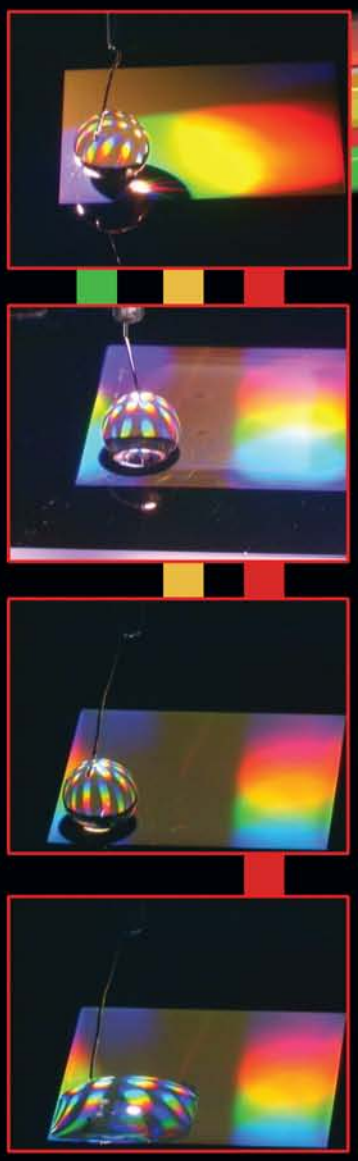
BIONANOTECHNOLOGY

GLOBAL PROSPECTS



Edited by

David E. Reisner

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Preface

Nature has been engaged in its own unfathomable and uncanny nanotechnology project since the dawn of life, billions of years ago. It is only recently that humans have developed their own tools to observe Nature as she assembles and manipulates structures so complex and purposeful so as to defy the imagination. No one would argue that all molecular biology is based on nanotechnology. After all, these structural building blocks composed of ordered elements are well within the 100-nanometer scale that is generally agreed upon as the physical dimensional ceiling below which nanotechnology processes occur. It is no wonder that man is now attempting to mimic Nature by building analogous structures from the bottom up.

A few words about the book title: The temptation to consider "nanobiotechnology" as a subset of biotechnology fails to pay homage to the gargantuan impact of the burgeoning nanotechnology field—a field in the throes of revolutionary growth. The word *nanobiotechnology* feels redundant, a bromide. In distinction, the term *bionanotechnology* connotes a rapidly evolving sector of the nanotechnology field that deals strictly with biological processes and structures. Many refer to this synthesis as "convergence." As will be demonstrated in this monograph, the seeds of bionanotechnology development have been planted. Commercial products will likely be on the marketplace well before the next edition appears. Many nanotech soothsayers predict that as time goes on, this convergence of biotechnology and nanotechnology will become a dominant focus area for technological innovation worldwide and will impact all of our lives on a daily basis.

Naturally, this is also an engineering book. One need not stretch the imagination very far to appreciate that Nature has fundamentally engineered life as we know it, culminating in our own species. This fact has not gone unnoticed on the part of nanotechnologists, who have begun in earnest to mimic Nature's fundamental engineering processes through invoking precise controls over her building blocks. Self-assembly, a key construct of nanotechnology, forms the backbone of biological processes. For example, exploiting DNA as scaffolding for the engineering of DNA-templated molecular electronic devices is an inspiring example of our newfound ability to insinuate our own design skills at the nanoscale level in living systems. Using this approach, it is possible to create self-assembling electronic circuits or devices in solution. Directed evolution based on repeated mutagenesis experiments can be conducted at the nanoscale level. Along these lines, the use of the solar energy conversion properties of bacteriorhodopsin for making thin-film memories, photovoltaic convertors, holographic processors, artificial memories, logic gates, and protein-semiconductor hybrid devices is astounding.

Quantum dots are tiny light-emitting particles on the nanoscale. They have been developed as a new class of biological fluorophore. Once rendered hydrophilic with appropriate functional groups, quantum dots can act as biosensors that can detect biomolecular targets on a real-time or continuous basis. Different colors of quantum dots could be combined into a larger structure to yield an optical bar code. Gold nanoparticles can be functionalized to serve as biological tags.

Nanomedicine is a burgeoning area of development, encompassing drug delivery by nanoparticulates, including fullerenes, as well as new enabling opportunities in medical diagnostics, labeling, and imaging. Quantum dots will certainly play a large role in nanomedicine. Years from now, we will laugh at the archaic approach to treating disease we presently take for granted, carried over from the twentieth century, relying on a single drug formulation to treat a specific disease in all people without acknowledging each individual's unique genetic makeup. Nanocoatings also play an important near-term role in the lifetime of medical devices, especially orthopedic prosthetics. Nanocrystalline hydroxyapatite is far less soluble in human body fluid than conventional amorphous material, thereby anticipating great increases in its service life.

It is not the intention to provide a comprehensive treatise on bionanotechnology, rather I hope to provide representative reporting on a wide variety of activity in the field from all corners of the planet (now that the "world is flat" it has corners). I have attempted to assemble chapters that are relevant to looming product opportunities and instructional for those readers interested in developing the bionanotech products of the future. To that end, I felt it appropriate to conclude the discussion with a chapter that reviews the patent landscape for bionanotechnology, which is presently in a state of great flux. Now more than ever, intellectual property is relevant to both the academic and corporate sectors, and as such, patents are being ascribed greater value and importance. Bionanotechnology commercialization will be driven by the increases in government funding as well as the expiration of more traditional drug patents.

Accumulating author contributions from experts scattered across the globe acquired a life of its own in the evolution of this book. As a Technology Pioneer at the Annual Meeting of the World Economic Forum (WEF) in Davos, Switzerland, I was privy to a worldview that few technologists are able to enjoy. Klaus Schwab, WEF's driving force, has observed that everywhere in society and business, the power is moving from the center to the periphery. This monograph is a testimonial to that paradigm shift. Authors have contributed from 15 different countries in cities from as far as Florianópolis, Mumbai, Ramat-Gan, Pretoria, Havana, Tehran, Glasgow, Shenyang, and Kiev, just to name a few. Of course, this diaspora of academic excellence is largely enabled by the most pervasive technological innovation of our time, the Web.

Chris Anderson has postulated a compelling new economics of culture and commerce, dubbed the "Long Tail," so named because in statistics, the tail of a $1/x$ power law curve is very long relative to the head. Long Tail economics is about the economics of abundance (not scarcity), and we now see quantum shifts in customer buying habits at Amazon, Netflix, and eBay, as well as shifts in content distribution at Wikipedia, Google, and the emerging "Blogosphere." This phenomenon is also playing out in scientific research across the globe, where the Long Tail has now made possible world-class creative technology advances that not long ago were unimaginable. This monograph is proof in spades of this paradigm shift. I dedicate this book to all the authors who gave their valuable time to create the contributions that fill this volume. Many of those authors delivered expert chapters in the face of severe obstacles, some even endured personal hardship and loss over the course of their writing. They know who they are, and I thank them. I dedicate this book to the singer, not the song.

David E. Reisner
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The Editor

David E. Reisner, Ph.D., is a well known entrepreneur in the burgeoning field of nanotechnology, having cofounded in 1996 two nanotech companies in Connecticut, Inframat® and US Nanocorp®. He has been the Chief Executive Officer of both companies since founding, which were recognized in Y2002–Y2005 for their fast revenue growth as Deloitte & Touche *Connecticut Technology Fast50 Award* recipients. In 2004, The Nano Group, Inc. was formed as a parent holding company for investment. Dr. Reisner and the cofounders were featured in *Forbes* magazine in 2004. He is also a Managing Director in Delta Capital Group.

Dr. Reisner has more than 175 publications and is an inventor on 10 issued patents. He is the editor for the "Bionanotechnology" section of the 3rd Edition of *The BioMedical Engineering Handbook*. He has written articles on the business of nanotechnology in *Nanotechnology Law & Business* as well as the Chinese publication *Science & Culture Review.*

Dr. Reisner served a 3-year term as a Technology Pioneer for the World Economic Forum and was a panelist at the 2004 Annual Meeting in Davos. He is on the Board of the Connecticut Venture Group and is Chairman of the Board of the Connecticut Technology Council. He was a National Aeronautics and Space Administration (NASA) *NanoTech Briefs* Nano50 awardee in 2006. For his efforts in the field of medical implantable devices, Reisner won the 1st Annual BEACON Award for Medical Technology in 2004. He is a member of the Connecticut Academy of Science and Engineering.

Reisner is a 1978 University Honors graduate from Wesleyan University and received his Ph.D. at MIT in 1983 in the field of chemical physics. He was recognized for his historic preservation efforts in 1994 when he received the Volunteer Recognition Award from the Connecticut Historical Commission and the Connecticut Trust for Historic Preservation. Dr. Reisner is known nationally for his expertise in vintage Corvette restoration and documentation.

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1 Nanotechnology in Stem Cell Biology and Technology*

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CONTENTS

1.1 INTRODUCTION

Nanotechnology is the science and engineering concerned with the design, synthesis, characterization, and application of materials and devices that have a functional organization in at least one dimension on the nanometer (nm) scale, ranging from a few to about 100 nm. Nanotechnology is beginning to help advance the equally pioneering field of stem-cell research, with devices that can precisely control stem cells (SCs) and provide nanoscaled-biodegradable scaffolds and magnetic tracking systems. SCs are undifferentiated cells generally characterized by their functional capacity to both self-renew and to generate a large number of differentiated progeny cells. The characteristics of SCs indicate that these cells, in addition to use in developmental biology studies, have the potential to provide an unlimited supply of different cell types for tissue replacement, drug screening, and functional genomics applications. Tissue engineering at the nanoscale level is a potentially useful approach to develop viable substitutes, which can restore, maintain, or improve the function of human tissue. Regenerating tissue can be achieved by using nanobiomaterials to convey signals to surrounding tissues to recruit cells that promote inherent regeneration or by using cells and a nanobiomaterial scaffold to act as a framework for developing tissue. In this regard, nanomaterials

* The authors would like to dedicate this chapter to the memory of Dr. Saeid Kazemi Ashtiani. He was a wonderful colleague, a great stem cell biologist, and an inspirational advocate of human stem cell research in Iran.

such as nanofibers are of particular interest. Three different approaches toward the formation of nanofibrous materials have emerged: self-assembly, electrospinning, and phase separation [1]. Each of these approaches is unique with respect to its characteristics, and each could lead to the development of a scaffolding system. For example, self-assembly can generate small-diameter nanofibers in the lowest end of the range of natural extracellular matrix (ECM) collagen, while electrospinning is more useful for generating large-diameter nanofibers on the upper end of the range of natural ECM collagen. Phase separation, on the other hand, has generated nanofibers in the same range as natural ECM collagen and allows for the design of macropore structures. Specifically designed amphiphilic peptides that contain a carbon alkyl tail and several other functional peptide regions have been synthesized and shown to form nanofibers through a self-assembly process by mixing cell suspensions in media with dilute aqueous solutions of the peptide amphiphil (PA) [2,3]. The challenges with the techniques mentioned above are that electrospinning is typically limited to forming sheets of fibers and thus limiting the ability to create a designed three-dimensional (3D) pore scaffold, and self-assembling materials usually form hydrogels, limiting the geometric complexity and mechanical properties of the 3D structure. Another class of nanomaterials includes carbon nanotubes (CNTs), which are a macromolecular form of carbon with high potential for biological applications due in part to their unique mechanical, physical, and chemical properties [4,5]. CNTs are strong, flexible, conduct electrical current [6], and can be functionalized with different molecules [7], properties that may be useful in basic and applied biological research (for review see [8]). Single-walled carbon nanotubes (SWNTs) have an average diameter of 1.5 nm, and their length varies from several hundred nanometers to several micrometers. Multiwalled carbon nanotube (MWNT) diameters typically range between 10 and 30 nm. The diameters of SWNTs are close to the size of the triple helix collagen fibers, which makes them ideal candidates for substrates for bone growth. As prepared CNTs are insoluble in most solvents, chemical modifications are aimed at increasing their solubility in water and organic solvents.

In this chapter, we aim to offer a basic understanding of this emerging field of SC nanoengineering based on the fusion of SCs, tissue engineering, and nanotechnology.

1.2 STEM CELLS AND TYPES

Although most cells of the body, such as heart cells or skin cells, are committed to conduct a specific function, a SC is an uncommitted cell that has the ability to self-renew and differentiate into a functional cell type [9–11]. Conventionally, SCs are classified as those derived either from embryo or adult tissues (Figure 1.1). Embryonic SCs, embryonic carcinoma cells, and embryonic germ cells are derived from the inner cell mass of blastocysts, teratocarcinoms, and primordial germ cells, respectively. These cells are pluripotent, because they have the ability to entirely colonize an organism and give rise to almost all cell types, except extracellular tissues (e.g., placenta). SCs found in adult organisms are referred to as adult SCs, and are present in most, if not all, adult organs [12]. They are considered multipotent, because they can originate mature cell types of one or more lineages but cannot reconstitute the organism as a whole. What determines SC potency is dependent to a large extent on the genetic makeup of the cell and whether it contains the appropriate genetic circuitry to differentiate to a specific cell type. However, the decision to differentiate or self-renew is often regulated by the SC microenvironment, also known as the SC niche. For example, changes in cytokine gradients, cell–cell, and cell–matrix contacts are important in switching "on" and "off" genes and gene pathways, thereby controlling the type of cell generated.

1.2.1 Embryonic Stem Cells

Embryonic stem cells (ESCs) from mice were first derived in 1981 from the inner cell mass (ICM) of developing mouse blastocysts [13,14]. Human ESCs were established by Thomson and

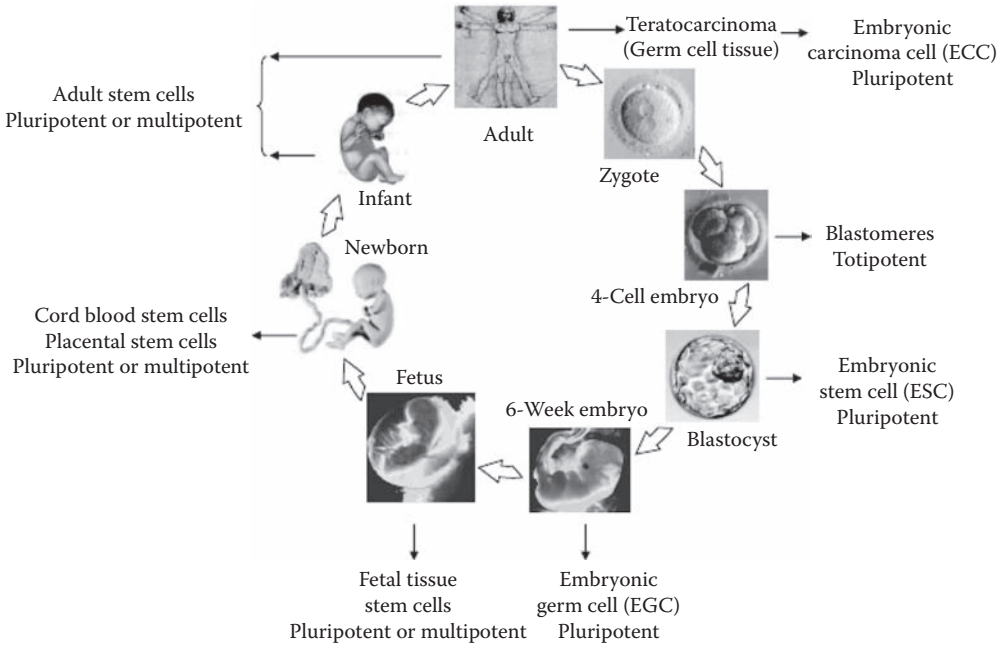

FIGURE 1.1 Origin of different stem cells. Stem cells at different developmental stages appear to have different capacities for self-renewal and differentiation.

colleagues in 1998 [15]. ESCs can be stably propagated indefinitely and maintain a normal karyotype without undergoing cell senescence *in vitro* when cultured in the presence of leukemia inhibitory factor (LIF) (in the case of the mouse) or over a layer of mitotically inactivated mouse embryonic fibroblasts (MEFs), in the monkey and human systems (Figure 1.2). Upon injection of mouse ESCs into blastocysts [16], their progeny is present in all tissues and organs, including the germ line of a chimeric individual (not shown in human ESC due to ethics) and can contribute in the formation of functional gametes [17]. The transmission of genetically manipulated ESCs *in vitro* can thus be passed into chimeric murine offspring and provide a useful approach for studying varying genetic aspects related to ESCs. Homologous recombination has become a useful transgenic approach for introducing selected mutations into the mouse germ line [16,18]. These mutant mice are useful animal models for studying gene function *in vivo* and for clarifying the roles of specific genes in all aspects of mammalian development, metabolic pathways, and immunologic functions.

Upon removal of ESCs from feeder layers and subsequent transfer to suspension cultures, ESCs begin to differentiate into 3D, multicellular aggregates, forming both differentiated and undifferentiated cells, termed embryoid bodies (EBs). Initiation of differentiation may also be induced following the addition of cells into two-dimensional (2D) cultures (i.e., on a differentiation inducing layer such as a matrix or feeder cells). EBs can spontaneously differentiate into different cells and the type of voluntary cells increased by addition of inducing substances or growth factors in their medium, including rhythmically contracting cardiomyocytes, pigmented and nonpigmented epithelial cells, neural cells with outgrowths of axons and dendrites, and mesenchymal cells (Figure 1.2) [19]. Recent studies have also demonstrated ESC differentiation into germ cells and more mature gametes, although significant unanswered questions remain about the functionality of these cells [20]. The derivation of germ cells from ESCs *in vitro* provides an invaluable assay both for the genetic dissection of germ cell development and for epigenetic reprogramming, and may one day facilitate nuclear transfer technology and infertility treatments.

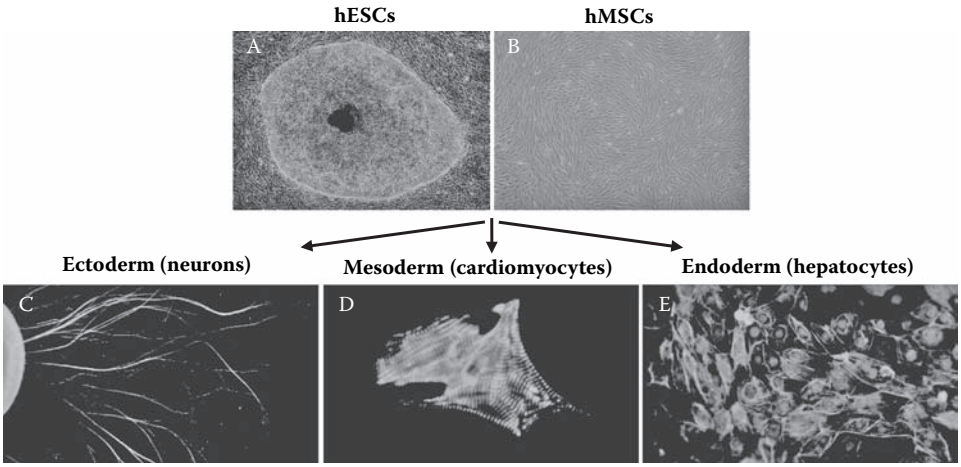

FIGURE 1.2 Morphology and derivatives of embryonic and adult stem cells. Phase-contrast microscopy of (A) a human embryonic stem cell (hESC) (Royan H5) colony cultured on mouse embryonic fibroblast feeder cells (see Baharvand, H., et al., *Dev. Growth. Differ.* 48, 117–128, 2006). (B) Human bone marrow mesenchymal stem cells (hMSCs). Immunocytochemistry of differentiated ESCs with (C) antineuron-specific tubulin III, (D) antialpha actinin, and (E) anticytokeratin 18. **(See color insert following page 112.)**

1.2.2 Adult Stem Cells

The ability of adult tissue such as skin, hemopoietic system, bone, and liver to repair or renew indicates the presence of stem or progenitor cells. The use of autologous or allogeneic cells taken from adult patients might provide a less difficult route to regenerative-cell therapies. In adult soma, SCs generally have been thought of as tissue specific and able to be lineage restricted and therefore only able to differentiate into cell types of the tissue of origin. However, several recent studies suggest that these cells might be able to break the barriers of germ layer commitment and differentiate *in vitro* and *in vivo* into cells of different tissues. For example, when bone marrow is extracted and the cells are placed in a plastic dish, the populations of cells that float are blood-forming SCs (hemopoietic SCs [HSCs]), and those that adhere are referred to as stromal cells [21], including mesenchymal stem or progenitor cells (MSCs, Figure 1.2) [22]. These cells can replicate as undifferentiated forms and have the potential to differentiate to lineages of mesodermal tissues, including bone, cartilage, fat, and muscle [23,24]. Moreover, transplanted bone marrow cells contribute to endothelium [25] and skeletal muscle myoblasts [26] and acquire properties of hepatic and biliary duct cells [27], lung, gut, and skin epithelia [28] as well as neuroectodermal cells [29]. Recently, bone marrow was shown as a potential source of germ cells that could sustain oocyte production in adulthood [30]. Furthermore, neural SCs (NSCs) may repopulate the hematopoietic system [31], and muscle cells may differentiate into hematopoietic cells [32].

Jiang and coworkers recently demonstrated a rare multipotent adult progenitor cell (MAPC) within MSC cultures from rodent bone marrow [33,34]. This cell type differentiates not only into mesenchymal lineage cells but also into endothelium and endoderm. Mouse MAPCs injected in the blastocyst contribute to most, if not all, somatic cell lineages including brain [33]. Furthermore, mouse MAPCs can also be induced to differentiate *in vitro* using a coculture system with astrocytes into cells with biochemical, anatomical, and electrophysiological characteristics of neuronal cells [35].

Umbilical cord blood (UCB) is a source of a population of pluripotent, mesenchymal-like SCs [36] and HSCs for transplantation. There are several reports of MSCs or somatic SCs with pluripotent differentiation potential from various sites in the umbilical cord [36–38]. For example, Buzanska and colleagues [39] reported recently that human UCB-derived cells attain neuronal and glial features *in vitro*. Thus, this tissue is a rich source of SCs that may be useful for a variety of

therapeutic purposes. This has led to the establishment of cord blood banks and the increased use of UCB for transplantation [40,41].

1.2.3 Differentiation of Stem Cells *In Vitro*

SCs are a useful tool for investigating methods relating to the extraction of specific cell types from mixed cell populations or heterogeneous teratomas and to perhaps study the differentiation events of precursor cells toward a particular cell lineage. Feasible methods that may help to achieve these include the addition of specific combinations of growth factors or chemical morphogens; changes in physical and geometrical properties of the microenvironment; coculture or transplantation of SCs with inducer tissues or cells; implantation of SCs into specific organs or regions; and overexpression of transcription factors associated with the development of specific tissues. However, to date, these strategies have not yielded a 100% pure population of mature progeny. Therefore, efficient protocols for purifying cell populations are required. Methods such as fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) allow such purification but are dependent on the cell type of interest expressing a surface marker that can be recognized by a fluorescent or magnetic microbead-tagged antibody, and to be fully effective, the marker needs to be cell-type specific. In most cases, these cell markers are not commercially available. Thus, sorting methods are reliant on genetic modification of the SCs, especially the ESCs, by tagging a lineage-specific promoter to a fluorescent marker. Alternatively, cells could be transduced with a drug-resistance gene instead of a marker to allow for preferential selection of subpopulations [19].

1.3 BEHAVIOR OF CELLS ON NANOBIOMATERIALS

Studies of the interactions between substrate topography and cells have encompassed a wide variety of cell types and substratum features, including grooves, ridges, steps, pores, wells, nodes, and adsorbed protein fibers. Grooves are the most common feature type employed in the study of the effects of surface structure on cells [42–45]. Typically, the grooves are arrayed in regular, repeating patterns, often with equal groove and ridge width. The cross sections of the groove are often of the square wave, V-shape, or truncated V-shape [46]. In general, investigations of grooved surfaces have revealed that the cells aligned to the long axis of the grooves [44,47] often with organization of actin and other cytoskeletal elements in an orientation parallel to the grooves [48,49]. The organization of cytoskeletal elements was observed to occur in some cases with actin and microtubules aligned along walls and edges [48,50]. Many studies have found that the depth of the grooves was more important than their width in determining cell orientation [51], because the orientation often increased with increasing depth but decreased with increasing groove width. Repeat spacing also played a role, with orientation decreasing at higher repeat spacing [52]. There are some studies investigating the behavior of cells on other synthetic features. Green and coworkers found that nodes of 2 μm and 5 μm resulted in increased cell proliferation compared to 10 μm nodes and smooth surfaces [53]. Campbell and von Recum found that pore size played a larger role than material hydrophobicity in determining tissue response [54]. The behavior of cells on sandblasted surfaces has been studied, although the observed trends seem less clear than those on controlled morphologies, such as grooves. In general, adhesion, migration, and ECM production were greater on rougher surfaces than on surfaces sandblasted with larger grain sizes [55,56]. Studies have also been performed in which protein patterns were used to guide cues for several cell types, including neural cells [57,58]. Isolated tracks were found to provide stronger guidance than repeated tracks [57]. Goodman et al. used polymer casting to replicate the topographical features of the ECM [59] and observed endothelial cells cultured on the ECM textured replicas spread faster and had appearance more like cells in their native arteries than did cells grown on untextured surfaces [59,60]. Advancements in electron beam lithography technology have allowed engineers to fabricate well-defined nanostructures down to a possible lateral feature size of 12 nm [61]. The ability to fabricate these nanofeatures has

enabled biologists to look at the effects of such features (which are of a similar size to those that surround a cell, for example, the 66 nm repeat of collagen) on SCs.

When a cell interacts with a biomaterial, it senses the surface topography and will respond accordingly. If a suitable site for adhesion is detected, focal adhesions and actin stress fibers are formed; later, microtubules are recruited, which stabilize the contact [61]. Recently, it was reported that regular nanotopography significantly reduces cell adhesion [62]. Gallagher et al. cultured fibroblasts onto nanopatterned ε-PCL (polycaprolactone) surfaces and showed that cell spreading is reduced compared with that on a planar substrate. Furthermore, cytoskeletal organization is disrupted as indicated by a marked decrease in the number and size of focal contacts [63]. Focal adhesion contacts are of great importance in signal transductive pathways [64]. The signal transductive events originating from focal contacts can affect the long-term cell differentiation in response to materials [61,65].

For scaffolds, it is generally agreed that a highly porous microstructure with interconnected pores and a large surface area is conducive to tissue ingrowth. For bone regeneration, pore sizes between 100 μm and 350 μm and porosities of more than 90% are preferred. For example, rat MSCs that were cultured on highly porous electrospun (PCL) nanofiber scaffolds migrated more rapidly and differentiated into osteoblasts in rotating bioreactors [66]. It is also believed that small fiber diameter and the overall porous structure aid in the adhesion and migration of cells into the scaffold [66].

Nanofibrous scaffolds formed by electrospinning are highly porous, have a high surface-area-to-volume ratio, and have morphological properties that are similar to collagen fibrils [67]. These physical characteristics promote favorable biological responses of seeded cells within these scaffolds, including enhanced cell attachment, proliferation, and maintenance of the chondrocytic phenotype [68,69].

Nanoparticles can also affect ECM properties and cell behavior. For example, carboxylated SWNT can be incorporated into type I collagen scaffolds. Living smooth muscle cells were also incorporated at the time of collagen gelation to produce cell-seeded collagen–CNT composite matrices. These cell-seeded collagen matrices can be further aligned through constrained gelation and compaction, as well as through the application of external mechanical strain [70].

1.4 EXTRACELLULAR MATRIX ENHANCEMENT

Tissues are complex and are typically organized into a well-defined, 3D structure in our bodies. This architecture contributes significantly to the biological functions in the tissues. Furthermore, it provides oxygen and nutrient support and spatial environment for the cells to grow [71]. In this respect, there are three key factors to be considered for the success of tissue regeneration: cells, scaffold, and cell-matrix (scaffold) interaction. The scaffold plays a pivotal role in accommodating the cells. An ideal scaffold for tissue engineering application should mimic the natural microenvironment of the natural tissue and present the appropriate biochemical and topographical cues in a spatially controlled manner for cell proliferation and differentiation. When a cell comes into contact with biomaterial, it will perceive the chemistry of a surface using integrin transmembrane proteins to find suitable sites for adhesion, growth, and maturation. *In vitro*, cells will readily translocate on the material surface to the sites of preferential attachment, and cells will produce distinct morphologies when motile and when adhered and entering the S-phase [72].

Tissue structure and function depend greatly on the arrangement of cellular and noncellular components at the micro- and nanoscale levels—featuring a higher specific surface and thus a higher interface area—in ECM [73]. In addition to providing a physical support for cells, the native ECM also provides a substrate with specific ligands for cell adhesion and migration, and regulates cellular proliferation and function by providing various growth factors. A well-known feature of native ECM is the nanoscaled dimensions of its physical structure. In a typical connective tissue, structural protein fibers such as collagen and elastin fibers, have diameters that range from several

tens to several hundred nanometers. The nanoscaled protein fibers entangle with each other to form a nonwoven mesh that provides tensile strength and elasticity, and laminin, which provides a specific binding site for cell adhesion, also exists as nanoscaled fibers in the ECM. The scaffold should therefore mimic the structure and biological function of native ECM as much as possible, both in terms of chemical composition and physical structure. It is reasonable to expect that an ECM-mimicking tissue-engineered scaffold will play a similar role to promote tissue regeneration *in vitro* as native ECM does *in vivo*. Accordingly, the design of nanofeatured tissue scaffolds is novel and exciting, opening a new area in tissue engineering. Work with ECM components has demonstrated that the physical presentation of these molecules affects morphology, proliferation, and morphogenesis of differentiated cells [74–76]. Culture on or within 3D as opposed to 2D arrays of matrix molecules promotes cellular phenotypes that display more *in vivo*-like structure and function [74,77,78].

These observations were made in the absence of added ECM, suggesting that the geometry of the ECM can influence cellular phenotype and function even in the absence of chemistry. The understanding of how the microenvironment can influence the cell behavior will aid the development of the next generation of scaffolds for tissue engineering and SC applications.

1.4.1 Proliferation of Stem Cells

To provide a more topologically accurate and reproducible representation of the geometry and porosity of the ECM/basement membrane for SC culture [79], Ultra-Web™ (Corning, New York), a commercially available 3D nanofibrillar and nanoporous matrix produced by depositing electrospun nanofibers composed of polyamide onto the surface of glass or plastic coverslips, was used. Within these scaffolds, mouse ESCs had enhanced proliferation with self-renewal in comparison with tissue culture surfaces independent of soluble factors such as LIF. Significantly, cells did not adhere to 2D films composed of polyamide, demonstrating the importance of the nanofibrillar geometry for SC proliferation. It is important to note that these proliferation measurements were performed in the presence of less than 5% of the original feeder MEFs, which remained during passage. Because MEFs normally provide cues that promote SC proliferation, these results suggest that the 3D nanofibrillar surfaces can compensate, at least in part, for the absence of MEFs, but standard tissue culture surfaces cannot perform the same synergistic or replacement function.

This was the first report in which ESCs were cultured on a defined synthetic 3D nanofibrillar surface that resembles the geometry of the basement membrane and in which a relationship is demonstrated between the 3D nanotopology, proliferation with self-renewal, upregulation of Nanog, a homeoprotein shown to be required for maintenance of pluripotency [80], the activation of the small GTPase Rac, and the activation of the phosphoinositide 3-kinase (PI3K)/AKT, components of the PI3K signaling pathway. SCs cultured on the 3D nanofibrillar surface maintained their ability to differentiate in the presence of differentiating factors such as retinoic acid. Because nanofibers influence cellular parameters such as cell shape, actin cytoskeleton, and fibronectin deposition [77,78], it is possible that they influence SCs both directly and indirectly by altering the phenotype of the feeder cells. Moreover, Ultra-Web was used to culture NIH-3T3 fibroblasts and normal rat kidney cells and observed dramatic changes in cellular morphology [77,78]. These observations more closely resembled their *in vivo* counterparts [77,78]. SCs cultured on the 3D nanofibrillar surface maintained their ability to differentiate in the presence of differentiating factors such as retinoic acid. Because nanofibers influence cellular parameters such as cell shape, actin cytoskeleton, and fibronectin deposition [77,78], it is possible that they influence SCs both directly and indirectly by altering the phenotype of the feeder cells. Moreover, Ultra-Web was used to culture NIH-3T3 fibroblasts and normal rat kidney cells, and dramatic changes were observed in cellular morphology that more closely resembled their *in vivo* counterparts [77,78].

Recent experiments using MSCs demonstrated an important role for mechanical cues in regulating SC fate [81]. Li et al. [68] were the first to examine the ability of an electrospun nanofibrous scaffold to support MSC proliferation. Jin et al. also reported that the nanofibrous scaffold