanism [70]. A typical procedure of generating nanofiber matrices includes phase separation-gelation, solvent extraction, freezing,

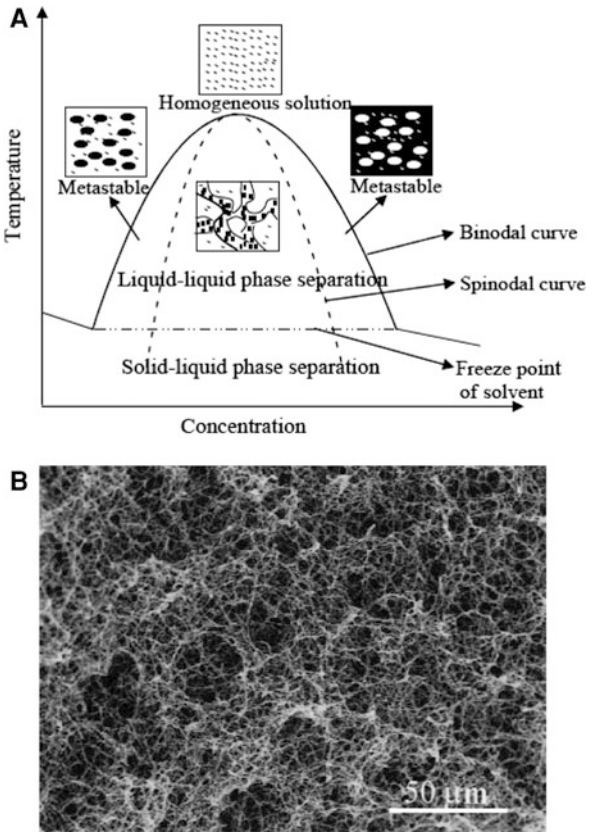

Fig. 5 Phase separation technique used to create nanofibrous matrix. **a** Schematic phase diagram of polymer solution [70] and **b** SEM micrograph of porous nanofibrous matrix made with liquid–liquid phase separation technique [68]. Adapted from [70] and [68]

and lyophilization. The diameters of fibers generated using this method range from tens to hundreds of nanometers. The phase separation-gelation is the most critical step of this process and important for the control over the porosity and fibrous morphology. Low gelation temperature favors the formation of the nanoscale fiber network. Low initial polymer concentration leads to smaller diameter fibers and a more porous network [70]. Based on this principle, many polymer–solvent systems that have similar phase diagrams may be used to generate nanofiber scaffolds with the phase separation technique.

One advantage of phase separation is the ease of incorporating macropores into the nanofiber scaffolds by inducing phase separation in the presence of salt or paraffin particles [71]. These porogen particles are leached-out after lyophilization by soaking the scaffold in appropriate solvents (e.g. water for salt particles and hexane for paraffin particles). This method generates both macroscale (250–425 μm) pores and nanoscale fibers [72]. Recently, this method has been extended to prepare a macroporous and nanofibrous scaffold from gelatin, which mimics both the nanotopography and chemical composition of type I collagen in the ECM. The gelatin nanofiber scaffold has a porosity of higher than 96%, well-connected

macropores, and nanofibrous pore wall structures. The nanofiber diameters ranged from 160 to 180 nm [73].

The phase separation method provides a convenient way to generate polymer nanofiber scaffolds without any special equipment; it is easy to scale up; and it is possible to control the porosity and macropore size of the scaffold. Thus far, only preparation of a randomly oriented nanofiber matrix has been reported with this method.

3.4 Comparison of Nanofiber Generation Methods

Electrospinning, self-assembly, and thermally induced phase separation techniques produce nanofibers with slightly different feature sizes and physical properties (Table 1). Electrospinning is the most versatile method and has been used to generate fibers from a wide range of materials. The phase separation method requires more stringent selection of the solvent–polymer pairing that allows spinodal liquid–liquid phase separation to occur. The self-assembly method has only been successful in creating nanofibers from peptide amphiphiles and β-sheet forming peptides. The diameter range of self-assembled nanofibers is typically much smaller than electrospun fibers and nanofibers prepared by solution phase separation. Self-assembled nanofibers are used in hydrogel forms and can be injected, whereas the other two types of fiber matrices are used as solid scaffolds. In addition, surface biofunctionalization techniques have to be specifically tailored for each type of matrix.

Table 1 Comparison of nanofiber matrices prepared by electrospinning, self-assembly and solution phase separation methods

Fiber preparation method	Electrospinning	Self-assembly	Solution phase separation
Materials choice	Wide range of natural and synthetic polymers, ceramic fibers can also be prepared from their precursors	Peptide amphiphiles β-sheet forming peptides	Selected natural and synthetic polymers
Fiber diameter	50 nm to 2 μm	5–20 nm	50–500 nm
Physical form	Solid fiber matrices	Soft hydrogels, injectable	Solid fiber matrices
Incorporation of macropores	Possible	Difficult	Easy to incorporate with porogens
Cell adhesion property	Surface adsorption or covalent conjugation of ECM proteins and other ligands	Direct peptide conjugation or through biotinylation scheme	Surface adsorption or covalent conjugation of ECM proteins and other ligands

4 Nanofibrous Matrices for Stem Cell Expansion

Due to their capacity for long-term self-renewal and ability to differentiate into multiple specialized progeny, stem cells have enormous potential for use in a variety of cellular therapies. In order to achieve therapeutic outcomes in most cases, a much larger number of stem cells is required than can be obtained from a patient, necessitating the use of ex vivo culture systems to expand the initial cell population. In addition to quantity, both the quality (maintaining stem cell phenotype and regeneration potential) and purity (free of contamination from other cell types and animal products) of the expanded stem cells are important. Certainly, robust and efficient methods for producing large populations of high quality stem cells are needed.

In recent years, researchers have engineered a variety of artificial culture systems for the efficient ex vivo expansion of stem cells. Because stem cells are extremely sensitive to their local microenvironment, such systems are designed to provide a well-controlled presentation of cellular, molecular, and physical cues. Here we describe how nanofiber scaffolds have been used to facilitate the undifferentiated expansion of a variety of stem cell types.

4.1 Nanofiber-mediated Expansion of Human Hematopoietic Stem Cells (HSCs)

Human HSCs constantly undergo self-renewal and differentiation within the bone marrow microenvironment, whereas in culture they tend to lose their self-renewal ability. It is believed that HSC self-renewal may be subject to regulation by the biochemical and topographical cues in the bone marrow niche [74–76]. Such biochemical cues include cell-secreted hematopoietic factors (HGFs) and contact with the stroma and ECM molecules [77]; whereas the topographical cues, likely originating from the 3D architecture of the bone marrow, can influence cell–cell and cell–substrate interactions. A successful HSC expansion strategy should ideally mimic these key characteristics of the bone marrow stem cell niche. Current HSC expansion methodologies have incorporated various components of the niche, and can be categorized into either a biological approach or a biomaterials approach.

The biological approach uses a stromal monolayer to provide biological signals to HSCs [76]. In the first few weeks of culture, an adherent layer of stromal cells comprised of fibroblasts, macrophages, adipocytes, endothelial cells and reticular cells is laid down, and serves as a substrate for the subsequent HSC culture. HSC self-renewal can be maintained for months through direct interactions between the HSCs and various elements of the stroma. Although these studies demonstrate that the stromal layer, to a large extent, can promote the self-renewal of human HSCs, it has a number of disadvantages. The stromal layer is fragile thus requires a rigid surface for support, and has a limited lifespan of about 6–8 weeks. More

importantly, its use for clinical applications poses logistical problems. First, the need to collect and then grow a layer of the patient's own stromal cells adds significantly to the time, cost, and complexity of the production of autologous HSCs. Second, it could be problematic to obtain healthy stromal cells from patients with other diseases. Third, the composition of stromal layers is poorly defined. It is difficult to harvest and culture the stroma in a reproducible fashion, which renders the expansion outcome less predictable. Allogeneic sources of stroma, although feasible, are unreliable. They pose potential infection risks and contain an insufficient quantity of primary stromal cells. An additional complication is that the stromal layer may produce negative regulators of hematopoiesis such as transforming growth factor TGF-β and chemokines [78, 79].

Due to these concerns, stromal-free culture has gradually emerged as an alternative approach, using various combinations of hematopoietic growth factors to substitute for the regulatory signals provided by the stroma [80, 81]. Stem cell factor (SCF) and Flt-3 ligand (FL) have been shown as the key growth factors for HSC expansion, because c-Kit and Flk-2/Flt-3—the tyrosine kinase receptors for SCF and FL, respectively—have been shown to transduce signals crucial for HSC development. Thrombopoietin (TPO), a ligand for c-Mpl and originally identified as a primary regulator for megakaryopoiesis, has also been shown to act synergistically with SCF and IL-3 to stimulate the expansion of primitive hematopoietic cells [82]. Simplicity is a major advantage of the growth factor-supplemented suspension culture. However, different growth factor and cytokine cocktails have only yielded limited success [74, 83]. This is probably because these suspension cultures lack other components of the unique regulatory microenvironment of bone marrow stroma.

The biomaterials approach aims to partially mimic the three-dimensional (3D) aspects of the bone marrow microenvironment. Several studies have indicated that the 3D culture microenvironment contributes to the regulation of HSC proliferation and differentiation. For example, Bagley et al. have shown that a 3D tantalum porous scaffold supports the maintenance of primitive $CD34^+CD38^-$ cells for up to 6 weeks and yields a 6.7-fold increase in colony-forming cells (CFCs) without supplementing cytokines in serum-free medium [84]. Li et al. cultured human cord blood-derived HSCs in a nonwoven polyethylene terephthalate (PET) mesh and obtained significantly higher numbers of $CD34^+$ cells and colony-forming cells (CFCs) after 7–9 weeks of culture in the scaffold compared with 2D substrate cultures [85].

Independent of physical topography, HSC substrate adhesion through β_1 integrins has been shown to be important for the regulation of HSC survival and homing to the niche [86]. Binding of integrin receptors to surface-coated fibronectin may contribute to improved homing of the expanded HSCs after transplantation, since the engraftment ability is lost if HSCs are cultured in suspension culture. The cell–substrate adhesion through integrin binding is also thought to prevent terminal differentiation in HSCs. Feng et al. prepared FN-conjugated PET fibrous scaffolds and investigated the effect of HSC adhesion in a 3D scaffold on cell expansion [87]. FN-conjugated PET fibrous scaffolds mediated higher cell expansion efficiency than FN-conjugated PET films or tissue culture polystyrene

during a 10-day expansion culture in serum-free medium. Human umbilical cord blood $CD34^+$ cells cultured in FN-conjugated scaffolds also gave the highest yield of long-term culture initiating cells compared with all other substrates.

The effect of nanoscale topography on HSC expansion was first demonstrated using a series of electrospun polyethersulfone (PES) fibrous matrices with various types of surface functional groups: carboxylic, hydroxyl, and amino groups in comparison with unmodified fibers [88]. The functionalized PES nanofibers had an average diameter of 529 ± 144 nm with a surface functional group density of 55 nmol/cm^2. Ex vivo culture of human cord blood-derived HSCs showed that surface amino groups in synergy with a nanofiber matrix significantly enhanced the expansion of total $CD34^+$ cells and colony-forming unit (CFU) cells. Moreover, HSC expansion efficiency on the aminated nanofiber matrix was much higher than on similarly aminated PES film surfaces. The CFU assay also confirmed that cells expanded on aminated nanofibers contained a higher frequency of CFU-GEMM cells, which represent more primitive progenitor cells, compared to the aminated PES film.

A striking difference was observed in HSC adhesion to aminated nanofibers versus aminated films. Following a 10-day expansion culture, relatively few cells ($\sim$22%) remained attached to the aminated film and most of the adherent cells were found at the edges of cracks and other film defects. In contrast, cells expanded on an animated nanofiber matrix formed abundant colonies, accounting for more than 45% of the expanded cells. All other conditions (including unmodified, carboxylated and hydroxylated nanofibers and films) yielded negligible levels of adherent cells. More importantly, a much higher fraction (45%) of the adherent cells cultured on aminated PES nanofibers maintained a $CD34^+$ phenotype after 10 days of culture, compared to 17% $CD34^+$ cells among the suspension fraction. The enrichment of $CD34^+$ cell population bound to the animated nanofiber scaffold suggests that substrate topographical cues can improve ex vivo expansion of HSCs through regulated cell adhesion. The clinical application of this technology was further verified by the successful engraftment of the expanded HSCs in NOD/SCID mice [52] and the enhanced neovascularization in NOD/SCID mice in a hind limb vascular injury model [89].

Culture of hHSCs in a collagen nanofiber matrix was performed in a self-assembled type I collagen nanofibrous hydrogel. Human cord blood-derived $CD34^+$ cells were cultured in this collagen type I nanofiber hydrogel in the presence of Flt3-ligand, stem cell factor, and interleukin 3 [90]. 7-day serum-free in vitro culture within the collagen nanofibrous gel produced an increased number of myeloid colony-forming units (CFC-U), although the total expansion factor of $CD34^+$ cells was slightly lower compared to the control suspension cultures, suggesting that collagen I scaffolds may improve the preservation of the multipotency of the $CD34^+$ cells.

It is worth noting that the diameter and elastic modulus of reconstituted collagen nanofibers are different than that of electrospun synthetic fibers. These factors may also contribute to the influence of the fibrous matrix on HSC adhesion strength and the ability to migrate, and, hence, on expansion efficiency.

4.2 Nanofiber-mediated Expansion of Neural Stem Cells (NSCs)

Current protocols for NSC expansion call for either co-culture with stromal cells or neurosphere cultures in growth factor-supplemented media [91–93]. NSCs and neurons provided by these methods are far from satisfactory. The former method poses a serious problem for clinical application due to contamination from xenogenic components; the second method is laborious and inefficient, as the generated neurospheres are heterogeneous and only a small fraction of expanded cells retain their primitiveness. Adult NSCs are associated with basal lamina in the subventricular zone of the lateral ventricle and the dentate gyrus subgranular zone of the hippocampus [94, 95]. Therefore, the nanofibrous topography intrinsic to the basal lamina may benefit the expansion and maintenance of NSCs in culture. Nisbet et al. cultured NSCs isolated from adult rats on electrospun polycaprolactone (PCL) fibers of 750 nm diameter with or without amino-group surface functionalization, and found that the aminated PCL fibers improved NSC expansion over unmodified PCL nanofibers in EGF, FGF-2 and heparin-supplemented medium or under fetal bovine serum-supplemented medium without growth factors [96]. Though amino-functionalization did not appear to impact rat NSC differentiation, the increased hydrophilicity of the PCL nanofibers enhanced cell adhesion, spreading and proliferation.

Laminin is known to promote NSC adhesion in vitro [10] as well as being a critical component of the mammalian basement membrane [97]. To this end, laminin-coated electrospun polyethersulfone (PES) fiber meshes with average fiber diameters of 283, 749 and 1,452 nm were used to investigate the role of nanofiber diameter on rNSC proliferation and differentiation [49]. It was found that rat NSC expansion increased with decreasing fiber diameter. Morphological investigations with scanning electron microscopy (SEM) revealed that rat NSCs on 283 nm fibers proliferated in a colony-like fashion with enhanced cell–cell contact. Conversely, rat NSCs cultured on TCPS were well spread and exhibited far less cell–cell interaction. These observations may point to the role of nanofiber topography in combination with laminin mediated cell–cell and cell–substrate interactions in regulating cell migration and proliferation.

4.3 Nanofiber-mediated Expansion of Embryonic Stem Cells (ESCs)

Until recently, ESCs were cultured exclusively on mouse embryonic fibroblasts, which served as a feeder layer crucial to proper maintenance. A commercially available 3D nanofibrous scaffold of electrospun polyamide nanofibers, known as Ultra-Web, was investigated for its effect on ESC proliferation and self-renewal [98]. ESC colonies were significantly larger in size for cells cultured on Ultra-Web compared to glass coverslips, while still expressing alkaline phosphatase, an

undifferentiated stem cell marker. Further mechanistic experiments revealed the critical involvement of Rac (a small GTPase), the PI3K/AKT signaling pathway, and the up-regulation of Nanog and c-Fos in nanofiber-mediated ESC proliferation.

4.4 Nanofiber-mediated Expansion of Mesenchymal Stem Cells (MSCs)

Electrospun collagen nanofibers have been evaluated in expansion of MSCs [99]. MSCs were expanded on two sets of collagen fibers with diameters ranging 200–500, and 500–1000 nm, respectively, and on tissue culture polystyrene (TCPS) plates, with the greatest proliferation observed on the 500–1000 nm collagen matrix. Concurrently, there was a decreased amount of vinculin-associated focal adhesion complexes for the large diameter fiber matrix. This suggests a possible involvement of cytoskeletal-linked signaling pathways in hMSC self-renewal, which could be influenced by the topography and mechanical properties of the nanofibrous collagen matrix, in contrast to the rigid 2D TCPS substrates.

The effect of nanofiber topography on MSC expansion was investigated in a similar fashion, examining self-assembled peptide amphiphile nanofiber hydrogels in place of electrospun scaffolds [100]. The peptide nanofibers were functionalized with an arginine-glycine-aspartic acid (RGD) peptide sequence to facilitate cell adhesion. MSCs isolated from the femurs of 3-week-old male Wistar rats were seeded in the peptide nanofiber hydrogels with or without the RGD sequence. The RGD containing PA nanofibers facilitated both significantly higher cell attachment and proliferation than either nanofibers without RGD ligands or TCPS plates. One possible advantage of the self-assembling nanofiber matrix lies with their potential as an in vivo proliferation platform, thereby providing a strategy for possible in vivo expansion before in situ differentiation and tissue regeneration.

5 Nanofiber Matrices for Differentiation of Stem Cells

In addition to facilitating stem cell expansion, nanofiber matrices can be designed to either passively or directly induce differentiation along specific lineages. These matrices are intended to provide provisional support and facilitate stem cell adhesion, differentiation, and tissue organization, either prior to or following implantation in vivo. Conventional approaches towards induction of stem cell differentiation in vitro have been restricted to treatment with a variety of molecular mediators, for example small molecules or growth factors. On the other hand, there is gathering evidence to indicate that stem cell differentiation is sensitive and responsive to the physical signals emanating from their microenvironment. For example, MSCs can be restricted into various geometries by engineering their

adhesive contacts to the substrate, resulting in a fate choice between adipogenic (constrained morphology) or osteoblastic (spread out morphology) lineages [101]. It was postulated that these effects were brought about by the associated changes in cytoskeletal arrangement and focal adhesion assembly imposed by the restrictions on cell spreading, and their subsequent impact on Rho/ROCK signaling. Additionally, other studies have emerged to indicate that stem cells can make fate decisions in response to substrates stiffness [15], mechanical stress [102, 103], electrical stimulation [104, 105], and so on. With this in mind, synthetic nanofibrous substrates can be fabricated that are capable of presenting such extracellular signals. In addition to controlling substrate topography, composition and mechanical properties, nanofibrous substrates can be used as a means of presenting surface-immobilized adhesive or signaling ligands, and controlled release of inductive growth factors. The ultimate goal is to be able to sufficiently recapitulate the signaling events involved in the adult stem cell niche or embryonic development, so as to engineer a more functional and well-integrated regenerated tissue.

5.1 Nanofiber-mediated Stem Cell Differentiation into Neuronal Lineages

Development of clinical interventions for the repair of the nervous system is an active area of interest as it is unable to spontaneously recover following traumatic insult or injury. In particular, regeneration in the spinal cord is particularly challenging due to the formation of the glial scar and presence of growth-inhibitory molecules. In contrast to the central nervous system, adult peripheral nerves retain a limited ability to regenerate, as Wallerian degeneration creates a more permissive microenvironment, allowing severed nerves to re-grow over limited distances (typically less than 5 mm). Even in instances of successful nerve re-connection, functional recovery is often less than desired due to the formation of improper axonal contacts and atrophy of target tissues following prolonged denervation [106]. Stem cell transplantation-based strategies for repairing the nervous system have been extensively explored, and shown to promote functional recovery in a variety of disease and injury models, including spinal cord [107] and peripheral nerves.

A number of groups have investigated the use of electrospun nanofibers as niches for the neural differentiation of stem cells. Most of these studies were focused on elucidating the effects of topographical cues presented by nanofiber scaffolds, particularly their influence on cell morphology, attachment, and neurite extension. Mouse ES cells were induced to differentiate via treatment with retinoic acid, and the resultant embryoid bodies were cultured on aligned and random electrospun PCL fiber scaffolds [108]. Neurite fields extending from EBs seeded onto aligned fibers showed directional extension along the axis of fiber alignment, and also had longer average length than neurites observed on random fibers. Interactions of EBs with random fiber topography also seemed to result in a higher proportion of cells that differentiated into astrocytes.

During Wallerian degeneration following peripheral nerve injury, Schwann cells respond to the loss of axonal contacts by dedifferentiation and proliferation within the injury site. The Schwann cells then re-form into bands of Büngner to guide regenerating nerve ends, then finally regain their myelinating phenotypes after axonal reconnection is complete [109, 110]. Chew et al. set out to establish the impact of aligned electrospun fibers on the maturation of these primary Schwann cells by evaluating changes in gene expression [111]. Fiber alignment was found to promote the up-regulation of the pro-myelination genes P0 and MAG as compared with random electrospun fibers and two-dimensional polymeric films, suggesting that fiber alignment was an effective cue in directing cells towards a more differentiated state. The authors hypothesized that this pro-differentiation effect was responsible for the significant functional recovery observed when polymeric conduits presenting an aligned fiber topography were used to guide regenerating axons following rat sciatic nerve injury [112].

In addition to fiber alignment as a potential neurotrophic cue, neural stem cells were demonstrated to be responsive to the dimensions of single electrospun fibers. In one study, the effect of electrospun fiber diameter and alignment on C17.2 mouse neonatal cerebellum stem cells was investigated [113]. Random and aligned mats of fibers with average diameters of 300 nm and 1.5 μm were electrospun from poly-L-lactide (PLLA). The average length of neurites extended from C17.2 cells was significantly longer when cultured on aligned nanofibers than on the other scaffolds, indicating that the fiber dimension in combination with fiber alignment helped to enhance C17.2 differentiation. Further evidence that stem cells are sensitive to electrospun fiber diameter was presented in the work of Christopherson et al. [49]. Rat adult hippocampal-derived NSCs were cultured on laminin-coated PES electrospun fibers and differentiation was induced by retinoic acid treatment. NSCs cultured on fibers with average diameter of 283 nm extended processes that were guided by the underlying nanofiber morphology and showed preferential differentiation into oligodendrocytes. NSCs cultured on larger diameter (average ~749 nm) fibers were restricted in their ability to spread and migrate, and consequently a higher proportion of neuronal progenitors were observed.

Nanofibrous scaffolds resulting from self-assembled peptide amphiphiles can be modified to present signaling epitopes particularly relevant to NSCs, for example epitopes from laminin, which is the predominant ECM protein within the NSC niche. This approach is advantageous over presenting laminin as a component of the scaffold, for example via coating or electrospinning [47], as a supraphysiological density of the signaling epitopes can be achieved. A peptide amphiphile nanofiber network incorporating the laminin-derived pentapeptide epitope IKVAV preferentially enhanced differentiation of embryonic mouse NSCs into neuronal over astrocytic lineage [61]; neuronal differentiation was observed in almost 50% of cells in nanofiber hydrogel, as compared with only 10% of cells cultured on either laminin or poly-D-lysine coated 2D substrates. This effect was attributed to the 1000-fold higher calculated density of IKVAV peptide that could be achieved in the PA gel as compared to a close-packed model of laminin coating.

Electrical stimulation was found to increase regeneration of both motor [114] and sensory [115] peripheral nerves via activation of genes associated with the cellular response to injury [116] as well as secretion of neurotrophic molecules [117]. Sensing of extrinsically applied electrical fields was found to be facilitated by transmembrane ion channels, which appear and become functional even at early stages of embryonic development [118]. Electroactive polymeric materials were investigated as substrates for exposing cells in vitro and in vivo to mild electric potential or current [119, 120], and enhanced neurite outgrowth was observed in PC12 cells seeded on polypyrrole films and subjected to a constant 100 mV potential [121]. Electrically conductive polymers can further be formed into three-dimensional architectures to integrate structural and electrical cues. Lee et al. coated aligned and random electrospun poly-L-lactide-*co*-glycolide (PLGA) fiber matrices with polypyrrole and subjected PC12 cells seeded on the meshes to a constant electrical potential [122], and showed that a higher proportion of stimulated cells extended neurites with a longer median length than cells that did not receive stimulation.

5.2 *Nanofiber-mediated Stem Cell Differentiation into Chondrogenic and Osteogenic Lineages*

MSCs, most commonly derived from bone marrow, are an attractive cell source for cell replacement therapy due to their relative ease of derivation and expansion, as well as well-established multilineage differentiation potential [123]. For the purposes of musculoskeletal tissue engineering, cell therapy must often be complemented by suitable scaffolding that supports cell migration, repopulation, and matrix deposition, while simultaneously maintaining the desired shape of the final tissue. MSCs have been cultured with a variety of scaffold types including hydrogels, foams, microfiber and nanofiber matrices [124–128]. These studies clearly established the compatibility of various scaffolding materials for MSC culture. However, whether biomimetic scaffolds can be designed that actively instruct cell fates remains an area of active exploration.

Repair of damaged cartilage is a particularly challenging problem because the avascular nature of the tissue offers limited access to both the nutrient supply as well as a cell source for repopulating the injury site. The high porosity inherent within nanofibrous scaffolds offers a microenvironment particularly suited to facilitating biological processes that require intimate cell–cell or cell–matrix contact. Nanofibrous matrices are a logical alternative to the current protocol for chondrogenic induction of MSCs, which calls for high density pellet culture to simulate cartilage development within the embryo [129, 130]. TGF-β1-mediated chondrogenic induction of hMSCs seeded into an electrospun PCL fibrous scaffold proved to be more effective than similar induction of hMSC pellets [125]. Although cartilaginous gene expression as measured by RT–PCR was at a similar extent in both culture systems, hMSCs in fibrous scaffolds consistently produced a

greater amount of sulfated glycosaminoglycans, possibly due to more available space within the scaffold for ECM accumulation. Interestingly, chondrocyte-like cells in the fibrous scaffold were also organized into a zonal arrangement that was somewhat reminiscent of native articular cartilage. In an extension of this work, it was also later demonstrated that this platform was amenable to the production of pre-shaped cartilaginous constructs without loss of chondrogenic differentiation potential [131]. Further studies revealed that the mechanical properties of this type of engineered cartilage could also be enhanced when MSC morphology was oriented in response to fiber alignment [132]. MSCs were cultured on aligned PCL fibers in an attempt to replicate the organization of the fibrocartilaginous menisci of the knee, where fibrochondrocytes are surrounded by a tight network of radially aligned collagen I filaments. After 10 weeks in culture, aligned fiber constructs had higher stiffness and modulus compared with random fiber constructs due to the more organized deposition of ECM in the former, even though overall levels of GAG and total collagen production were comparable.

One strategy for designing biomimetic scaffolds for the osteogenic niche is to incorporate ceramics or minerals with similar composition of the inorganic component of the native bone in order to promote osteoinduction and osteointegration between native bone and the scaffold [133, 134]. Bioceramics such as calcium phosphate or hydroxyapatite have demonstrated excellent biocompatibility and bioactivity both in vitro and in vivo [135, 136]. These ceramics can be blended with or used to coat nanofibrous scaffolds in order to ameliorate the intrinsically brittle and fragile nature of the inorganic components. One approach is to incorporate or coat electrospun fibers with calcium phosphate as a means of achieving mineralization in vitro via hydroxyapatite deposition on the fiber surface following immersion in simulated body fluid. Calcium phosphate can be loaded into the polymer solution as aerosolized nanoparticles or crystals prior to electrospinning [137, 138], or simply coated onto mats of nanofibers [139]. These mineralized scaffolds did not have any adverse effects on biocompatibility, as evidenced by their ability to support continued proliferation and osteogenic differentiation of hMSCs. In a study performed by Lee et al., hydroxyapatite encapsulation into PLGA nanofiber matrix resulted in increased alkaline phosphatase activity as well as osteogenic gene expression and further scaffold mineralization through calcium deposition [140].

Osteoblasts and osteoclasts that comprise the cellular component of bone interact with proteins in their niche in a number of ways, such as integrin-mediated binding to osteopontin and bone sialoprotein [141]. Using self-assembling peptides, a nanofiber matrix can be prepared that presents short peptide signaling motifs that underlie these bone matrix interactions [142]. Peptide sequences tested were osteogenic growth peptide ALK [143, 144], the osteopontin cell adhesion motive DGR, and a synthetic sequence containing two RGD cell adhesion motifs. Preosteoblastic MC3T3-E1 cells were cultured on the various hydrogel nanofiber scaffolds and induced to differentiate into bone. The highest degree of osteogenic differentiation was observed in nanofiber matrices presenting the dual-RGD motif, with the highest alkaline phosphatase activity as well as secretion of osteocalcin,

although all motif-presenting scaffolds showed greater differentiation than the control nanofiber matrix without any signaling sequence. These results directly implicate the importance of cell–scaffold adhesive interactions in mediating osteogenic differentiation.

5.3 *Nanofiber-mediated Stem Cell Differentiation into Myogenic Lineage*

Formation of mature skeletal muscle requires the fusion of skeletal myoblasts into bundles of muscle fibers. Although muscle cells are considered to be post-mitotic and terminally differentiated, evidence exists to suggest that injured muscle tissue retains a limited capacity for regeneration. This is believed to occur via activation of a normally quiescent population of progenitor cells known as satellite cells that normally reside below the basal lamina of muscle fibers. Upon activation, satellite cells proliferate, undergo myogenic differentiation to form myoblasts that eventually give rise to new muscle fibers, or fuse with existing damaged fibers [145, 146]. Although the biochemical [147, 148] and biophysical features [149, 150] of the satellite cell niche have been extensively examined elsewhere, less is understood about the effects of the structural features of the niche on myoblast differentiation. Designing nanofiber scaffolds intended to simulate the structure and organization within the basal lamina is one strategy for engineering muscle tissue adopted by several research groups.

The ability of the underlying substrate topography to dictate cell morphology is the driving principle behind the use of aligned electrospun fibers as a scaffold for the regeneration of muscle fibers in vitro. The densely packed, unidirectional fibrous substrate closely mimics the organization of native muscle tissue. A working hypothesis is that satellite cell alignment induced by the substrate can promote myoblast differentiation and facilitate eventual fusion into new muscle fibers. Choi et al. demonstrated that culture on aligned PCL fibers resulted in highly oriented F-actin organization in myotubes [151]. Although the width of myotubes was approximately the same on both random and aligned fibers, myotubes on aligned fibers were on average approximately twice as long as myotubes formed on random fibers [152]. A separate study showed that C2C12 myoblasts cultured on aligned polyesterurethane fibers experienced up-regulation of myogenin and myosin heavy chain (MHC) gene expression, concomitant with the formation of putative multinucleated myotubes in highly parallel arrays, which was not observed on random fibers [153]. The inductive potential of topographical alignment beyond merely facilitating myotube formation and fusion was also suggested in a study by Dang et al., in which hMSCs cultured on aligned hydroxybutyl chitosan fibers showed up-regulation of the myogenic genes collagen IV, desmin, Pax-3, Pax-7 and myogenin compared with hMSCs cultured on films [154].

Electrospun fibers comprised of a blend of poly-L-lactide-*co*-caprolactone (PLCL) and polyaniline (PAN) were used as an electroactive scaffold to facilitate

myoblast differentiation into myotubes [155]. PAN is a conducting polymer and was previously shown to support cardiac myoblast proliferation while also retaining its conductive properties under tissue culture conditions [156]. PLCL was blended with PAN to fabricate scaffolds due to the intrinsic lack of mechanical rigidity and stability of PAN alone. C2C12 myoblasts cultured on PAN-containing nanofiber matrix under differentiation conditions showed significantly higher quantities of myotube formation as compared with myoblasts on cultured on matrix without PAN; increasing the proportion of PAN in the matrix from 15 to 30% also resulted in formation of larger myotubes. Furthermore, expression of the myogenic genes myogenin, troponin T and myosin heavy chain were up-regulated in myoblasts cultured on an electroactive nanofiber matrix. Although the cells were not subject to electrical stimulation during the culture period, it was hypothesized that the electrical conductivity of the substrate may facilitate better cell–cell communication. This study implies the importance of exposing skeletal muscle cells to electrical impulses to enhance muscle regeneration.

6 Nanofibrous Matrices for Stem Cell Delivery

Stem cell-based therapy offers tremendous potential for the repair and regeneration of tissues damaged by injury or disease. Using animal models, several groups have shown that they can achieve a significant level of functional recovery simply by injecting the stem cells intravenously [157, 158] or directly into the tissue of interest [159–161]. A basic premise of this strategy is that tissue-specific signals emanating from local microenvironments, both from somatic cells and ECM, direct the implanted stem cells to differentiate towards specific lineages. Despite these promising results, however, bone marrow transplantation remains the only stem cell-based therapy with any real clinical success for humans. Among the reasons indicated for the lack of success with other stem cell therapies are the large-scale cell loss, cell death, and poor engraftment of transplanted stem cells, and the poor control over stem cell fate after implantation [162]. Likely, the local microenvironments present in damaged or diseased tissues do not provide the proper signals for stem cell retention, survival, and differentiation.

To improve the efficacy of stem cell-based therapies, a variety of delivery scaffolds are being developed with the aim of providing microenvironmental support for implanted stem cells [162]. Such scaffolds act as synthetic analogs of the ECM, providing adhesion cues to maintain the viability of implanted cells and retain them at the implant site. Scaffolds may also be designed to encourage stem cell differentiation towards specialized lineages and to provide structural support for the newly developing tissue. They can be seeded with cells prior to implantation (in the case of solid scaffolds) or mixed with the cells and spontaneously assembled in situ (in the case of nanofiber hydrogels).

To date, a limited number of nanofibrous scaffolds have been developed for in vivo stem cell delivery. Electrospun fibers have been used to deliver stem cells for various tissue regeneration applications. Li et al. reported the use of electrospun PCL scaffolds for articular cartilage repair in a swine model [163]. The scaffolds were seeded with either allogenic chondrocytes or xenogenic human MSCs, and maintained in culture for a period of 3 weeks prior to implantation into 7 mm full-thickness cartilage defects created in the distal weight-bearing surface of femoral condyles. At 6 months post-implantation, the MSC-seeded constructs showed the most complete repair of the defects, reproducing a smooth hyaline-like cartilage surface with mechanical properties similar to native hyaline cartilage.

Hashi et al. demonstrated the advantage of using a tubular PLLA nanofiber scaffolds to improve the efficiency of MSC-based vascular tissue engineering in a rat model [164]. After seeding MSCs onto flat, electrospun nanofiber sheets and allowing the cells to attach for 1 day, the authors wrapped the sheets around 0.7 mm mandrels and bound them by sutures to maintain their tubular shape. The cell-seeded constructs were then removed from the mandrels and cultured for a period of 2 days prior to implantation, resulting in tubular scaffolds with embedded and circumferentially aligned MSCs. Similar acellular scaffolds were also prepared as controls. The scaffolds were implanted by suturing them to the common carotid artery using an end-to-end anastomosis procedure. At 2 months post-implantation, large numbers of endothelial cells and smooth muscle cells were present in both the MSC-seeded and acellular scaffolds, and complete endothelialization was observed. However, only the MSC-seeded constructs were non-thrombogenic and they showed excellent long-term patency compared to acellular scaffolds. Surprisingly, the authors attributed the presence of endothelial cells and smooth muscle cells in MSC-seeded constructs solely to recruitment from the surrounding host tissue, even though it is possible that the implanted MSCs may also differentiated into both cell types in situ.

Due to the ease of cell delivery by injectable hydrogels, self-assembled nanofiber hydrogels have also been explored as an artificial matrix to improve the retention, survival, and differentiation of implanted stem cells. Using a β-sheet forming peptide nanofiber hydrogel, Davis et al. showed that a nanofiber hydrogel, when injected into the wall of the left ventricle of adult mice, created a favorable microenvironment within the myocardium for recruitment of host endothelial cells and smooth muscle cells [165]. They also tested the delivery of undifferentiated ESCs to myocardium using this injectable hydrogel, with injected ESCs spontaneously differentiating into cardiac myocytes after 2 weeks, likely due to signals emanating from the myocardial microenvironment. Introducing a local release function of growth factors in the hydrogel may significantly improve the regeneration outcome. This can be achieved by entrapping a sustained release system inside the hydrogel or by immobilization of growth factors to the peptide nanofibers. Davis et al. have achieved local and sustained release of insulin-like growth factor 1 (IGF-1), known to increase cardiac stem cell growth, by using a "biotin-streptavidin sandwich" scheme [66]. Using this method, IGF-1 was loaded without

interfering with the self-assembly of peptide nanofibers, and had controlled release for 28 days to the local myocardial tissue. This IGF-1-loaded hydrogel, when injected together with cardiomyoctes, resulted in reduced cardiomyocyte apoptosis and improved restoration of systolic function after experimental myocardial infarction.

The peptide amphiphile nanofiber hydrogel has also been shown as an effective delivery vehicle for stem cells. Cell survival and proliferation in the peptide amphiphile hydrogel can be enhanced by incorporating cell adhesion ligands. Webber et al. constructed a binary nanofiber hydrogel with a mixture of RGDS-conjugated and unconjugated peptide amphiphiles [166]. The luciferase-expressing bone marrow mononuclear cells (BMNCs) were encapsulated in the nanofiber hydrogel and injected subcutaneously in a mouse model. At 4 days post-injection, the cells implanted within the RGDS-containing nanofiber hydrogel showed a significant increase in bioluminescence (315%) when compared to the initial baseline. This increase in bioluminescence was also significantly greater than cells implanted in hydrogel without RGDS ligand (127%) and with saline (147%). This study indicated that cell adhesion cues in nanofiber matrix are crucial to support BMNC survival and proliferation in vivo.

7 Summary

Nanofibrous matrices prepared by electrospinning, solution phase separation, and self-assembly provide a unique set of substrates that can be used to partially recreate stem cell niches for the expansion, differentiation and delivery of stem/progenitor cells. Nanotopography presented by these nanofiber matrices has been shown to significantly influence stem cell adhesion, migration, proliferation and differentiation. These matrices provide great opportunities to modulate cellular behavior and tissue regeneration at the nanometer scale, the same scale where molecular signaling transduction occurs at the cell–cell and cell–substrate interfaces.

Integrating the biochemical cues and topographical cues is an important engineering consideration in designing the nanofiber matrix. A nanofiber matrix without any cell adhesion ligands or other biochemical cues is less likely to exert significant influence to stem cells cultured in the matrix. Specific cell adhesion signals should be an integral component for future design of nanofiber matrix; and the capability to enhance the local stability, concentration and presentation of growth factors relevant to stem cell control will be an important feature of the artificial stem cell niches. In addition, the optimal configuration of topographical cues (diameter, alignment, pore structure, mechanical properties, etc.) and biochemical cues—including matrix bound and supplemented factors—needs to be tailored for each cell type. Similarly, the set of effective cues for expansion and differentiation are likely to be different.

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