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NANOFIBER TECHNOLOGY FOR CONTROLLING STEM CELL FUNCTIONS AND TISSUE ENGINEERING

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2.1 INTRODUCTION

Nanotechnology is an upcoming yet promising technology with respect to the development of well-established products. It holds the potential to create new products with novel characteristics and functions in a wide range of applications.

Micro and Nanotechnologies in Engineering Stem Cells and Tissues, First Edition. Edited by Murugan Ramalingam, Esmaiel Jabbari, Seeram Ramakrishna, and Ali Khademhosseini.

Application of nanotechnology in life sciences research, particularly at the cellular level, sets the stage for an exciting role of nanotechnology in nanomedicine for health care. The potential medical applications are predominantly in detection, diagnostics (disease diagnosis and imaging), monitoring, and therapeutics. The availability of more durable and better prosthetics and new drug-delivery systems are of great scientific interest and give hope for cancer treatment and minimum invasive treatments for heart disease, diabetes, and other diseases.[1] Nanofibers are potentially recent additions to materials in relation to tissue engineering (TE). Tissue engineering is the application of knowledge and expertise from a multidisciplinary field to develop and manufacture therapeutic products that use the combination of matrix scaffolds with viable human cell systems or cell responsive biomolecules derived from such cells for the repair, restoration, or regeneration of cells or tissue damaged by injury, disease, or congenital defects.[2]

Tissues in the body are made up of cells and insoluble materials present between the cells known as the extracellular matrix (ECM). ECM is composed of various biomacromolecules secreted by surrounding cells and is responsible for the structural support and tensile strength of the tissue. It provides a substrate for cell adhesion and migration and regulates cellular differentiation. The interaction between cells and ECM is mediated by the process of biorecognition whereby the transmembrane protein receptors on the cell membrane combine specifically with specific ligands in the ECM, triggering a series of events in the signal transduction cascade within the cells and eventually influencing their gene expression. For example, growth factors such as fibroblast growth factor combine with their receptors on cell surfaces and stimulate their proliferation and differentiation.[3]

Recently, nanofiber-based scaffolds are being explored as scaffolds for tissue engineering applications. TE is an interdisciplinary field of research whereby diverse cell-based and cell-free strategies are being investigated in the quest for a sustainable therapeutic for refurbishment of organ functionality. Essentially, TE is an attempt at bringing about repair by mimicking nature. It is aimed at boosting the low regenerative capacity of the damaged myocardium by applying principles of engineering, material chemistry, and cell biology. The classical strategy used in tissue engineering is the provision of external help in the form of biomaterials and biomolecules with properties bearing close resemblance to their natural counterparts. However, owing to the uniqueness of each organ, the quest for optimal biomaterials and an efficient strategy for TE remain persistent. A bioengineered construct is desired to possess certain essential characteristics, such as appropriate physical and mechanical properties, ready adherence, nontoxicity, nonantigenicity, noninvasive applicability, and ability for complete integration with the host.[4,5] An ideal polymeric scaffold satisfies several structural and chemical features: (1) a three-dimensional architecture with a desired volume, shape, and mechanical strength;[6] (2) a highly porous and well-interconnected open pore structure to allow high cell seeding density and tissue ingrowth; (3) chemical compositions such that its surface and degradation products are biocompatible, causing minimal immune or inflammatory responses;[7] and (4) their degradation rate finely tuned in a pattern that it provides sufficient support until the full regrowth of impaired tissues. Several scaffold fabrication techniques, namely, electrospinning (random, aligned, vertical, and

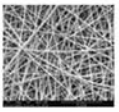
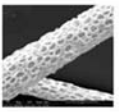
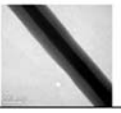
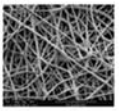
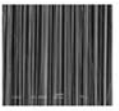
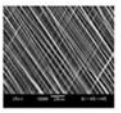
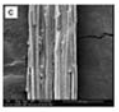
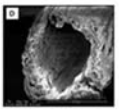
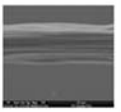
Composition	Natural • Collagen • Chitosan • Silk • Alginate	Synthetic • PCL • PLLA • PLGA • PHBV	Blended • Natural + synthetic • Natural + synthetic + coating
Morphology	Solid 	Porous 	Core-shell 
Assembly	Random 	Aligned 	Layered 
	Yarn 	Hollow yarn 	Fiber bundle 

FIGURE 2.1 Different types of electrospun fibers. PCL = poly(ε-caprolactone); PHBV = poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); PLGA = poly(lactic-*co*-glycolic acid); PLLA = poly(L-lactic acid).

core shell nanofibers), self-assembly, phase separation, melt-blown, and template synthesis, are used for the preparation of nanofibrous materials (Fig. 2.1). This makes designing of nanofibrous scaffolds an important technique for designing synthetic and natural nanofibers in tissue engineering. It is highly advantageous to have an artificial ECM that promotes cell adhesion and that can be assimilated by the body as the new tissue regenerates. For regeneration of tissues, cell adhesion has been proven beneficial and can be achieved by suitable modifications of biomaterial surface chemistry such as addition of arginine–glycine–aspartic acid (RGD) moieties or growth factors for cell attachment or chemotactic recruitment. Attempting to fabricate artificial ECM, each approach has its own unique characteristics and has the potential to accommodate cells and guide their growth for tissue regeneration.[8] However, electrospinning is the most widely accepted technique; it seems to be demonstrating most promising results for tissue engineering applications.[9] Nanotechnology is also extended as drug-delivery and drug-targeting systems. Owing to the smallness of nanomaterials, they have the ability to be delivered into the human body with ease. They migrate through cell membranes beneath a critical size and are able to pass and develop nanoscaled ferries, which transport high potential pharmaceutics precisely to their destination.[10] Nanostructured biodegradable polymeric materials act as alternative candidates used to promote a new concept of chemotherapy that may include sustained chemotherapy, controlled and targeted chemotherapy, personalized chemotherapy, and chemotherapy across the various physiological drug barriers such as the gastrointestinal barrier for oral chemotherapy

and the blood–brain barrier for brain tumors.[11] Currently, there is a huge demand for controlled-release polymer systems, and the worldwide annual market exceeds $60 billion. Electrospinning has developed into a versatile technique to fabricate polymeric nanofiber matrices, and the ability to incorporate bioactive therapeutic molecules without adversely affecting their structural integrity and biological activity using the mild electrospinning process has generated significant interest in polymeric nanofiber-based drug release patterns by changing the mode of encapsulation as well as by varying the matrix polymer.[12]

2.2 FABRICATION OF NANOFIBROUS SCAFFOLDS BY ELECTROSPINNING

Electrospinning generates a nonwoven mat of polymeric nanofibers from an electrostatically driven jet of polymer solution. The basic elements of an electrospinning system involve (1) a high-voltage supplier (2–40 kV), (2) a source electrode and grounded collector electrode, and (3) a capillary tube with a needle of small diameter. Electrospinning may be carried out with polymer solution as well as polymer melt for fabrication of nanofibers. The morphology and fiber diameter of the electrospun nanofibers can be controlled by varying the parameters, such as applied electric field strength; spinneret diameter; distance between the spinneret and the collecting substrate; temperature; feeding rate; humidity; air speed; and properties of the solution or melt, including the type of polymer, and polymer molecular weight, such as surface tension, conductivity, and viscosity, depending not only on the temperature but also on the concentration of the sample.[13] The advantage of an electrospun nanofibrous scaffold includes an extremely high (favorable) surface-to-volume ratio, appropriate porosity, and malleability to conform to a wide variety of sizes, textures, and shapes of superior architecture[14] (Fig. 2.2).

In addition, scaffold composition and fabrication can be controlled to confirm desired properties and biofunctionalities. The design and development of nanofibrous scaffolds for tissue engineering approaches involve the understanding of biological processes that are mainly aimed at a conducive to ECM. Many studies were also focused on the understanding and evaluations of several cell-scaffold interactions.[15] Interaction between the stem cells and nanofibers are crucial in a cell-scaffold matrix while using them for different tissue engineering applications. Because the nanofibrous scaffolds are highly porous and the pore size is smaller than the normal cell size, scaffolds might inhibit cell migration. Despite this, studies showed the capability of nanofibrous meshes to infiltrate cells. Cells entering into the matrix through amoeboid movement to migrate through the pores can push the surrounding fibers aside to expand the pore. Scaffolds constructed from naturally occurring proteins, such as collagen, allows much better infiltration of cells into the scaffold than the synthetic polymeric nanofibrous scaffolds.[16] The low-molecular-weight peptides (tripeptide and tetrapeptide) found in ECM proteins, such as laminin, fibronectin, collagen, and vitronectin, are found to modulate the cell behavior to a higher extent. Immobilizations of these

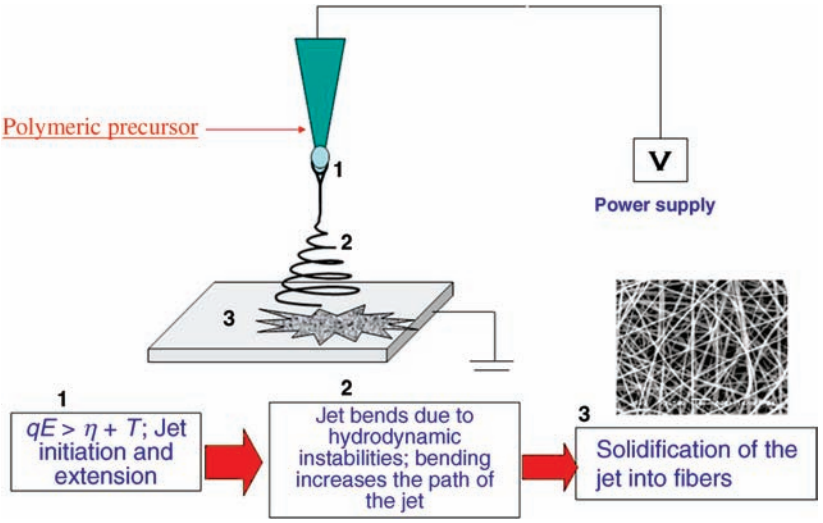


FIGURE 2.2 Schematics of the electrospinning process. The experimental setup consists of a high-voltage power supply, a spinneret, and a collector. The three processes—formation of tailor cone (1), bending caused by various instabilities (2), and collection of solid samples (3)—are shown. The qE is the electrostatic force, η is the viscosity, and T is the surface tension. Conventionally, electrospinning produces a fiber cloth consisting of randomly oriented nano- or microfibers, a typical SEM image of which is also shown.

biological motifs on synthetic biomaterial surfaces are also studied by few researchers so as to increase the bioactivity of the scaffolds. Moreover, the dynamic architecture of the fibers allows cells to adjust according to the pore size and grow into the nanofiber matrices.[17] For many tissue engineering applications, nanofiber modifications are therefore necessary to achieve the required scaffold properties. Polymer blending, coelectrospinning, multilayering and mixing for nanofiber production or cross-linking, surface modifications, and coating of the scaffold can improve the stability and biocompatibility of the scaffold. Multilayering electrospinning is a subset of the electrospinning process that involves sequential electrospinning of polymers on the same collector. This produces multilayered meshes with hierarchically ordered layers made from particular fibers. For example, a trilayered electrospun mesh composed of type I collagen, styrenated gelatin, and segmented polyurethane was prepared; a bilayered tubular construct composed of a thick segmented polyurethane microfiber mesh as an outer layer and a thin type I collagen nanofiber mesh as an inner layer was also fabricated.[18] Alternatively, in a multicomponent mixing electrospinning process, different polymers are simultaneously electrospun from different syringes under special conditions. The produced fibers are mixed on the same collector, resulting in the formation of a mixed fiber mesh (e.g., mixed electrospun fiber mesh composed of segmented polyurethane and polyethylene oxide). Specific

cells are sensitive to pore sizes, and hence high importance is given to polymeric scaffolds with greater porosity. Such porous membranes may be created by phase separation methods.[19] The phase separation method is based on the thermodynamic demixing of a homogenous solution of polymer in solvent into polymer-rich and polymer-poor phases by exposure to another immiscible solvent or by cooling the solution below the glass transition temperature (T_g) of the polymer. Fabrication of scaffolds is influenced by processing variables such as the polymer type, concentration, solvent, and temperature. This method allows the generation of three-dimensional (3D) porous networks within the scaffolds with higher control over porosity and morphology.[20,21]

Physical patterning techniques such as reactive-ion etching and polymer molding allow creation of microgrooves for designated cellular orientations. Patterned surfaces are advantageous as TE scaffolds because they serve better cellular attachment, migration, and orientation.[22] Soft lithographic techniques have been used to generate exquisite control over protein and cells in spatially defined patterns. Such patterning has been shown to regulate the temporal and spatial distribution of biomolecules and has been performed to direct explicit cell behavior and functions. Patterning is also carried out using methods such as imprint lithography, photo or electron beam lithography, and microcontact printing. These methods aid in constructing geometrically designed substrates suitable for cellular interaction on a nanoscale. Imprint lithography method uses a silicone rubber stamp inked with molecules to transfer the agent and develop grids, honeycomb networks, dots, and patterns.[23] These patterns mimic the basement membrane structures of nanometer-sized pores that define mechanical cues that aid specific cell type. However, nanoimprint lithography is capable of creating patterns of submicron 10 nm features with simpler equipment and convenient processing steps.[24] The TE scaffolds designs may be tailored to have specific nanotopographical patterns based on the specific tissue needs by application of the various methods available.

2.3 STEM CELLS: TYPE, ORIGIN, AND FUNCTIONALITY

Stem cells are cells of mammalian origin that possess two specific distinct characteristics: self-renewal and the potential to differentiate into several cell type. In a developing embryo, stem cells can differentiate into all the specialized cells but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. In an adult, stem cells act as a repair and replenish system for all cell types. Stem cells are broadly classified as ESCs, obtained from inner cell mass of an embryo and adult stem cells (ASCs), that are obtained from adult tissues (Fig. 2.3). Stem cells can be cultured *in vitro* and transformed into specialized cell types with characteristics consistent with cells of various tissues such as bone, cartilage, muscle, or nerve after being acquired from an embryo or adult. Recent research demonstrates the development of ESCs such as cells from adult somatic cells by transfusion of pluripotent genes. These are called induced pluripotent stem (iPS) cells.

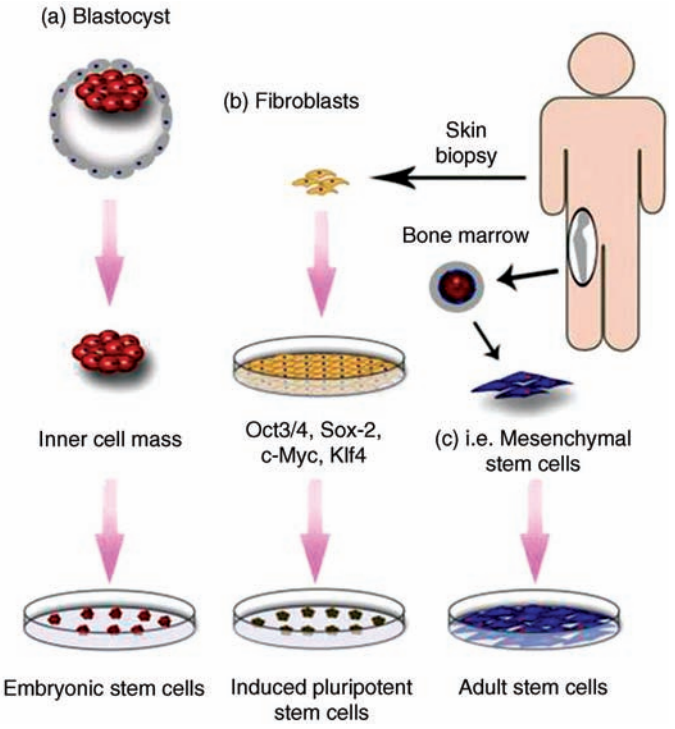


FIGURE 2.3 Stem cell biogenesis. (a) Embryonic stem cells, derived from the inner mass of the blastocysts, are pluripotent cells that may differentiate toward all cell types. (b) Induced pluripotent stem cells generated *in vitro* from somatic cells overexpressing Oct3/4, Sox2, c-Myc, and Klf4. (c) ASCs are created during ontogeny (e.g., bone marrow mesenchymal stem cells) and persist within the niche in most adult animal tissues and organs. Reproduced with permission from Ref. [44].

2.3.1 Mesenchymal Stem Cells

The term mesenchymal stem cells (MSCs) was used by Caplan in 1991 to describe the adherent cells derived from bone marrow that were capable of self-proliferation as well as differentiation into different lineages of connective tissue.[25] However, the identification of these cells dates back to 1867, when Cohnheim identified these cells as nonhematopoietic cells. Traditionally, MSCs were thought to reside in both blood and bone marrow.[26] However, recent researches provide evidence of MSCs in diverse tissue and organs such as lung, adipose tissues, amniotic fluid, umbilical cord, periosteum, dental pulp, hair follicle, thymus, and trabecular bone.[27,28] MSCs give rise to connective tissues of various origin such as bone (osteogenic), cartilage (chondrogenic), and fat (adipogenic).[29] MSCs are also worthy of giving rise to several other tissues of mesodermal (myocyte, osteocyte, endothelium, cardiomyocyte), ectodermal (neuronal), and endodermal (hepatic, pancreatic, respiratory epithelium) lineages.[30] MSCs constitute approximately 2–3% of the total nuclear

cell fraction of the bone marrow. Bone marrow–derived MSCs pose advantage in regenerative medicine because they are naturally poised to generate a particular tissue, which might consist of several cell types such as adipocytes, chondrocytes, osteoblasts, tenocytes, myoblasts, and neurocytes.[31] MSCs express CD44, CD73, CD90, and CD105 receptors while lacking hematopoietic stem cell markers such as CD34 and CD45. MSCs exhibit low expression of major histocompatibility complex (MHC) class I and are negative for MHC class II antigens.[32] Various studies have shown that *in vitro* expanded MSC preferentially home to sites of tissue damage, where they enhance wound healing, support tissue regeneration, and restore the bone marrow microenvironment.[33] However, the exact signaling events that drive MSCs toward this repair mechanism are unknown. MSCs have been applied as therapeutic agents for tissue repair owing to their immunomodulatory properties.[34] All of these properties of MSCs make them an ideal cell source for tissue engineering.[35]

2.3.2 Embryonic Stem Cells

ESCs are isolated from the inner mass of blastocyst cells.[36] Under defined conditions, ESCs are capable of propagating themselves indefinitely. This allows ESCs to be employed as useful tools for both research and regenerative medicine, because they can produce limitless numbers of themselves for continued research or clinical use.[37] Human ESCs are known to express antigens such as octamer binding protein (Oct-4), Nanog, alkaline phosphatase, LIN28, rex-1, crypto/TDGF1, SOX2, and stage-specific embryonic antigen (SSEA) 3 and 4. They also show high levels of telomerase activities.[38,39] It is understood that Oct-3/4 along with SOX2 and Nanog play a crucial role in the process of self-renewal,[40] whereas genes such as Klf4 and c-Myc are involved with maintenance of pluripotency.[41] Because of their plasticity and potentially unlimited capacity for self-renewal, ESC therapies have been proposed for regenerative medicine and tissue replacement after injury or disease. Diseases that could potentially be treated by pluripotent stem cells include a number of blood- and immune system-related genetic diseases, cancers, and disorders; juvenile diabetes; Parkinson's disease; blindness; and spinal cord injuries. Besides the ethical concerns of stem cell therapy, ESCs face certain major technical challenges such as histocompatibility and graft-versus-host disease.

2.3.3 Induced Pluripotent Stem Cells

A few years ago, a completely new class of stem cells was introduced by Takahashi and Yamanaka.[42] The group demonstrated that uptake of genes such as Oct-3/4, Sox2, c-Myc, and Klf4 induces pluripotent properties in somatic cells. These reprogrammed cells were termed iPS cells.[42] Currently, many researchers are actively studying the generation of iPS cells from various sources and trying to improve the experimental procedures.[43] iPS cells are similar to natural pluripotent stem cells, such as ESCs, in many respects, including the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and

differentiability, but the full extent of their relation to natural pluripotent stem cells is still being assessed.[44] Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatments for humans.[45]

2.4 STEM CELL–NANOFIBER INTERACTIONS IN REGENERATIVE MEDICINE AND TISSUE ENGINEERING

Research in the area of drug delivery and tissue engineering witnessed huge progress because of their unlimited potential to improve human health. Drug delivery and tissue engineering are closely related fields, in which both drug delivery vehicles and tissue-engineered scaffolds need to be biodegradable and biocompatible. Controlled drug delivery strategies not only increase the efficacy of drugs but also maximize patient compliance, enhancing the ability to use poorly unstable/soluble and toxic drugs.[46] Such highly selective and effective therapeutic and diagnostic modalities can have a dramatic impact in medicine. Electrospun nanofibrous scaffolds were used as a carrier for both hydrophilic and hydrophobic drugs, in which the modulation of scaffold composition, morphology, and porosity are primarily carried out for a controlled drug release.[47] In tissue engineering approaches, it is important to recapitulate proper function and organization of native tissues as much as possible, which is usually done by mimicking tissue properties at nanoscale. ECM provides a natural web of tissue-specific and organized nanofibers support and maintains the cell microenvironment. Cells reside in a unique complex environment, and hence scaffolds for tissue engineering approaches maintain and regulate cell behavior[48] (Fig. 2.4). The design and fabrication of these substrates will require either a surface is naturally adhere to ECM molecules or that reproduces high-affinity binding sites for cell-associated receptors to reproduce the natural tissue organization observed in the pancreas, liver, and cartilage. Moreover, the utilization of electrospun nanofibrous scaffolds as cell delivery vehicles has been substantially increased in recent years owing, in part, to the physical similarities between nanofibrous scaffolds and ECM found in native tissues.[49] Such approaches might even be used to regulate and replicate *in vitro* cellular environment for stem cell differentiation.

2.4.1 Skin

Chronic wounds present a worldwide growing health and economic problem because of a steadily increasing number of patients, high morbidity and risk of amputations, unsatisfactory results of existing therapies, and heavy socioeconomic burden. Patients with 50% total body surface area (TBSA) full-thickness wounds have only 50% of undamaged skin left, which could be used for split-thickness skin harvesting. Donor sites would add to the total wound size, resulting in a wound area covering 100% of the body.[50] These donor sites heal with some scarring and may be very painful; hence, an

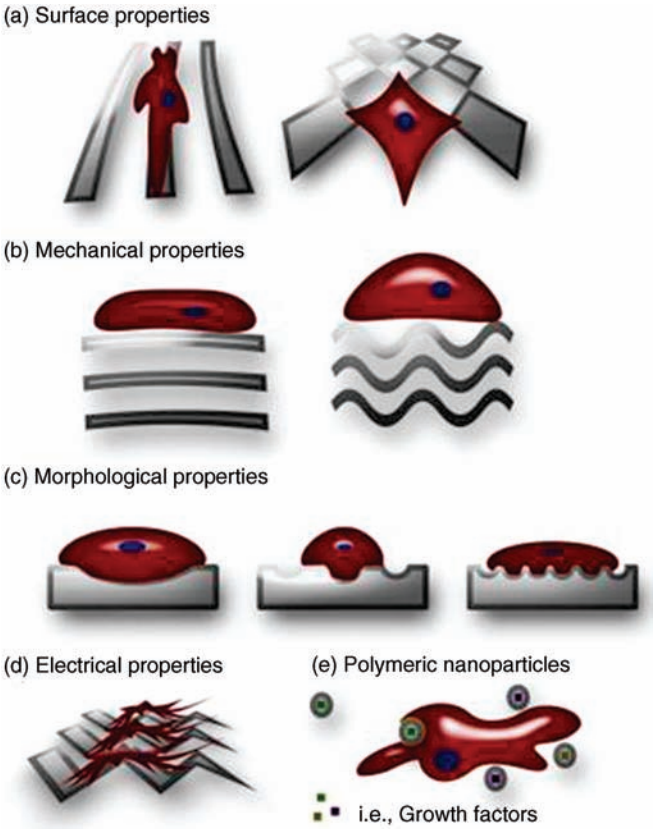


FIGURE 2.4 Scaffold properties. (a) Surface properties. The surface topography could drive cell adhesion, proliferation, migration, and differentiation. (b) Mechanical properties. Stem cells respond to the mechanical properties of the substrate on which they are growing, thus changing their fate. (c) Morphological properties. Scaffold morphologies for stem cell biomaterial interaction may vary in terms of interconnectivity, pore size, and shape. (d) Electrical properties. Electrical properties of the substrates are important issues in biomaterial–cell interaction. (e) Polymeric nanoparticles. Different smart nanosystems, nanoparticles, and nanoshells can be developed based on biodegradable polymers. Biodegradable nanosystems allow improvement of the therapeutic value of several water-soluble and nonsoluble bioactive molecules by improving bioavailability, solubility, and retention time. Reproduced with permission from Ref. [48].

additional analgesic pharmacological load is required for skin regeneration. Alternate lifesaving approaches in the treatment of extensive full-thickness wounds, in which donor sites for split-thickness skin grafts (SSG) harvesting are not available, include the use of cultured autologous keratinocytes, bioengineered skin substitutes, or both.[51] Significant progress has been made recently in the development and clinical use of these products. The most common skin injuries or skin wounds are categorized on the basis of the depth of the skin injury: epidermal or full-thickness skin wounds. Skin can

regenerate itself from minor epidermal injury. However, when the injury is a full-thickness skin wound (loss of both epidermis and dermis), the damaged skin cannot regenerate spontaneously. Natural repair of wound healing is slow compared with the rapid wound cover needed to reduce the time of wound healing.[52] Aligned and random fibrous scaffolds fabricated with fiber diameters down to 100 nm range with a wide range of pore sizes for the scaffolds.[53] These fiber mats have large surface areas available to interact with the cell surfaces and varying levels of porosity that enable differing amounts of cellular infiltration. Porosity and a ratio of high surface area to volume of the mats also facilitate diffusion into 3D structures, aiding in mass transfer. Nanofibrous scaffolds not only serve as carriers for the delivery of drugs but are also used as scaffolds for engineering skin, bone, cartilage, and vascular and neural tissue engineering.[54] Nanofibers can be electrospun in various patterns depending on the applications such as random, aligned, core shell, yarn, and fiber bundle. The mechanical properties of tissue engineering scaffolds are of the utmost importance in order for them to adequately perform their function. Various polymeric nanofibers have been investigated as a novel wound dressing material and as hemostatic devices. The high surface area of nanofiber matrix allows oxygen permeability and prevents fluid accumulation at the wound site. On the other hand, the small pore size of nanofibrous matrix efficiently prevents bacterial penetration, making them ideal candidates for wound dressings, where dressings for human wounds aimed to protect, removal of exudates, inhibition of exogenous microorganism invasion, and improved appearance. Systemic transplantation and local implantation of MSCs are promising treatment methods for skin wounds, especially for chronic wounds. The mechanisms by which BM-MSCs participate in cutaneous wound healing is by either differentiating into phenotypes of various damaged cells or by enhancing the repair process by creating a microenvironment that promotes the local regeneration of cells endogenous to the tissue. Nanofibrous scaffolds have been recently used in the field of tissue engineering because of their nanosize structure, which promotes cell attachment, function, proliferation, and infiltration. Recently, Wu et al. proved that BM-MSC-treated wounds exhibited significantly accelerated wound closure with increased reepithelialization, cellularity, and angiogenesis. Nanofibers have also been shown to enhance infiltration of stem cells.[55] Their results demonstrated that hMSCs isolated from human BM can differentiate into epithelial-like cells and may thus serve as a cell source for TE and cell therapy of epithelial tissue. Parenteau-Bareil et al. (2011) proved collagen–chitosan porous scaffolds mimicking the ECM of natural proteins for tissue engineering dermis.[56] To induce epithelial differentiation, they cultured MSCs using epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF)-II. Jin et al. (2011) showed the comparative scanning electron microscope (SEM) images of differentiated MSCs of epidermal phenotype and undifferentiated MSCs grown on collagen/poly(L-lactic acid)-*co*-poly (ε-caprolactone) (Coll/PLACL) nanofibrous scaffolds[57] (Fig. 2.5). SEM images of MSCs seeded with normal growth medium on the Coll/PLACL scaffold attached and remained undifferentiated with a fibroblastic phenotype. However, with time in culture, MSCs grown on Coll/PLACL nanofibrous scaffolds using epidermal induction medium acquired polygonal and round morphologies, and no cobblestone pattern clusters were

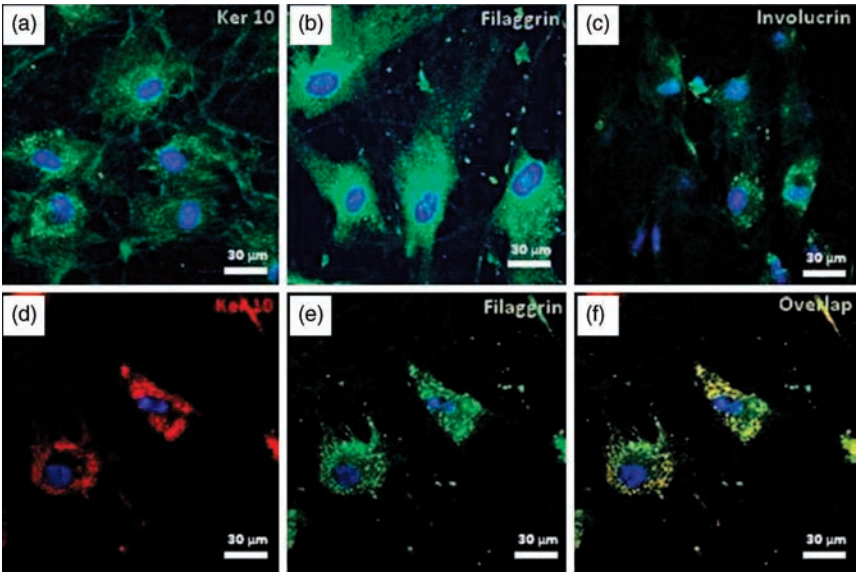


FIGURE 2.5 Laser scanning confocal microscope (LSCM) images of MSC grown in epidermal induction medium on Coll/PLLCL nanofibers expressing Ker 10 (a), filaggrin (b), and involucrin (c). Double staining for Ker 10 (d) and filaggrin (e) and (f) overlay image of (d) and (e) after 15 days of cell culture. Reproduced with permission from Ref. [57].

found on the Coll/PLACL nanofibrous scaffolds. The study suggest that the electrospun Coll/PLACL nanofibers supported the differentiation of MSCs in the presence of growth factors, thereby creating the possibility of cell–scaffold transplantation of a construct with differentiated keratinocytes to the sites of skin injury. Kobayashi and Spector (2009) investigated the clinical effects of mechanical stress on the behavior of BM-MSCs in a collagen type I/glycosaminoglycan scaffold matrix for 1 week under cyclic stretch loading conditions.[58] Their results suggested that mechanical stress may affect the proliferation and differentiation of stem cells and, subsequently, the wound healing process via interactions between the stem cells and scaffold matrix. Adipose-derived stem cells (ADSCs) secrete various growth factors that control and manage damaged neighboring cells, and this has been identified as an essential function of ADSCs. ADSCs stimulated both collagen synthesis and migration of dermal fibroblasts, which improved wrinkling and accelerated wound healing in animal models.[59,60]

Novel cost-effective electrospun nanofibrous scaffolds are established for wound dressing and allogeneic cultured dermal substitute through the cultivation of human dermal fibroblast for skin defects. A combination of growth factors together with the porous structure of the scaffolds might substantially improve the skin regeneration efficacy. This can be achieved by a simple incorporation of growth factors during the scaffold preparation, either with an electrospinning process or obtaining a controlled release of growth factors via a coaxial electrospinning technique.[61]

2.4.2 Cardiac

Myocardial infarction occurs when supply of oxygen and nutrients to the cardiac muscle is impaired, usually because of occluded coronary arteries. As a result, massive cell death occurs in the affected heart region.[62] Besides life-threatening arrhythmia, damage of muscle tissue in the left ventricle can cause dysfunction and remodeling in terms of progressive dilation, imparting structural changes that culminate in the formation of noncontractile fibrotic scar tissue.[63,64] Hence, the damage incurred to the heart wall is beyond recall because the myocardial tissue has limited regeneration capacity.[65,66] Although the body compensates for left ventricular (LV) remodeling initially, mismatch of mechanical and electrical properties of scar with native myocardium ultimately affects the functioning of the heart, leading to chronic heart failure, whereby the heart cannot pump adequate blood for all metabolic activities of the body.[67] Many intriguing modes of regenerating injured myocardium have emerged over time with pioneering research in a variety of technologies, including cell therapy using various cell types, injection of biomaterials, bioengineered patches, 3D construct implantation, and even bioreactor-treated implants.[68–70] In native tissue, cell growth and structural development is supported by the ECM. Lack of an appropriate microenvironment in scarred myocardium might be a plausible reason for the colossal loss and ineffective homing of injected cells. To enhance cell attachment, proliferation, and differentiation, it is necessary to mimic some of the nanostructure of the natural ECM. Scaffolds with nanoscaled architecture provide larger surface area to adsorb proteins and provide more binding site to cell membrane receptors, unlike microscale and flat surfaces.[71] This makes nanofabrication of biomaterials for myocardial regeneration is an attractive strategy. Traditionally, a cardiomyocyte has been considered terminally differentiated in response to injury. However, recent evidence raises the possibility that a natural system of myocyte repair exists. According to this study, fewer than 50% of cardiomyocytes are exchanged during a normal life span. This system appears to be inadequate in face of an ischemic or heart failure insult and its treatment.[12] Ultrafine woven nanofibers having ECM-like topography can be achieved by electrospinning of biomaterial or self-assembly of certain peptides via noncovalent interactions.[72,73] A versatile, biodegradable *in vitro* construct made of poly(ε-caprolactone) (PCL) nanofibers and cardiomyocytes was reported by Shin et al. (2004).[74] Being able to foster cellular ingrowth, it was proposed to be more desirable than 3D construct in patch application.[74] The bioengineered cardiac tissue structure and function, chemistry, and geometry of the provided nano- and microtextured using poly(lactic-*co*-glycolic acid) (PLGA) nanofibers were later demonstrated. Thereafter, nanofibers of blended and conductive polymers were shown to be potential choices in MTE.[75,76] Recently, coaxial electrospun poly(glycerol–sebacate) (PGS) nanofibers were fabricated, opening up new horizons in MTE owing to its resemblance to elastin fibers.[77] Recently, Mukherjee et al. showed that suitable cell–material interactions on the nanoscale can stipulate organization on the tissue level and yield novel insights into cell therapeutic science while providing materials for tissue regeneration.[78] Inspired by microscopic analysis of the ventricular organization

in native tissue, we fabricated a scalable, nanotopographically controlled *in vitro* model of nanoscale poly(L-lactic acid)-*co*-poly(ε-caprolactone)/collagen biocomposite scaffold of nanofibers measuring 594 ± 56 nm to mimic the native myocardial environment for freshly isolated cardiomyocytes from rabbit heart and specifically underlying ECM architecture to address specificity of underlying matrix in overcoming challenges faced by cellular therapeutics. Guided by nanoscale mechanical cues provided by the underlying random nanofibrous scaffold, the tissue constructs displayed anisotropic rearrangement of cells, characteristic of the native cardiac tissue. Surprisingly, cell morphology and growth and the expression of an interactive healthy cardiac cell population were exquisitely sensitive to differences in the composition of nanoscale scaffolds that features of the surrounding ECM.[79] Ravichandran et al. fabricated PGS/gelatin core/shell fibers and gelatin fibers alone by electrospinning for cardiac tissue engineering. In these PGS/gelatin core/shell fibers, PGS used as core polymer to impart the mechanical properties and gelatin as a shell material to achieve

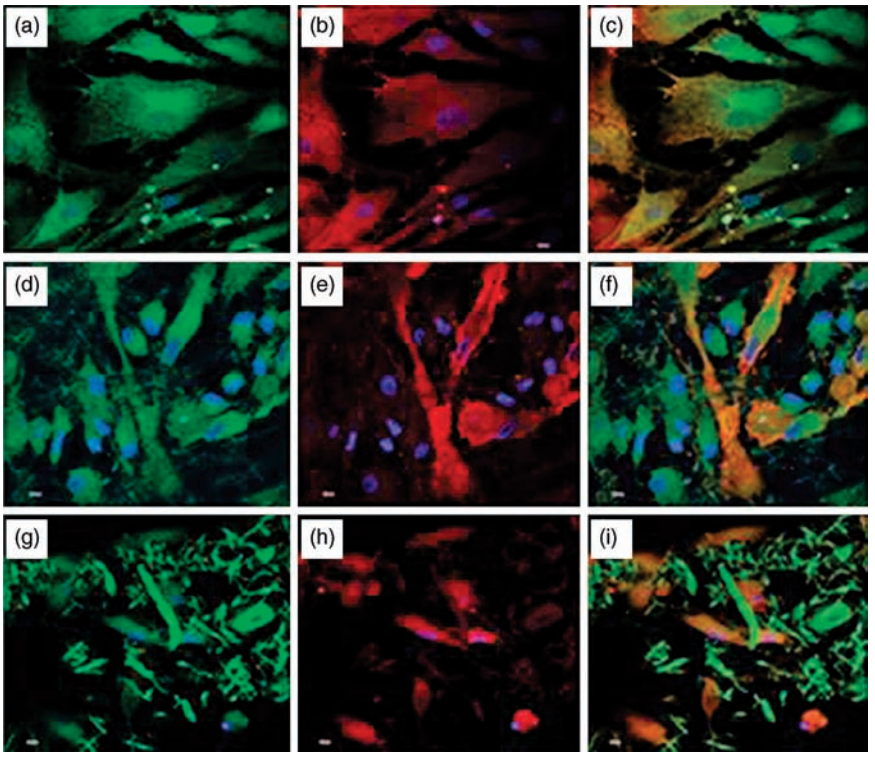


FIGURE 2.6 Core/shell (PGS/gelatin) fibrous structure for regeneration of myocardial infarction. Dual immunocytochemical analysis for the expression of MSC marker protein CD 105 (a, d, g) and cardiac marker protein actinin (b, e, h) in the coculture samples and the merged image showing the dual expression of both CD 105 and actinin (c, f, i); on the TCP (a, b, c), gelatin nanofibers (d, e, f), and PGS/gelatin core/shell fibers (g, h, i) at 60× magnification. Nucleus stained with DAPI. Reproduced with permission from Ref. [80].

favorable cell adhesion and proliferation. The study demonstrated that PGS/gelatin core/shell fibers, having good potential biocompatibility and mechanical properties for fabricating nanofibrous cardiac patch, favor differentiation MSC into the cardiac lineage[80] (Fig. 2.6). It is likely that the structure and function of the *in vivo* cardiac tissue are regulated by much smaller nanoscale cues provided by the ECM, which is responsible for extensive control over cell and tissue function.[81] Thus, biomaterials with controlled bioactivity could be potentially designed to respond and enhance the regenerative capability of myocytes or exogenous cells to adjust the myocardial mechanical load for myocardial tissue engineering.

2.4.3 Bone and Cartilage

Bone and cartilage tissue regeneration remains an important challenge in the fields of orthopedic and craniofacial surgery. Every year, millions of people around the world have bone defects arising from trauma, tumors, biochemical disorders, and abnormal skeletal development; the worst scenario is that many die because of insufficient bone and cartilage replacements.[82] Cell-based therapies such as autologous chondrocyte transplantation (ACT) has been used clinically since 1987 to treat full chondral thickness defects. Nearly 12,000 patients with full chondral thickness defects have benefited from ACT worldwide.[4] Currently, more than 250,000 knee and hip replacements are performed in the United States each year for end-stage disease joint failure, and many other patients have less severe cartilage damage.[83] The emerging trend in recent decades is the use of nanofibrous scaffolds as synthetic ECM with which cells interact before forming a new tissue. These nanofibrous scaffolds are capable of providing the desired support needed for cell adhesion, proliferation, and differentiation.[84] The osteoinductive and osteoconductive properties that are vital for mineralization and bone growth, various kind of material used for the preparation of scaffolds. The scaffold should be biocompatible and biodegradable, and the rate of biodegradation should match the rate of formation of the new tissues. It should be highly porous and should allow nutrient transport and tissue ingrowth. Several cell types have been reported for increased proliferative ability on nanofibrous scaffolds than control tissue culture plate (TCP). Osteoblasts, when seeded on nanofibrous scaffolds, have shown increased proliferation within 7–12 days of culture[85] because an increase in proliferation reduces the scar tissue formation, which eventually reduces the surgical necessities to remove scar tissue. Nanofibers enhanced the proliferation and differentiation of many cell types, including neural progenitors,[86] hepatocytes,[87] and osteoblasts.[88] Nanofibrous scaffolds also have the ability to rescue cells from regression, promoting them to a more immature phenotype during expansion culture.[89] The key attachment proteins such as fibronectin, vitronectin, and laminin have been found to adsorb to nanofibrous scaffolds 2.6–3.9 times higher than the solid-walled (SW) scaffolds.[90] A variety of natural and synthetic biodegradable materials have been used for the fabrication of nanofibrous scaffolds in tissue engineering, but the main disadvantage in these synthetic scaffolds is the lack of biological recognition sites on their surface; in other words, they are noninformational scaffolds. Various groups have tried to modify the

surface of scaffolds to increase cell–surface interactions, eventually increasing the rate of mineralization.[91,92] Human ESC-derived embryoid body cells were cultured in the presence of osteogenic supplements such as ascorbic acid and beta-glycerophosphate (BGP) for 14 days, and dexamethasone was added to this medium for another 24 h. The stimulated cells were further seeded onto poly(lactic acid) (PLA) scaffolds and implanted subcutaneously to the back of immunodeficient mice for 5 weeks. Discrete areas of mineralization were observed, and osteocalcin was expressed by the implanted cells.[93] The cell–cell interactions and bone morphogenic proteins secreted by primary bone-derived cells stimulated human embryonic stem cells (hESCs) into osteogenic lineages in a direct coculture system.[94] Cell extracts derived from hESC-derived osteogenic cultures induced undifferentiated hESCs into osteogenic lineage.[95] Electrospun nanofibrous scaffolds have been successful in supporting the maintenance of chondrocyte phenotype and chondrogenic induction of stem cells.[96] These nanofibrous scaffolds have given hope for cartilage tissue-engineering applications. Chondrocytes seeded on electrospun scaffolds have shown increased proliferation within 3 weeks of culture than the controls. Increased chondrocytes proliferation, differentiation, and attachment have been studied in nanofibrous scaffold by Li et al.[97] The differentiation of stem cells to chondrocytes on nanofibrous scaffold was comparable to an established cell pellet culture. It was advantageous to use nanofibers rather than a cell pellet system, owing to their better mechanical properties, oxygen–nutrient exchange, and ease of fabrication. Cheng et al. reported that human cartilage cells attached and proliferated on hyaluronic acid nanocrystals homogeneously dispersed in PLA and that collagen fibers of 110–1.8 mm diameter supported chondrocyte growth and infiltration.[98] Chondrogenesis of MSCs was supported on 3D porous aqueous-derived silk scaffolds, forming cartilage-like tissue with spatial distribution of cells and ECM, with expression of chondrogenic genes, and zonal architecture resembling the native

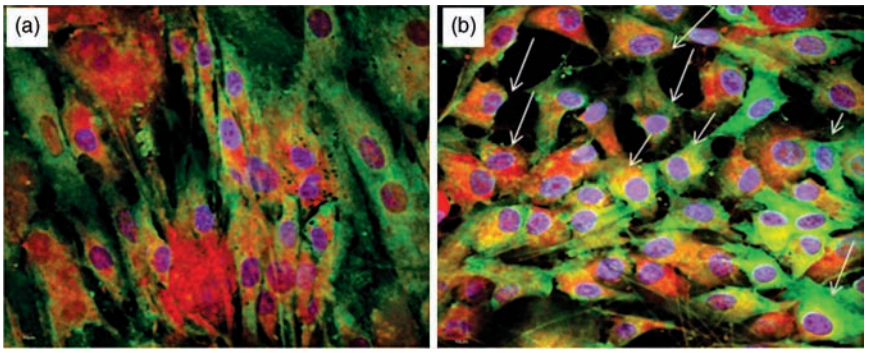


FIGURE 2.7 Confocal microscopy image of PLLA nanofibers (a) and PLLA/PBLG/Col/n-HA nanofibers (b) showing dual expression of both ADSC specific marker protein CD 105 and osteoblasts specific marker protein osteocalcin. *Arrows* indicate the characteristic cuboidal morphology of osteoblasts shown by the ADSCs that have undergone osteogenic differentiation on the PLLA/PBLG/Col/n-HA nanofibers at 60× magnification. Reproduced with permission from Ref. [102].

tissue.[99,100] Chondrogenesis was improved in silk scaffolds compared with collagen scaffolds in terms of cell attachment, metabolic activity, proliferation, ECM deposition, and glycosaminoglycan (GAG) content.[101] However, the biggest challenge with using nanofibrous scaffolds is the intrinsically small pore size of the fibers, which limits infiltration and migration of the seeded cells and affects cell distribution in the scaffold. This limitation can be overcome by changing the cell-seeding procedures on the scaffold for cartilage. Smart materials like PLLA/PBLG/Col/n-HA scaffolds elicit therapeutic effects by incorporating bio-signaling molecules within the nanofibers, such as proteins and genes, hold great promise as scaffolds for bone tissue engineering with drug delivery applications (Fig. 2.7).

2.4.4 Neural

Neural diseases represent a very complicated and significant clinical problem; for example, in the United States alone, about 250,000–400,000 people are living with spinal cord injury, and nearly 13,000 additional people sustain spinal cord injuries each year. Peripheral nerve lesions are serious injuries, affecting 2.8% of trauma patients annually, leading to lifelong disability.[103] Allograft and xenografts have certain disadvantages such as disease transmission and immunogenicity. The other disadvantages of autograft nerve repair systems include the loss of function at the donor nerve graft site and mismatch of damaged nerve and graft dimensions. TE offers promising strategies and provides viable alternatives to surgical procedures for harvested tissues and implants.[104] Many researchers have attempted to regenerate nerve tissue by combining scaffolds with MSCs, and it has also been shown that the chemical composition of scaffolds influences the differentiation of MSC to nerve cells. Prabhakaran et al. compared the potential of hMSCs for *in vitro* neuronal differentiation on poly(L-lactic acid)-*co*-poly(ε-caprolactone)/collagen (PLCL/collagen) and PLCL nanofibrous scaffolds. Many researchers have attempted to regenerate nerve tissue by combining scaffolds with MSCs, and it has also been shown that the chemical composition of scaffolds influences the differentiation of MSC to nerve cells.[105] Prabhakaran et al. compared the potential of hMSCs for *in vitro* neuronal differentiation on PLCL/collagen and PLCL nanofibrous scaffolds.[106] MSCs have been shown to have an important regenerative potential after transplantation into the stumps of transected sciatic nerves. Lopes et al. evaluated the regeneration of peripheral nerve using a tubular nerve guide of resorbable collagen filled with MSCs. Their results showed that a biodegradable collagen tube filled with MSCs induced better regeneration of peripheral nerve fibers across a nerve gap than a collagen tube without cells.[107] Oliveira et al. fabricated PCL conduits for regeneration of transected mouse median nerves and investigated the effect of MSCs on nerve regeneration by seeding MSCs on PCL nerve conduit before grafting of PCL conduits.[108] Hou et al. differentiated MSCs into cells expressing characteristic markers of Schwann cells and used PLGA nerve conduit along with differentiated MSCs for bridging a 10 mm long sciatic nerve defect.[109] Lee et al. constructed nanoscale ridge/groove pattern arrays using UV-assisted capillary force lithography on polyurethane acrylate (PUA) and showed that the nanoscale ridge/groove pattern

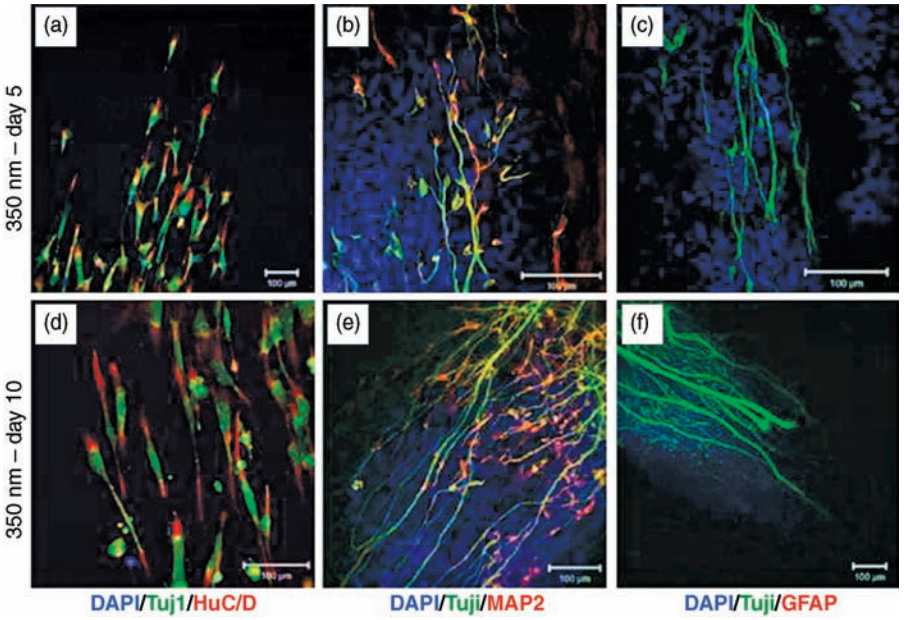


FIGURE 2.8 Immunofluorescence staining of human embryonic stem cells (hESCs) with neural and glial markers. (a, d) hESCs were immunolabeled for DAPI, Tuj1, and HuC/D. (b, e) hESCs were immunolabeled for DAPI, Tuj1, and MAP2. (c, f) hESCs were immunolabeled for DAPI, Tuj1, and GFAP. hESCs cultured for 5 days (a, b, c) and 10 days (d, e, f) on the 350 nm ridge/groove pattern arrays. Reproduced with permission from Ref. [110].

arrays can rapidly and efficiently induce the differentiation of hESCs into neuronal lineages even in the absence of differentiation-inducing agents[110] (Fig. 2.8). Functionalizing biomaterials with bioactive molecules such as ECM-derived cell adhesive molecules to impregnate guiding cues on the scaffolds is an emerging research interest and can provide an instructive extracellular microenvironment for neural regeneration.

2.5 CONCLUSIONS

Nanotechnology has the potential to change medical research dramatically with advances in cell-based technologies. Tissue engineering is the promising therapeutic approach that combines cells, biomaterials, and microenvironmental factors to induce differentiation signals into surgically transplantable formats and promote tissue repair, functional restoration, or both. One obstacle can be identified as the scaffolds play an important role as the ECM, but they are often unable to create the exact or correct microenvironment during the engineered tissue development to promote the accurate *in vitro* tissue development. The emerging and promising next generation of engineered tissues relies on producing scaffolds with an informational

function, such as material containing a growth factors sequence that facilitates cell attachment, proliferation, and differentiation that is far better than noninformational polymers. Stem cell-based tissue engineering has matured from its original goal of prolonging or replacing; it now involves customized systems are designed to achieve specific spatial and temporal control in tissue engineering applications. The new generation of tissue engineering systems incorporates "smart" biosensing functionalities and will enable unaided *in vivo* feedback control. To advance the biotechnological and especially biomedical nanotechnology applications of polymer nanofibers from the perspective to commercialized stages, collaborative interdisciplinary research involving surgeons, material scientists, biologist, physiologists, clinicians, and engineers is required. One may believe that continual research and development in this field not only shortens the distance to a practical application in the listed areas but also open up other new opportunities for polymer nanofibers in drug delivery and tissue engineering applications.

ACKNOWLEDGMENTS

The authors would like to acknowledge financial support from NRF-Technion (Grant No.: R-398-001-063-592), Division of Bioengineering (National University of Singapore), and the Nanoscience and Nanotechnology Initiative (National University of Singapore).

REFERENCES

1. Logothetidis S. Nanotechnology in medicine: the medicine of tomorrow and nanomedicine. Hippokratia 2006;10:7–21.
2. Venugopal J, Low S, Choon AW, Ramakrishna S. Interaction of cells and nanofiber scaffolds in tissue engineering. J Biomed Mater Res B Appl Biomater 2008;84:34–48.
3. Ma Z, Ramakrishna S. Nanostructured extracellular matrix. In: *Encyclopedia of Nanoscience and Nanotechnology*. Vol. 7. California, USA: American Scientific Publishers 2004, pp. 641–655.
4. Cannizzaro SM, Padera RF, Langer R, Rogers RA, Black FE, Davies MC, Tendler SJ, Shakesheff KM. A novel biotinylated degradable polymer for cell-interactive applications. Biotechnol Bioeng 1998;58:529–535.
5. Wang DA, Ji J, Sun YH, Shen JC, Feng LX, Elisseeff JH. *In situ* immobilization of proteins and RGD peptide on polyurethane surfaces via poly(ethylene oxide) coupling polymers for human endothelial cell growth. Biomacromolecules 2002;3:1286–1295.
6. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials 2000;21:2529–2543.
7. Peter SJ, Miller MJ, Yasko AW, Yaszemski AG, Mikos AG. Polymer concepts in tissue engineering. Appl Biomater 1998;43:422–427.
8. Smith LA, Ma PX. Nano-fibrous scaffolds for tissue engineering. Colloids Surf B Biointerfaces 2004;39:125–131.

9. Zhang YZ, Huang ZM, Xu X, Lim CT, Ramakrishna S. Preparation of core–shell structured PCL-r-gelatin bi-component nanofibers by coaxial electrospinning. Chem Mater 2004;16:3406–3409.
10. Peng T, Cheng YL. PNIPAAm and PMAA co-grafted porous PE membranes: living radical co-grafting mechanism and multi-stimuli responsive permeability. Polymer 2001;42:2091–2100.
11. Yokoyama M. Drug targeting with nano-sized carrier systems. J Artif Organs 2005;8:77–84.
12. Langer R, Tirrell DA. Designing materials for biology and medicine. Nature 2004;428:487–492.
13. Zhang YZ, Su B, Lim CT, Venugopal J and S. Ramakrishna. Biomimetic and bioactive nanofibrous scaffolds by electrospinning of composite materials. Int J Nanomed 2007;2 (4):623–638.
14. Loscertales IG, Barrero A, Marquez M, Ganan-Calvo AM. Micro/nano encapsulation via electrified coaxial liquid jets. Science 2002;295:1695–1698.
15. Szentivanyi A, Chakradeo T, Zernetsch H, Glasmacher B. Electrospun cellular microenvironments: understanding controlled release and scaffold structure. Adv Drug Delivery Rev 2011;63(4–5):209–220.
16. Kolácná L, Bakesova F, Varga F, Kostáková E, Plánka L, Necas A, Lukás D, Amler E, Pelouch V. Biochemical and biophysical aspects of collagen nanostructure in the extracellular matrix. Physiol Res 2007;56(Suppl):S51–S60.
17. Stitzel J, Liu J, Lee SJ, Komura M, Berry J, Yoo JJ, Atala A. Controlled fabrication of a biological vascular substitute. Biomaterials 2006;27:1088–1094.
18. Kolacna L, Bakesova F, Varga F, Pelouch V. Biochemical and biophysical aspects of collagen nanostructure in the extracellular matrix. Physiol Res 2007;56 (Suppl 1): S51–60.
19. Miller C, Shanks H, Witt A, Rutkowski G, Mallapragada S. Oriented Schwann cell growth on micropatterned biodegradable polymer substrates. Biomaterials 2001;22: 1263–1269.
20. Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 2006;27:3413–3431.
21. Guarino V, Ambrosio L. The synergic effect of polylactide fiber and calcium phosphate particle reinforcement in poly epsilon-caprolactone-based composite scaffolds. Acta Biomater 2008;4:1778.
22. Gadegaard N, Martines E, Riehle MO, Seunarine K, Wilkinson CDW. Applications of nano-patterning to tissue engineering. Microelectron Eng 2006;83:1577.
23. Truskett VN, Watts MPC. Trends in imprint lithography for biological applications. Trends Biotechnol 2006;24:312.
24. Guo LJ. Recent progress in nanoimprint technology and its application. J Phys D Appl Phys 2004;37:R123–R141.
25. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9(5):641–650.
26. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. Cytotherapy 2009;11 (4):377–391.
27. Alhadlaq A, Mao JJ. Mesenchymal stem cells: isolation and therapeutics. Stem Cells Dev 2004;13:436–448.

28. Le Blanc K, Pittenger M. Mesenchymal stem cells: progress toward promise. Cytotherapy 2005;7:36–45.
29. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211.
30. Bottai D, Fiocco R, Gelain F, Defilippis L, Galli R, Gritti A, Vescovi LA. Neural stem cells in the adult nervous system. J Hematother Stem Cell Res 2003;12:655.
31. Beyer Nardi N, da Silva Meirelles L. Mesenchymal stem cells: isolation, *in vitro* expansion and characterization. Handb Exp Pharmacol 2006;174:249–282.
32. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 1999;181:67–73.
33. Ries C, Egea V, Karow M, Kolb H, Jochum M, Neth P. MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood 2007;109:4055–4063.
34. Le Blanc K. Immunomodulatory effects of fetal and adult mesenchymal stem cells. Cytotherapy 2003;5:485–489.
35. Lu L, Shen RN, Broxmeyer HE. Stem cells from bone marrow, umbilical cord blood and peripheral blood for clinical application: current status and future application. Crit Rev Oncol Hematol 1996;22:61–78.
36. Richards M, Tan SP, Tan JH, Chan WK, Bongso A. The transcriptome profile of human embryonic stem cells as defined by SAGE. Stem Cells 2004;22:51–64.
37. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998;282 (5391):1145–1147.
38. Chambers I, Smith A. Self-renewal of teratocarcinoma and embryonic stem cells. Oncogene 2004;23:7150–7160.
39. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. Nat Biotechnol 2000;18: 399–404.
40. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 2003;113:631–642.
41. Judson RL, Babiarz JE, Venere M, Blelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 2009;27:459–461.
42. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.
43. Sendtner M. Stem cells: tailor-made diseased neurons. Nature 2009;457:269–270.
44. Martino S, D'Angelo F, Armentano I, Kenny JM, Orlacchio A. Stem cell-biomaterial interactions for regenerative medicine. Biotechnol Adv 2011;30:338–351.
45. Zhao T, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature 2011;474 (7350):212–215.
46. Singh S. Nanomedicine-nanoscale drugs and delivery systems. J Nanosci Nanotechnol 2010;10(12):7906–7918.
47. Kumbar SG, Nair LS, Bhattacharyya S, Laurencin CT. Polymeric nanofibers as novel carriers for the delivery of therapeutic molecules. J Nanosci Nanotechnol 2006;6 (9–10):2591–2607.

48. Ashammakhi N, Ndreu A, Piras A, Nikkola L, Sindelar T, Ylikauppila H, Harlin A, Chiellini E, Hasirci V, Redl H. Biodegradable nanomats produced by electrospinning: expanding multifunctionality and potential for tissue engineering. J Nanosci Nanotechnol 2006;6:2693–2711.
49. Burger C, Hsiao BS, Chu B. Nanofibrous materials and their applications. Annu Rev Mater Res 2006;36:333–368.
50. MacNeil S. Progress and opportunities for tissue-engineered skin. Nature 2007;445:874–880.
51. Pham C, Greenwood J, Cleland H, Woodruff P, Maddern G. Bioengineered skin substitutes for the management of burns: a systematic review. Burns 2007;33:946–957.
52. Yannas IV, Kwan MD, Longaker MT. Early fetal healing as a model for adult organ regeneration. Tissue Eng 2007;13:1789.
53. Venugopal J, Low S, Choon AT, Ramakrishna S. Interaction of cells and nanofibrous scaffolds in tissue engineering. J Biomed Mater Res 2008;84B:34–48.
54. Venugopal J, Prabhakaran MP, Low S, Choon AT, Ramakrishna S. Nanotechnology for nanomedicine and delivery of drugs. Curr Pharma Design 2008;14:2184–2200.
55. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007;25:2648–2659.
56. Parenteau-Bareil R, Gauvin R, Berthod F. Comparative study of bovine, porcine and avian collagens for the production of a tissue engineered dermis. Acta Biomater 2011;7 (10):3757–3765.
57. Jin G, Prabhakaran MP, Ramakrishna S. Stem cell differentiation to epidermal lineages on electrospun nanofibrous substrates for skin tissue engineering. Acta Biomater 2011;7 (8):3113–3122.
58. Kobayashi M, Spector M. *In vitro* response of the bone marrow-derived mesenchymal stem cells seeded in a type-I collagen-glycosaminoglycan scaffold for skin wound repair under the mechanical loading condition. Mol Cell Biomech 2009;6:217–227.
59. Kim WS, Park BS, Sung JH, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci 2007;48 (1):15–24.
60. Sasaki M, Abe R, Fujita Y, et al. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 2008;180(4):2581–2587.
61. Zhang YZ, Venugopal J, Huang ZM, Lim CT, Ramakrishna S. Characterization of the surface biocompatibility of the electrospun PCL-collagen nanofibers using fibroblasts. Biomacromolecules 2005;6:2583–2589.
62. Zwaan C, Daemen M, Hermens W. Mechanisms of cell death in acute myocardial infarction: pathophysiological implications for treatment. Netherland heart J 2001;9:30–44.
63. Cairns JA, Connolly SJ, Gent M, Roberts R. Post-myocardial infarction mortality in patients with ventricular premature depolarizations. Canadian Amiodarone Myocardial Infarction Arrhythmia Trial Pilot Study. Circulation 1991;84:550–557.
64. Uusimaa P, Risteli J, Niemelä M, Lumme J, Ikäheimo M, Jounela A, Peuhkurinen K. Collagen scar formation after acute myocardial infarction: relationships to infarct size, left ventricular function, and coronary artery patency. Circulation 1997;96:2565–2572.

65. Pasumarthi KB, Field LJ. Cardiomyocyte cell cycle regulation. Circ Res 2002;90: 1044–1054.
66. Rubart M, Field LJ. Cardiac regeneration: repopulating the heart. Ann Rev Physiol 2006;68:29–49.
67. Baig MK, Mahon N, McKenna WJ, Caforio AL, Bonow RO, Francis GS, Gheorghiade M. The pathophysiology of advanced heart failure. Heart Lung 1999;28:87–101.
68. Jawad H, Ali NN, Lyon AR, Chen QZ, Harding SE, Boccaccini AR. Myocardial tissue engineering: a review. J Tissue Eng Regen Med 2007;1:327–342.
69. Leor J, Amsalem Y, Cohen S. Cells, scaffolds, and molecules for myocardial tissue engineering. Pharmacol Ther 2005;105:151–163.
70. Bär A, Haverich A, Hilfiker A. Cardiac tissue engineering: "reconstructing the motor of life." Scand J Surg 2007;96:154–158.
71. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science 2005;310:1135–1138.
72. Murugan R, Ramakrishna S. Nano-featured scaffolds for tissue engineering: a review of spinning methodologies. Tissue Eng 2006;12:435–447.
73. Venugopal J, Ramakrishna S. Applications of polymer nanofibers in biomedicine and biotechnology. Appl Biochem Biotechnol 2005;125:147–158.
74. Shin M, Ishii O, Sueda T, Vacanti JP. Contractile cardiac grafts using a novel nanofibrous mesh. Biomaterials 2004;25:3717–3723.
75. Li M, Guo Y, Wei Y, MacDiarmid AG, Lelkes PI. Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. Biomaterials 2006;27:2705–2715.
76. Li M, Mondrinos MJ, Chen X, Gandhi MR, Ko FK, Lelkes PI. Co-electrospun poly (lactide-*co*-glycolide), gelatin, and elastin blends for tissue engineering scaffolds. J Biomed Mater Res A 2006;79:963–973.
77. Yi F, LaVan DA. Poly(glycerol sebacate) nanofiber scaffolds by core/shell electrospinning. Macromol Biosci 2008;8:803–806.
78. Mukherjee S, Gualandi C, Focarete ML, Ravichandran R, Venugopal JR, Raghunath M, Ramakrishna S. Elastomeric electrospun scaffolds of poly(L-lactide-*co*-trimethylene carbonate) for myocardial tissue engineering. J Mater Sci Mater Med 2011;22(7):1689–1699.
79. Mukherjee S, Venugopal JR, Ravichandran R, Ramakrishna S, Raghunath M. Evaluation of the biocompatibility of PLACL/collagen nanostructured matrices with cardiomyocytes as a model for the regeneration of infarcted myocardium. Adv Funct Mater 2011;21 (12):2291–2300.
80. Ravichandran R, Venugopal JR, Sundarrajan S, Mukherjee S, Ramakrishna S. Poly (Glycerol sebacate)/gelatin core/shell fibrous structure for regeneration of myocardial infarction. Tissue Eng Part A 2011;17(9–10):1363–1373.
81. Lim JY, Hansen JC, Siedlecki CA, Hengstebeck RW, Cheng J, Winograd N, Donahue HJ. Osteoblast adhesion on poly(L-lactic acid)/polystyrene demixed thin film blends: effect of nanotopography, surface chemistry, and wettability. Biomacromolecules 2005; 6:3319.
82. Marlovits S, Zeller P, Singer P, Resinger C, Vecsei V. Cartilage repair: generations of autologous chondrocyte transplantation. Eur J Radiol 2006;57:24–31.

83. Elisseeff J. Injectable cartilage tissue engineering. Expert Opin Biol Ther 2004: 4:1849–1859.
84. Ravichandran R, Liao S, Ng CCh, Chan CK, Raghunath M, Ramakrishna S. Effects of nanotopography on stem cell phenotypes. World J Stem Cells 2009;1:55–66.
85. Chen VJ, Smith LA, Ma PX. Bone regeneration on computer-designed nano-fibrous scaffolds. Biomaterials 2006;27:3973–3979.
86. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science 2004;303:1352–1355.
87. Semino CE, Merok JR, Crane GG, Panagiotakos G, Zhang S. Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds. Differentiation 2003;71:262–270.
88. Woo KM, Jun JH, Chen VJ, Seo J, Baek JH, Ryoo HM, Kim GS, Somerman MJ, Ma PX. Nano-fibrous scaffolding promotes osteoblast differentiation and biomineralization. Biomaterials 2007;28:335–343.
89. Gupta D, Venugopal J, Mitra S, Giri dev VR, Ramakrishna S. Nanostructured biocomposite substrates by electrospinning and electrospraying for the mineralization of osteoblasts. Biomaterials 2009;30:2085–2094.
90. Woo KM, Chen VJ, Ma PX. Nano-fibrous scaffolding architecture selectively enhances protein adsorption contributing to cell attachment. J Biomed Mater Res 2003;67:531–537.
91. Cui W, Li X, Zhou S, Weng J. *In situ* growth of hydroxyapatite within electrospun poly (DL-lactide) fibers. J Biomed Mater Res 2007;82:831–841.
92. Gao J, Niklason L, Langer R. Surface hydrolysis of poly(glycolic acid) meshes increases the seeding density of vascular smooth muscle cells. J Biomed Mater Res 1998;42:417–424.
93. Bielby RC, Boccaccini AR, Polak JM. *In vitro* differentiation and *in vivo* mineralization of osteogenic cells derived from human embryonic stem cells. Tissue Eng 2004;10:1518.
94. Ahn SE, Kim S, Park KH. Primary bone-derived cells induce osteogenic differentiation without exogenous factors in human embryonic stem cells. Biochem Biophys Res Commun 2006;340:403.
95. Heng BC, Toh WS, Pereira BP. An autologous cell lysate extract from human embryonic stem cell (hESC) derived osteoblasts can enhance osteogenesis of hESC. Tissue Cell 2008;40:219.
96. Meinel L, Hofmann S, Karageorgiou V, Zichner L, Langer R, Kaplan D, Vunjak-Novakovic G. Engineering cartilage-like tissue using human mesenchymal stem cells and silk protein scaffolds. Biotechnol Bioeng 2004;88:379–391.
97. Li WJ, Tuli R, Okafor C, Derfoul A, Danielson KG, Hall DJ, Tuan RS. Biomaterials 2005;26:599–609.
98. Cheng L, Zhang SM, Chen PP, Huang SL, Cao RR, Zhou W, Liu J, Lou QM, Gong H. Fabrication and characterization of nano-hydroxyapatite/poly (D,L-lactide) composite porous scaffolds for human cartilage tissue engineering. Key Eng Mater 2006; 309–311:943.
99. Wang Y, Blasioli DJ, Kim HJ, Kaplan DL. Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. Biomaterials 2006;27:4434.

100. Wang Y, Kim UJ, Blasioli DJ, Kim HJ, Kaplan DL. *In vitro* cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. Biomaterials 2005;26:7082.

101. Hofmann S, Knecht S, Kaplan DL, Merkle HP. Cartilage-like tissue engineering using silk scaffolds and mesenchymal stem cells. Tissue Eng 2006;12:2729.

102. Ravichandran R, Venugopal J, Sundarrajan S, Mukherjee S, Ramakrishna S. Precipitation of nanohydroxyapatite on PLLA/PBLG/Collagen nanofibrous structures for the differentiation of adipose derived stem cells to osteogenic lineage. Biomaterials 2012; 33(3):846–855.

103. Tran PA, Zhang L, Webster TJ. Carbon nanofibers and carbon nanotubes in regenerative medicine. Adv Drug Deliv Rev 2009;61:1097.

104. Schmidt CE, Leach JB. Neural tissue engineering: strategies for repair and regeneration. Annu Rev Biomed Eng 2003;5:293–347.

105. Wang L, Wang ZH, Shen CY, You ML, Xiao JF, Chen GQ. Differentiation of human bone marrow mesenchymal stem cells grown in terpolyesters of 3-hydroxyalkanoates scaffolds into nerve cells. Biomaterials 2010;31:1691.

106. Cho YI, Choi JS, Jeong SY, Yoo HS. Nerve growth factor (NGF)-conjugated electrospun nanostructures with topographical cues for neuronal differentiation of mesenchymal stem cells. Acta Biomater 2010;6:4725.

107. Lopes FRP, Camargo de Moura Campos L, Dias Corrêa J, Jr., Balduino A, Lora S, Langone F, Borojevic R, Blanco Martinez AM. Bone marrow stromal cells and resorbable collagen guidance tubes enhance sciatic nerve regeneration in mice. Exp Neurol 2006;198:457.

108. Oliveira JT, Almeida FM, Biancalana A, Baptista AF, Tomaz MA, Melo PA, Martinez AM. Mesenchymal stem cells in a polycaprolactone conduit enhance median-nerve regeneration, prevent decrease of creatine phosphokinase levels in muscle, and improve functional recovery in mice. Neuroscience 2010;170:1295.

109. Hou SY, Zhang HY, Quan DP, Liu XL, Zhu JK. Tissue-engineered peripheral nerve grafting by differentiated bone marrow stromal cells. Neuroscience 2006;140:101.

110. Lee MR, Kwon KW, Jung H, Kim HN, Suh KY, Kim K, Kim KS. Direct differentiation of human embryonic stem cells into selective neurons on nanoscale ridge/groove pattern arrays. Biomaterials 2010;31:4360.