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SYNTHETIC ENROUTES TO ENGINEER ELECTROSPUN SCAFFOLDS FOR STEM CELLS AND TISSUE REGENERATION

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5.1 INTRODUCTION

Nanotechnology is one of the rapidly growing scientific disciplines involved in the development of materials with nanoscale dimensions, and it aims at resolving many of the diseases related to organ damage. Nanotechnology for tissue engineering application focuses on the role of extracellular matrix (ECM) in cell patterning, migration, proliferation, and differentiation.[1] Tissue growth or regeneration is achieved by stimulation assisted by cells or drug or growth factor loaded matrix at the damage tissue site. Matrix suitable for tissue regeneration should satisfy a few criteria such that it should be physically stable within the implanted site of injury, direct and control tissue growth, be biodegradable *in vivo*, and should not produce toxic metabolic byproducts. Various biomimetic tissue engineering scaffolds are made from natural and synthetic polymers possess certain optimal mechanical strength and form a sponge type or nanofibrous matrix or hydrogel architecture.

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To engineer such complex and multifunctional scaffolds, many developments in the field of nanotechnology were evolved to create porous, nanometer-sized nanofiber scaffolds so as to determine the fate of the cells, allow regulation of specific protein expression patterns, and encourage cell-specific scaffold remodeling. These nanotechniques can modulate surface topography down to submicron or nanometer range, and they include methods such as nanoscale surface pattern fabrication, electrospinning, and self-assembly fabrication.[2] Incorporating biological signals in the form of growth factors, angiogenic factors, cell surface receptors, drug entities, reactive oxygen species, and spatial cues can further influence cell proliferation, migration, differentiation, and 3D organization.

Nanofibrous scaffolds are ideal for the purpose of tissue regeneration because their dimensions are similar to components of ECM and mimic its fibrillar structure, providing essential cues for cellular organization and survival function. Electrospinning is one of the most important promising techniques for designing polymer nanofibers for tissue engineering applications. Tissue engineering is a multidisciplinary area of research and clinical application that aims for the repair, replacement, or regeneration of cells, tissues, or organs to restore impaired function owing to congenital defects, disease, trauma, or aging. The principle of tissue engineering and regenerative medicine is the application opt biomaterial scaffolds to produce living structures with sufficient size and function to improve human lives. The native ECM is a complex arrangement of proteins and polysaccharides such as collagen, hyaluronic acid, proteoglycans, glycosaminoglycans, and elastin, and electrospinning produces nanofibers with ECM mimicking molecules and architecture This chapter discusses "electrospinning process" (Fig. 5.1) as a novel method for engineering scaffolds for stem cells and tissue regeneration. Scaffolds made of natural proteins and carbohydrate materials have poor mechanical properties, and in

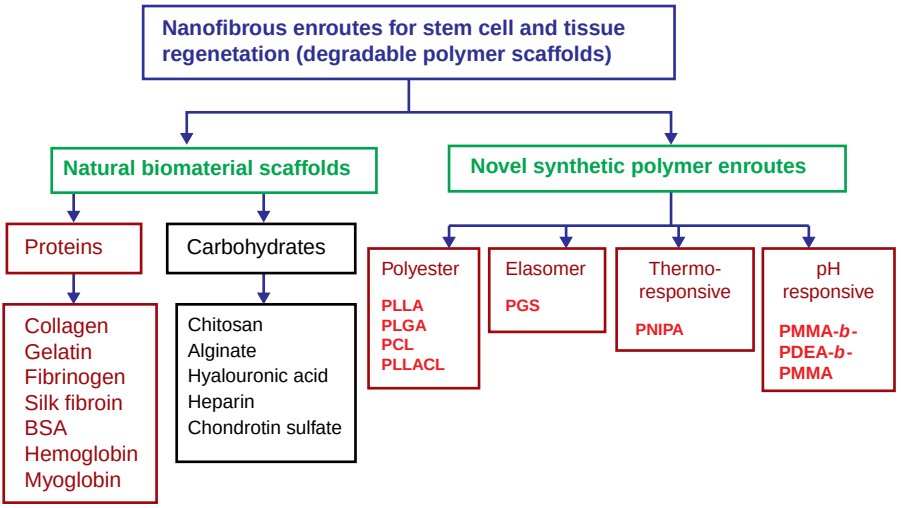

FIGURE 5.1 Biodegradable nanofiber scaffolds for tissue engineering applications.

most cases, they cannot be applied for tissue engineering. Cross-linking is carried out by many researchers to maintain the structural integrity of the construct.

To improve the stability of the natural protein[3–24] or carbohydrate-based scaffolds and to reduce the biodegradation rate of the scaffolds, cross-linking becomes inevitable. The details of electrospun cross-linked polymeric scaffolds used for tissue regeneration are also provided in this chapter.

5.1.1 Electrospun Nanofibrous Scaffolds for Tissue Engineering

Electrospinning has been recognized as an efficient and well-established technique capable of producing nanofibers by electrically charging a suspended droplet of polymer melt or solution.[25–32] Various polymers, including synthetic ones such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-*co*-glycolic acid) (PLGA), polystyrene, polyurethane (PU), polyethylene terephthalate (PET), and poly(L-lactic acid)-*co*-poly(ε-caprolactone) (PLACL), and biological materials, such as collagen, gelatin, and chitosan, have been successfully electrospun to obtain fibers with diameters ranging from 3 nm to 5 μm. Different parameters control the electrospinning process, including the solution properties, applied voltage, solution flow rate, humidity, and temperature. Using a simple and inexpensive setup, this technique not only provides an opportunity for control over the thickness and composition of nanofibers but also controls fiber diameter and porosity of the electrospun nanofiber meshes. Typically, nanofibers are collected as random, and aligned nanofibers with improved mechanical stability and degradation properties are also produced for specific applications. Whereas deposition of nanofibers on a static plate produces randomly oriented nanofibrous (100–650 nm) scaffolds, aligned nanofiber (250–650 nm) mats are fabricated using a rotating cylinder or disk collector with a sharp edge as shown in Fig. 5.2a and b. Coaxial electrospinning is a modification or extension of the traditional electrospinning technique with a major difference being a compound spinneret used. Using the spinneret, two components are fed through different coaxial capillary channels and are integrated into core-shell structured composite fibers to fulfill different application purposes. For example, bioactive composite scaffolds are fabricated using collagen (imparting bioactivity) as the shell and PCL (synthetic polymer) as the core (Fig. 5.2c).

Core-shell structured nanofibers (360–400 nm) prepared by coaxial electrospinning, have the advantages of being able to control the shell thickness and manipulate overall mechanical strength and degradation properties of the resulting composite nanofibers without changing their biocompatibility. Alternatively, core-shell structured composite nanofibers are functionalized for potential use in drug or growth factor encapsulation and release and development of highly sensitive sensors and tissue engineering applications. 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.

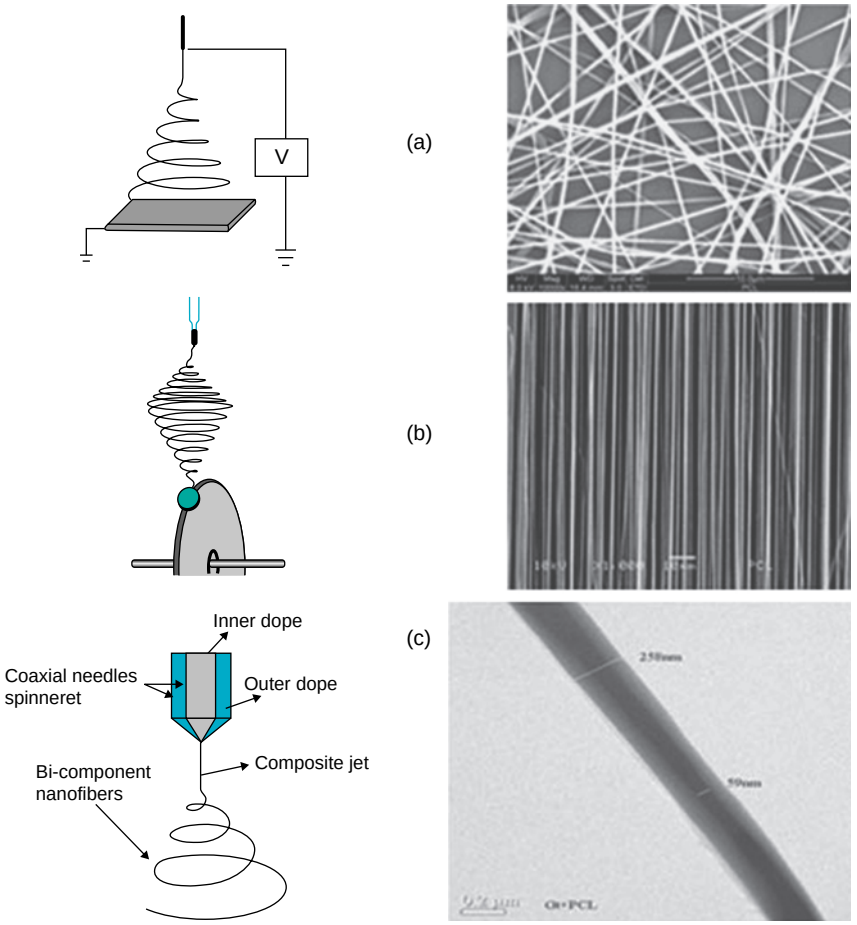

FIGURE 5.2 Schematics of electrospinning. (a) Random nanofibers produced by static collector. (b) Aligned nanofibers produced on a disk collector in a rotating wheel. (c) Coaxial electrospinning model for producing core-shell nanofibers.

Tissue engineering involves scaffolds or matrices to provide support for cells in order to express new ECM. The biocompatibility of scaffold materials actively participates in the signaling process for the requirement of safe degradation and provides a substratum for cell migration into the defect sites of the tissue. Potential applications of electrospun nanofibers for stem cell differentiation are envisioned in the fields of skin, bone, cartilage, blood vessels, cardiovascular diseases, nerves, and soft tissues.

5.1.2 Electrospun Nanoparticle Incorporated Natural Polymeric Scaffolds

5.1.2.1 Collagen Collagen is a fibrous protein found in animals, especially in the flesh and connective tissues of mammals. It is the most abundant protein in

mammals, constituting up to 35% of the whole-body protein content commonly created by fibroblast cells. Collagen is mostly found as elongated fibrils in fibrous tissues such as tendons, ligaments, and skin and is also abundant in corneas, cartilage, bone, blood vessels, the gut, and intervertebral discs. Collagen is a major ECM component that possesses a fibrous structure with fibrils of varying diameters (50–500 nm). This fibrils influence cell behavior by allowing cell attachment to the nanofeatured collagen matrix. Cells seeded on this nanofibrous matrix tend to maintain their normal phenotype and guided growth along the fiber orientation.

The motif behind the biomimetic nanostrategies is to dictate, control, and fabricate the morphology and composition of developed biomaterials. Nanoparticles are incorporated into natural or synthetic polymers to create functional polymeric composites suitable for tissue regeneration. Inorganic hydroxyl apatite (HAp) is being dispersed with preferential orientation so as to enhance bone tissue regeneration. HAp has inorganic crystalline nature same as that of natural bone and is biocompatible, bioactive, and osteoconductive in nature. Collagen and HAp biocomposite is a native ECM mimic and has the potential of replacing diseased skeletal bones. Because of potential biomedical applications, many studies report on the fabrication of bone-mimicking biocomposites of HAp and bioactive organic components such as collagen, gelatin, chondroitin sulfate, chitosan, and amphiphilic peptide.[33–36]

High levels of type I collagen and several noncollagenous proteins (e.g., osteopontin, bone sialoprotein, osteocalcin) constitute bone tissue. Collagen scaffolds get easily biodegraded and resorbed by the body and facilitate excellent attachment to cells. However, their mechanical properties are relatively low ($E \sim 100$ MPa), especially with respect to bone ($E \sim 2$–5 GPa),[37] and they are therefore highly cross-linked or found in composites, such as collagen–glycosaminoglycans for skin regeneration[38] or collagen–HAp for bone remodeling.[39] The advantage of the collagen and HAp devices in comparison with the synthetic PLGA devices[40] is that the biocomposite significantly inhibit the growth of bacterial pathogens, which is often associated with prosthesis. Although electrostatic co-spinning of nano HAp, and collagen improved the mechanical properties of the scaffold, much has to be done to exactly mimic the complex native nanostructured architecture of the bone. Collagen supported cell adhesion and proliferation, and HAp acted as a seed for biomineralization of osteoblasts in bone tissue regeneration.[41] The biocomposite of collagen and nanoHAp[42,43] is bioactive, osteoconductive, and osteoinductive and is a natural choice for bone grafting because it mimics the bone components. Bonelike orientation of c-axes of HAp nanocrystals with regular alignment along collagen fibrils are also fabricated.[44] The collagen–HAp composite, designed to simulate bone tissue, is produced using atelocollagen to reduce antigenicity by condensing $Ca(OH)_2/H_3PO_4$ suspension.[45]

Thus, electrospun nanofibrous collagen provides a native bonelike environment in the presence nanocrystalline HAp, enhancing regeneration of bone tissue or differentiation of stem cells into bone tissue.

5.1.2.2 Gelatin Gelatin is a protein obtained from the partial hydrolysis of collagen extracted from skin, bone, cartilage, ligaments, and so on. Gelatin is used as an alternative source of collagen to design tissue engineering scaffolds, mainly because of the lack of availability and high cost of collagen. Composite scaffolds of gelatin with other biodegradable synthetic polymers have been well adopted by many researchers. Moreover, these composite scaffolds with excellent biocompatibility, improved mechanical, and physical and chemical properties overcome the obstacles associated with the use of single natural polymers.[46] Interaction between cells and the scaffold material depends on various physicochemical properties of the material and particle size and surface properties that include topography, roughness, surface energy, and wettability.

Three-dimensional nanofiber-gelatin–apatite composite scaffolds were fabricated by Liu et al.[47] to mimic both the nanoscale native architecture and chemical composition of natural bone ECM. With a new thermally induced phase separation and porogen-leaching technique, these 3D nanofibrous gelatin scaffolds with well-defined macropores were designed. The inorganic HAp deposited all along the 3D porous structure is ideal for controlling surface topography and chemistry within complex nanostructures. And it was shown that these scaffolds have excellent biocompatibility and mechanical properties with enhanced osteoblast adhesion, proliferation, and differentiation suitable for bone tissue engineering.

5.1.2.3 Silk Fibroin Silk fibroin is considered as the most promising natural fibrous protein replacement for collagen in bone tissue engineering because of its biocompatibility, slow biodegradation, and excellent mechanical properties. In the past few years, two natural silk sources (e.g., silkworm silk *Bombyx mori* and spider dragline silk *Nephila clavipes*) have been processed for making nanofibers via electrospinning.[48–51] To improve the electrospinnability of silk protein solutions and to avoid potential influences of hazardous organic solvents such as hexafluoroisopropanol,[48] hexafluoroacetone,[49] and formic acid[51] toward the biocompatibility of the scaffolds, an all-aqueous electrospinning was attempted by Jin et al.[50] by blending silk fibroin with PEO at a ratio from 1 : 4 to 2 : 3. Methanol treatment of the electrospun scaffold renders water insolubility of the scaffold because of the structural conformational change into native β-sheet structure. Silk-based biocomposite nanofibers of HAp and bone morphogenetic protein 2 (BMP-2) were fabricated by Li et al.,[52] and an enhanced bone formation was observed by culturing with human bone marrow-derived mesenchymal stem cells (hMSCs). It was observed that the inclusion of BMP-2 and HAp with electrospun silk fibroin nanofibers resulted in the highest calcium deposition and upregulation of BMP-2 transcript levels compared with other electrospun silk-based scaffolds.

5.1.2.4 Chitosan Chitosan, an amino polysaccharide derived from the structural biopolymer chitin exists abundantly in crustacean shells (e.g., crabs) and plays a key role as that of collagen in higher vertebrates. Chitosan retains a number of salient features such as structural similarity to glycosaminoglycan found in bone, osteoconductivity, excellent biocompatibility, tailorable biodegradability,

low immunogenicity, and better mechanical properties[53–55] and at low cost. However, it is poorly electrospinnable and forms aggregates with non-electrospinnable HAp nanoparticles. Therefore, formulating a robust chitosan solution to generate nanofibrous HAp–chitosan biocomposite scaffolds is difficult. Because of these limitations in electrospinning of chitosan,[56,57] there are only a few reports on nanofibrous hydroxyapatite (HA)–chitosan composites for bone tissue engineering. Using ultrahigh-molecular-weight poly(ethylene oxide) (UHMWPEO) as a support polymer, Zhang et al.[58] fabricated composite chitosan nanofibers by a modified two-step approach.[59] In short, an *in situ* co-precipitation synthesis route was designed to overcome the problem of nanoparticles agglomeration and electrospinning process was carried out for the preparation of HAp–chitosan nanocomposite nanofibers with a higher (30 wt%) loading of HAp nanoparticles. It was confirmed with electron diffraction and X-ray diffraction analysis that the acetic acid used for chitosan dissolution had minor or no influence on the crystallinity of HAp nanoparticle incorporated within the nanocomposite nanofibrous structure. Bone regeneration ability of the scaffold was assessed on these HAp–chitosan nanocomposite nanofibrous scaffolds, and the results confirmed that the scaffolds had significantly enhanced bone formation compared with the pure chitosan scaffold.

5.2 SYNTHETIC ENROUTES

Multiple procedures and method combinations are used for the successful fabrication of a nanofibrous construct for stem cell or tissue regeneration. The scaffold needs to be stable in culture media; hence, natural polymeric scaffolds have limitations in direct application, highlighting the need for cross-linking of the electrospun natural protein-based scaffolds, which makes it stable during incubation in culture media.

5.2.1 Chemistry of Cross-Linking

Cross-linking is the process of chemically joining two or more molecules by a covalent bond. Cross-linking of proteins or carbohydrates depends on the availability of particular chemical groups that are capable of reacting with the specific kinds of functional groups that exist in proteins.

Despite the complexity of protein or carbohydrate structure, four major functional groups constitute for the vast majority of cross-linking and chemical modifications:

1. *Primary Amine Functionality ($-NH_2$):* The amine group exists at the N-terminus of each polypeptide chain and in the side chain of some amino acid residues.
2. *Carboxyl Groups (–COOH):* The carboxylic acid group exists at the C-terminus of each polypeptide chain and in the side chains of some amino acid residues.

3. *Sulfhydryl Functional Group (–SH):* The thiol group often helps in disulfide bond formation and exists in the side chain of cysteine amino acid.
4. *Carbonyl Functional Group (–CHO):* The aldehyde groups, which are often associated with carbohydrates and glycoproteins, are formed by oxidation.

When interconnected via the cross-linkers, these residues become stable toward degradation with improved mechanical strength. Glutaraldehyde solutions or vapors have been commonly used to cross-link protein-based and amino group containing carbohydrate scaffolds. The glutaraldehyde cross-linking technique is not expensive but efficiently cross-links over a variety of distances and reacts with many of the amino groups. The extent of cross-linking in an electrospun scaffold is directly proportional to the percentage of glutaraldehyde present in the cross-linking solution. The degree of nanofiber scaffold cross-linking increases as the percentage of glutaraldehyde present in the electrospinning solution increases but attains a maximum point where further no cross-linking can occur. However, some cytotoxity and calcification issues are associated with the glutaraldehyde cross-linker. Other methods for nanofibrous scaffold cross-linking are carbodiimide–ethanol techniques. Carbodiimide is a zero-length cross-linker with nominal potential cytotoxity issues and can be used to modulate material properties similar to glutaraldehyde. Genipin is a natural material cross-linker as a substitute for gluteraldehyde, carbodiimide, and isocyanate cross-linkers because of the cytotoxicity associated with these materials. Chitosan cross-links with ring-opening polymerization of a genipin double bond and the nucleophilic attack of chitosan on genipin. Despite its less cytotoxicity compared with other cross-linkers, there are only a few reports available with genipin cross-linking because of its high cost. Thus, there exists a demand for a new effective, nontoxic, economic cross-linker.

5.2.2 Elastomeric Scaffolds

An elastomer is a polymer that is elastic in nature and it has a relatively low Young's modulus and high yield strain compared with other synthetic and natural polymers. Elastin constitutes the natural elastomeric material present in various tissues of the human body. Many well-known elastomeric polymers, such as PU and biodegradable polyester urethane urea (PEUU), have been tried as cardiac patches, are biodegradable (poly(glycerol sebacate) [PGS]), and so on. Fong and Reneker[60] have electrospun styrene–butadiene–styrene triblock copolymer so as to fabricate elastomeric nanofibers with 100 nm diameters. Artelon (polyurethane urea elastomer) was electrospun[61] to obtain degradable nanofibers with an average diameter of 750 nm, and the biocompatibility studies were carried out using human fibroblasts. Stankus et al.[62] have electrospun biodegradable PEUU nanofiber scaffolds with tensile strengths ranging from 2.0 to 6.5 MPa and breaking strains from 850 to 1700% depending on the material axis, especially for regeneration of smooth muscle cells (Fig. 5.3). PGS, a tough biodegradable elastomer,[63] is being used in soft tissue engineering. PGS–gelatin nanofibrous scaffolds were fabricated by electrospinning

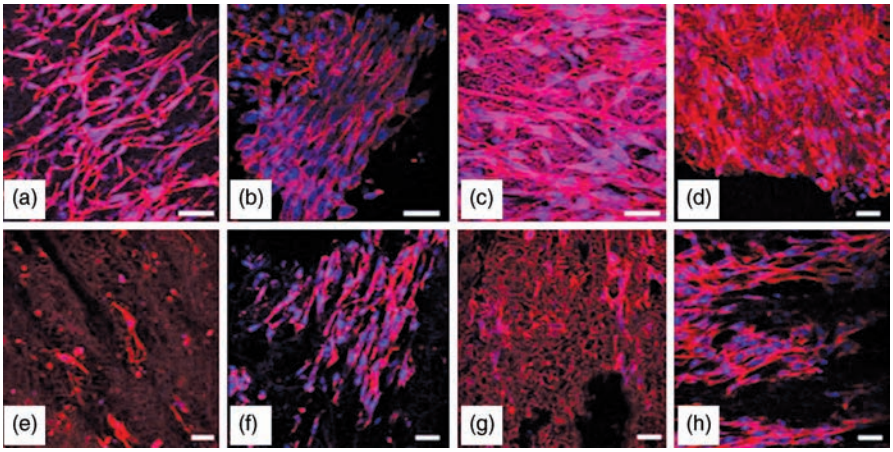

FIGURE 5.3 Fluorescent micrographs of SMC microintegrated e-PEUU constructs after 1 day of static culture (a), day 4 of perfusion culture (b), day 4 of perfusion culture (c), day 7 of perfusion culture (d), day 4 of static culture (e), high cell number surface image of day 4 of static culture (f), day 7 of static culture (g), and high cell number surface image of day 7 of static culture (h). Scale bar = 40 μm, red = f-actin and e-PEUU, blue = nuclei. Reproduced with copyright permission from Ref. [62].

to produce a unique ECM-like topography and were suggested as a potential biomaterial for myocardial infarction.[64]

5.2.3 pH Responsive Polymers

pH-sensitive or -responsive polymers are materials that respond to the changes in the pH of its surrounding medium. These polymers swell or collapse depending on the pH; this behaviour is exhibited because of the presence of acidic or basic functionality in the polymer chain. For example, whereas polyacrylic acid (PAA) is acidic in nature but swells at basic pH, chitosan with its basic amino groups swells if acidic changes occur in its surroundings. This pH-mediated response of the polymers is useful for the release of drug molecules or growth factors encapsulated within these polymers. Therefore, the application of these polymers will be enormous so as to work under physiological pH conditions. The phenomenon behind the swelling behaviour is the volume transition associated with the ionized state of the polyelectrolyte from the neutral state. Thus, all acidic polymers are base sensitive (polymethacrylic acid, xylan, etc.) and basic polymers (PEI, poly aniline, etc.) are acid sensitive. Amonodisperse triblock copolymer of poly(methyl methacrylate)-*block*-poly[2-(diethylamino)ethyl methacrylate]-*block*-poly(methyl methacrylate) ($PMMA_{273}$-*b*-$PDEA_{688}$-*b*-$PMMA_{273}$) was synthesized[65] via group transfer polymerization as a pH-responsive system and electrospun 35% of the copolymer to obtain the pH-sensitive scaffold. Wang et al.[66] fabricated electrospun pH-responsive γ-PGA

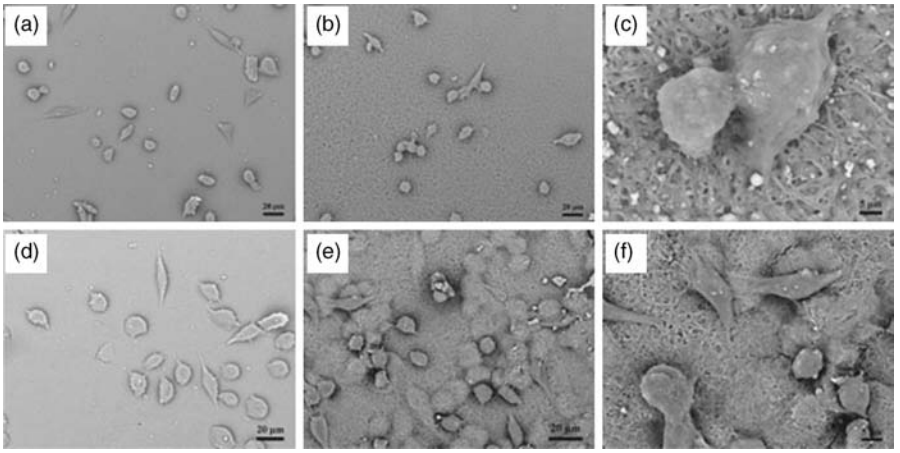

FIGURE 5.4 SEM micrographs of fibroblasts attached onto cover slips (a) and electrospun γ-PGA nanofibers formed using 5 wt% TFA as a solvent (b), respectively, after 8 h of culture. (c) High magnification image of (b). (d and e) SEM micrographs of fibroblasts proliferated onto cover slips and electrospun γ-PGA nanofibers formed using 5 wt% TFA as a solvent, respectively, after 3 days of culture. (f) High magnification image of (e). Reproduced with copyright permission from Ref. [66].

nanofibers that have an excellent biocompatibility to promote the cell adhesion and proliferation (Fig. 5.4).

5.2.4 Thermo-Responsive Polymer Fabrication and Engineering

Thermo- or temperature-responsive polymers respond to temperature change by expansion of dimension or size. This character of these polymers is used for the release of drug molecules incorporated or encapsulated drug from within the polymer. Poly(*N*-isopropylacrylamide) (PNIPAm) is a temperature-responsive polymer that can be synthesized from NIPAm monomer. It can be made to a 3D hydrogel architecture when cross-linked with *N,N′*-methylene-bis-acrylamide (MBAm) or *′-N, N′*-cystamine-bis-acrylamide (CBAm). In the presence of water when heated above 32 °C, it undergoes a swollen hydrated state to a shrunken dehydrated state by a reversible lower critical solution temperature phase transition, losing about 90% of its mass, by expelling its liquid contents at human body temperature. Thus, the polymer is useful for tissue engineering applications and in drug delivery. Azarbayjani et al.[67] have electrospun a series of nanofibrous membranes from poly(vinyl alcohol) (PVA) and PNIPAm blends to develop a sustained topical delivery of levothyroxine (T_4). These nanofiber mats were suggested as promising carriers for keeping the drugs concentrated on the skin over a prolonged period with reduced systemic uptake. Similar applications of PNIPAm in tissue engineering are well known, but the applications of PNIPAm nanofibers with tissue engineering applications is yet to be extensively studied.

5.2.5 Modified Electrospinning Processes

5.2.5.1 Simultaneous Electrospinning and Electrospraying HAp nanoparticles were electrosprayed on PLACL–gelatin nanofibers to produce PLACL–gelatin–HAp scaffolds with controlled morphology for application in bone tissue engineering. Gupta et al.[68] used a simultaneous electrospraying and electrospinning (Fig. 5.5) concept and fabricated PLACL–gelatin–HAp nanofibers and compared their mechanical and cellular properties with blend electrospun PLACL–gelatin–HAp scaffolds.

Electrospun PLACL–gelatin–HAp (blend) nanofibers had a drawback of trapping HAp inside the nanofibers (diameter, 198 ± 107 nm), but the HAp nanoparticles were found uniformly sprayed forming a layer of HA on the surface of the other PLACL–gelatin–HAp scaffold (diameter, 406 ± 155 nm). The tensile stress for HAp electrosprayed scaffold was higher than PLACL–gelatin–HAp (blend) scaffold because the electrospraying of HAp nanoparticles resulted in superficial dispersion of HAp nanoparticles. A significant increase in hFOB proliferation was observed on the HAp electrosprayed scaffold compared with the PLACL–gelatin–HAp (blend) nanofibers after 15 days of cell seeding. Furthermore, the electrosprayed scaffolds showed 50% higher biomineralization than the PLACL–gelatin–HAp (blend), thus proving the versatility of the electrospraying method compared with the blend technique with respect to scaffold design for bone tissue engineering. Jayasinghe and coworkers[69,70] have electrosprayed jurkat cells and assessed for their viability by

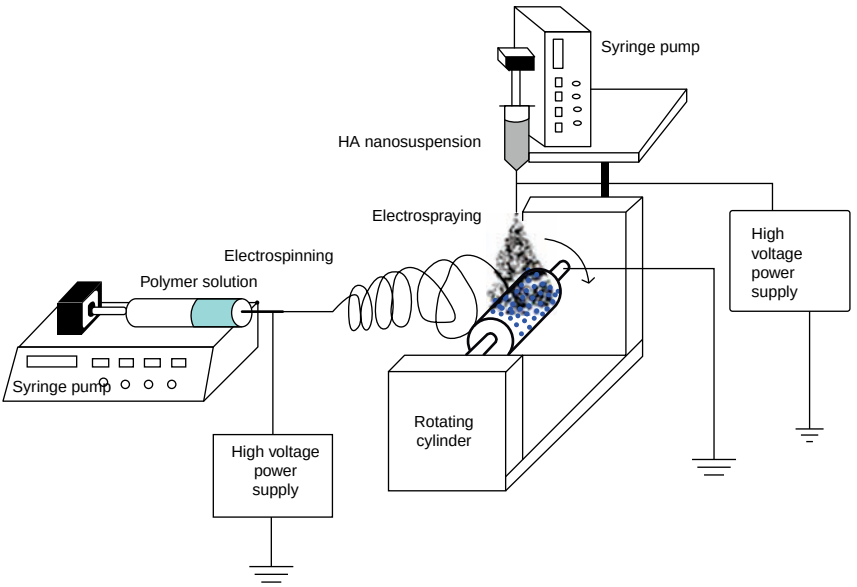

FIGURE 5.5 Schematic representation of simultaneous electrospraying and electrospinning.

way of trypan blue staining. This methodology of bioelectrospraying[71] is said to have a wide range of applications spanning from bio-analytics and diagnostics to the possible creation of synthetic tissues for repairing and replacing damaged or aging tissues to the targeted and controlled delivery of personalized medicine through experimental or medical cells or genes.

5.2.5.2 Coaxial Electrospinning Coaxial electrospinning is a method of electrospinning in which the core polymer is encapsulated by another polymer that forms the shell of the electrospun nanofibers because of electrostatic voltage applied via the shell polymer. In this method, only the shell polymer is electrospun in principle; the core polymer is just dragged inside the shell, and in most cases, it is a nonspinnable material polymer. In a tissue or stem cell regeneration perspective, it is a novel method for the development of controlled release of encapsulated growth factor or related differentiating material for the stem cells. Sahoo et al.[72] have shown the growth factor delivery via core shell nanofibers with PLGA as shell material and basic fibroblast growth factor (bFGF) as core. They realized that the material ensures sustained release of growth factors up to 2 weeks. They observed the nanofibrous scaffold enhanced cell attachment, proliferation, and fibroblastic differentiation of bone marrow stem cells, which they further confirmed with increased collagen production and upregulated gene expression of specific ECM proteins. Su et al.[73] have demonstrated the controlled release of BMP-2 and dexamethasone using core–shell PLLACL–collagen nanofibers for bone tissue engineering applications (Fig. 5.6). Similar approach with respect to stem cell differentiation is yet to be extensively explored.

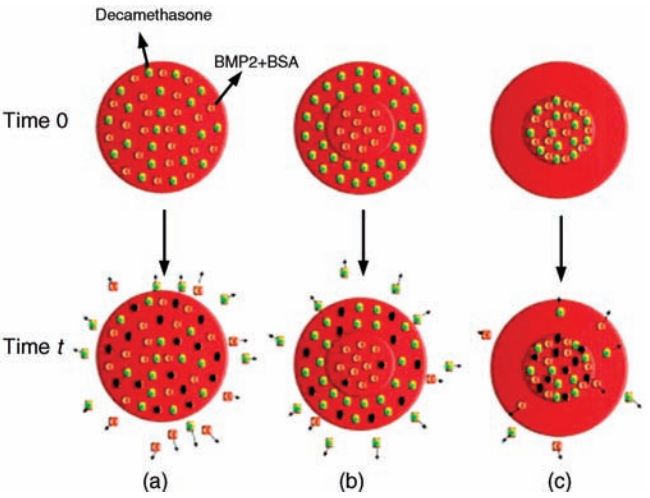

FIGURE 5.6 Schematic representation of the release of DEX and BMP2 from electrospun nanofibers (a, b, and c). Reproduced with copyright permission from Ref. [73].

5.3 NOVEL NANOFIBROUS STRATEGIES FOR STEM CELL REGENERATION AND DIFFERENTIATION

Stem cells are biological cells found in all multicellular organisms and have the capacity to self-renew; they divide via mitotic cell division and differentiate into diverse specialized cell types (tissue or organ). In mammals, there are two broad types of stem cells, embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. During development of an embryo, stem cells differentiate into many different types of specialized cells, and they also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. Another type is adult stem cells, which are undifferentiated cells found along with the differentiated cells in an organ or tissue, which can renew themselves and can differentiate to yield major specialized cell types of organ or tissue.

In mature organisms, stem cells and progenitor cells act as a repair system in the body, replenishing matured tissues. These adult stem cells maintain and repair the tissues in which they constitute. They can be collected from tissues such as adipose tissue, bone marrow, mammary tissue, central nervous system, olfactory bulb, and so on. Transdifferentiation ability has also been demonstrated by adult stem cells (i.e., they can switch their specific developmental lineage to another cell type of a different lineage).[74] However, the molecular mechanism that drives transdifferentiation is not clearly understood. Stem cells have the unique property of self-renewal without differentiation if appropriate biological and physical induction conditions are provided. In the context of tissue engineering, the use of stem cells has the following advantages compared with engineered tissue constructs: (1) they have high proliferative capacity, (2) they provide excellent regenerative capability that will likely lead to desired integrity and functionality of the engineered construct, (3) they make it possible to contemplate multifunctional tissue constructs (e.g., osteochondral tissue), and (4) they reduce or eliminate tissue rejection or failure.

Although the application of living cell therapy is associated with challenges, stem cells constitute the functional elements of tissue engineering and regenerative medicine.[75] The following are the prerequisites for researchers and clinicians to work out the success in cell-based treatments. For transplantation practices, stem cells must be reproducibly made to (1) differentiate into the desired cell types; (2) survive in the recipient after transplantation; (3) integrate into the surrounding tissue after transplantation; (4) function appropriately for the duration of the recipient's life; and (5) avoid harming the recipient in any way.

Researchers are working in the direction of minimizing or avoiding the problem of immune rejection of regenerated tissues with different research strategies. The most commonly studied stem cells are the bone marrow stem cells, especially the MSCs and hematopoietic stem cells (HSCs). Under controlled conditions, the MSCs have the ability to differentiate into cell lineages[76] such as osteoblasts, chondrocytes, cardiomyocytes, and fibroblasts. The *in vitro* cell culture of hMSCs, proliferation and differentiation into tissue specific cell phenotype such as

chondrogenic, osteogenic, adipogenic, and myogenic cells with the application of a biological or physical stimuli, is well understood and established.[77–80] The hMSCs have enormous therapeutic potential for treatment of damaged or diseased tissue; the complexity of events associated with such transformation of these precursor cells leaves many unanswered questions about morphologic, structural, proteomic, and functional changes in stem cells. Thus, there exist a need for better understanding of hMSC behavior that would allow more effective approaches to cell expansion *in vitro* and differentiation to a specific phenotype. Hence, there is a need for favorable scaffolds and engineering for hMSCs to orient, adhere, proliferate, and differentiate.

The multilineage differentiation potential of MSCs on 3D PCL nanofibrous scaffolds was demonstrated by Li et al.[81] They tested the ability of the scaffold to support and maintain multilineage differentiation of bone marrow-derived hMSCs *in vitro* by culturing in different differentiation media such as adipogenic, chondrogenic, or osteogenic and found the PCL scaffold as the promising one. The differentiation potential of MSCs into hepatocytes was observed by Kazemnejad et al.[82] on PCL–collagen–polyethersulfone scaffolds. The ability of the differentiated hepatocyte cells to produce albumin, urea, serum glutamic, pyruvic, transaminase, and serum oxaloacetate aminotransferase on the scaffolds further confirms the supporting role of the nanofibrous scaffolds. The osteoblastic differentiation potential of MSCs on poly(L-lactic acid) (PLLA)–collagen nanofibers was demonstrated by Schofer et al., who identified the advantages together with disadvantages of more stable PLLA–collagen fibers with respect to osteoblastic differentiation.[83] *In vitro* differentiation of MSCs into cardiac cells is commonly carried being out by exposure to 5-azacytidine, a DNA demethylating agent.[84] Expression of many cardiac specific genes and peptides was observed.[85] Recently, Nerurkar et al.[86] observed improved cellular ingress into electrospun scaffolds by adopting dynamic culture of MSCs on aligned PCL nanofibrous scaffolds. This dynamic culture modification for MSC culture has increased cellular infiltration and facilitated the use of aligned electrospun scaffolds for tissue engineering. In our laboratories, we studied the neuronal differentiation potential of hMSCs on PLCL–collagen scaffolds. The results of our study showed the neuronal phenotype of MSC differentiated cells together with the expression of nerve proteins such as NF200 and nestin.[87] Thus, with a better understanding of the behavior of MSCs on electrospun nanofiber scaffolds, a "stem cell–scaffold construct" might find real application in regenerative medicine curing various human diseases.

The transplantation of embryonic stem cells (ESCs) for the treatment of peripheral nerve injuries and possibly spinal cord injuries has also been demonstrated.[88,89] Functionalized electrospun nanofibrous scaffold with growth factors was found to enhance the differentiation of ESCs into neurons and oligodendrocytes.[90] Xie et al.[91] demonstrated that the ESCs are differentiated into neural cell lineages guided by electrospun nanofibrous scaffolds. They also found the ESCs to promote and direct neurite outgrowth. The novel strategy of using a combination of electrospun scaffolds together with ESC-derived neural progenitor cells might lead to better nerve repair. Lam et al.[92] immobilized bFGF or epidermal growth factor (EGF) onto

aligned PLLA nanofibers using heparin as the adapter molecule and elucidated the effect of growth factors on ESC differentiation into neural cells with significant promotion of axonal growth. Immobilization of bFGF and EGF in aligned nanofibers was successfully carried out by these researchers to promote neural tissue regeneration. Nuria and Carlos[93] proposed that 3D cell culture on self-assembling peptide nanofibrous scaffold could provide a unique microenvironment permissive to promote the differentiation of mouse ESCs into osteoblast-like cells while maintaining their own regenerative capacity. Kamal et al.[94] fabricated 3D polyamide fibrillar surfaces for the self-renewal of mouse ESCs through mechanism involving Rac and P13K/AKT signaling, thus exhibiting the role of nanostructural scaffold morphology for ESC proliferation. Optimization of a suitable nanostructure or microenvironment is the requirement for efficient differentiation of ESCs in 3D scaffold structures further led to the research on scaffold pore size, increasing mechanical stiffness, increasing the cell seeding density, co-culturing with stromal cells,[95,96] and so on. Hashemi et al.[97] have demonstrated very recently the promotion of stemness and pluripotency (Fig. 5.7) with collagen-grafted polyethersulfone (PES) 3D nanofibrous scaffold culturing mESCs.

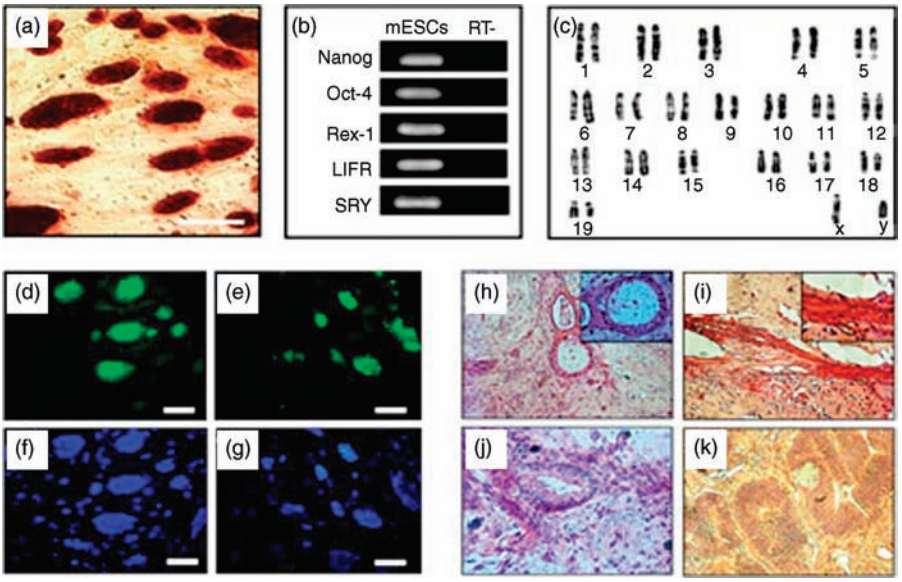

FIGURE 5.7 Characterization of the mESCs cultured on MEF in the presence of LIF after 10 passages: alkaline phosphatase assay (a); RT-PCR analysis of expression of embryonic stem cell–specific genes (b); Giemsa-banded karyotype of an embryonic stem cell showing a normal 40 XY karyotype (c); immunofluorescence staining of OCT-4 (d, f) and SSEA-1 (e, g); histologic analysis of teratoma-derived from mESCs, gutlike structures, muscle cells, secretory epithelium, and neural rosettes (h–k). Reproduced with copyright permission from Ref. [97].

Human umbilical cord blood (UCB) stem cells[98] are an alternative source of hematopoietic precursors for allogeneic stem cell transplantation in children with inborn errors or malignant diseases. HSCs, originating from bone marrow, are used for the treatment of many bloodborne and other diseases, including sickle cell anemia, thalassemia, aplastic anemia, leukemia, metabolic disorders, and certain genetic immunodeficiencies.[99] The cord blood stem cells show a higher proliferative capacity and expansion potential. Allogeneic stem cell transplantation is limited because of the lack of suitable bone marrow donors and the risk of graft-versus-host diseases. The percentage of stem cells is higher in cord blood than in the bone marrow, and the main merits of UCB stem cells over the other stem cell sources are (1) easy to recover, (2) no health risks for the mother or newborn, (3) immediate disposition at the cryobank, (4) low incidence of rejection of the transplant, (5) high cellular plasticity, (6) low possibilities of transmission of viral diseases, (7) low cost of the procedure, and (8) easy possibilities to create cord blood banks so as to store samples.

Transplantation protocols into adults is limited because of the low number of progenitors in cord blood harvest and due to this, expanding HSCs *ex vivo* to get sufficient number of cells for transplantation became a need. Several studies have demonstrated[100] the application of nanofibrous scaffolds for enhancement of cellular responses such as cell adhesion and cell phenotype maintenance. Researches on the influence of nanotopographical cues and biochemical cues on the nanofiber surface and their synergistic influence toward HSC adhesion, proliferation and phenotypic maintenance are also established. The highest expansion efficiency of CD34+, CD45+ cells, and colony-forming unit potential was observed in surface-aminated electrospun nanofibrous scaffolds compared with the unmodified, surface-hydroxylated, surface-carboxylated[101] nanofibrous scaffolds. Amino groups were conjugated as spacers to nanofiber surfaces, and it was found[102] that the cell–substratum interaction dictated the HSC–progenitor cell proliferation and self-renewal in cytokine supplemented expansion. Aminated nanofiber scaffolds and PCL–collagen nanofiber scaffolds were found to enhance the HSC–substrate adhesion and proliferation of progenitor cells. This formed the basis for research on specific cell adhesion molecules such as fibronectin in combination with the nanofiber substratum toward HSC adhesion and expansion *ex vivo* to solve various diseases.

Unrestricted somatic stem cells (USSCs) were seeded on electrospun PES nanofiber mats with plasma treatment and collagen grafting, and their biocompatibility and application in tissue engineering was investigated. Imam et al.[103] observed the infiltration of stem cells into the collagen grafted nanofibers after 7 days of cell culture, thus making collagen-grafted PES nanofibers an ideal candidate to form 3D structures in tissue engineering. They further observed that the PES–collagen nanofibers[104] have the highest capacity to support osteogenic differentiation and infiltration of stem cells into the 3D nanostructure, which they confirmed via assessment of osteogenic markers and histologic examination. Results from their study concluded that the PES–collagen scaffolds could act as a potential 3D bone graft with capacity for bone healing and regeneration *in vivo*.

5.4 CONCLUSIONS

Scaffolds that mimic the natural ECM are considered the most ideal scaffolds for tissue or stem cell regeneration. Bioengineers aim for the development of suitable substrates for tissue regeneration using various and ultimate nano- or microtechnologies. To find a synthetic solution to the natural scaffold materials, many groups use nanofibrous scaffold comprising various novel features such as cross-linking, surface modification, growth factor inclusion or sustained release, drug or antioxidant inclusion, and nanostructural modifications in fiber alignment. We have identified and provided in this chapter the summary of the above-stated research works so as to provide an overall outlook. Designing an optimized biomimetic ECM scaffold is an achievable task with better understanding of the chemistry of the scaffold and its structure and pattern along with the biochemical signals associated with stem cell differentiation and proliferation.

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