Biomaterials and Nanotechnology for Tissue Engineering

Edited by
Swaminathan Sethuraman
Uma Maheswari Krishnan
Anuradha Subramanian

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Contents

Foreword

It is my pleasure to write the foreword for *Biomaterials and Nanotechnology for Tissue Engineering* for CRC Press. This book contains 14 chapters on the applications of nanotechnology in biomaterials and tissue engineering. This book aims to provide an overview of the rationale involved in the choice of materials for regeneration of different tissues and the future directions in this fascinating area.

This book begins by discussing nanotechnology approaches to tissue engineering. It then covers tissue engineering of connective tissue, such as regeneration of bone tissue, cartilage, and ligaments. This book continues with sections covering bioengineering of skin, and tissue engineering-based functional restoration of blood vessels. It then looks at the engineering of the liver and pancreas. From there, the interesting area of neural regeneration is discussed, followed by tissue engineering therapies for ocular regeneration, and then dental applications. This book concludes by examining image-guided tissue engineering, a discussion of tissue engineered medical products and finally looks at regulatory challenges and ethics.

In summary, *Biomaterials and Nanotechnology for Tissue Engineering* examines many timely topics and should be a useful book for scientists in these important areas.

Robert Langer
MIT

Preface

Advancements in nanotechnology have resulted in the emergence of engineered biomaterial constructs that have ushered in a new era of regenerative medicine in the healthcare sector. Search for ideal scaffold materials has become indispensable in regenerative medicine owing to variations between soft, hard, and interfacial tissues in addition to patient-specific requirements. Fine-tuning the physical, chemical, and biological properties of biomaterials can improve the scaffold performance since it is tailored to restore the diseased tissues. Customizing the properties of tissue constructs is essential for translation of these materials from lab to clinic to meet the growing demand for tissue-specific and patient-specific scaffolds. The current paradigm in the field of tissue engineering is to mimic the native extracellular matrix through patterning intricate hierarchical nano-dimensional features in microarchitecture by various approaches, thereby providing an ideal *milieu* to facilitate tissue progression. The advent of sophisticated technologies, such as rapid prototyping, has promoted tissue engineering as a viable treatment alternative for patients suffering from tissue and organ failure. This book consolidates the progress made in scaffold materials, tissue-specific strategies, fabrication approaches, and development of tissue-engineered medical products. A separate discussion on safety concerns on the use of nanostructured materials has also been included. Section I provides an overview on the various nanotechnology approaches adopted in tissue engineering. Tissue-specific strategies for regeneration of skeletal, skin, neural, ocular, vascular, pancreatic, and hepatic tissues have been detailed in Sections II, III, and IV. The various facets of laser-assisted bioprinting for tissue engineering have been discussed in Section V, while Section VI elaborates on translation of tissue-engineered commercial products for clinical use and also deals with the safety aspects of nanomaterials and the challenges involved.

Section I briefly outlines the various approaches such as self-assembly, electrospinning, and layer-by-layer techniques to fabricate biomimetic tissue scaffolds with hierarchical organization of nano–micro structures as well as nanodiagnostics and nanoscale drug delivery systems. The influence of surface nanotopography on the cell fate processes has also been elaborated in this section. Section II deals with the biomedical applications of natural and synthetic polymers toward skeletal tissue engineering. Regenerating bone tissue remains a challenge, as it requires appropriate mechanical properties with adequate porous vascularizable architecture in addition to osteoconductive, osteointegrative, and osteoinductive properties. This section also highlights various strategies to fabricate nanofibers and their applications in bone regeneration apart from a discussion on emerging technologies to address the limitations of current bone grafts. A description of ideal properties for cartilage tissue constructs, choice of biomaterials, and surface modifications employed in the design of cartilage scaffolds is also provided in Section II.

The current scenario in biomaterial-based tissue engineering on the regeneration of specific sensory tissues namely skin, nerve, and ocular has been discussed in Section III. Chapter 6 on skin regeneration discusses in-depth on the integration of mechanical, biochemical stimuli apart from use of novel materials of natural and synthetic origin with appropriate topography for functional regeneration of skin and its appendages. Emerging strategies such as stem cell therapy, mi-RNA delivery, melanocyte incorporated skin, photosynthetically activated wound healing, and skin-on-a-chip are also discussed. Injuries

to the central nervous system and peripheral nervous system can lead to permanent disabilities. A detailed discussion highlighting recent advances in the use of biomaterials and nanotechnology for central nerve repair and peripheral nerve repair has been provided in Chapter 7. Application of tissue engineering principles in ophthalmology to overcome challenges such as graft rejection, infection, inflammation, and vision impairment has been the focus of Chapter 8. This chapter deals with a wide range of biomaterials for opthalmological applications and also on recent innovations in the regeneration of various ocular components such as cornea, retina, lachrymal gland, as well as replacement strategies for lens and vitreous fluid.

The progress in tissue engineering strategies for the repair and reconstruction of various functional tissues, such as blood vessels, pancreas, and liver involved in the transportation, metabolism, and synthesis, respectively, are elaborated in Chapters 9, 10, and 11, respectively. Developing vasculature using biomaterials and decellularized matrices and scaffold-free approaches such as cell sheet conduits, 3D bioprinting, as well as the clinical success of tissue-engineered blood vessels are outlined in this section. Engineering bioartificial pancreas using Islet cells and biomaterials, clinical trials, and current challenges in clinical applications has been described in Chapter 10. Chapter 11 in this section elaborates on the various therapeutic interventions, starting from conventional cell and biomaterial-based approaches, extracorporeal devices toward organogenesis for the treatment of acute and chronic liver diseases and failures.

The emergence of three-dimensional rapid prototyping of tissues and organs ensures the precise spatio-temporal positioning of biological and physical components. Section V discusses the rapidly evolving laser-assisted bioprinting field for tissue engineering that can potentially reconstruct the native system. The fundamentals of laser-induced forward transfer, underlying principles, and the materials employed have been elaborated along with descriptions of applications such as bioprinting of biomaterials, cells, DNA, peptides, and *in vivo* bioprinting with clinical implications.

Section VI discusses the progress of tissue-engineered medical products, which represents innovative technologies, materials, and treatments aiming to address unmet clinical needs—ranging from musculoskeletal applications to nerve and cardiovascular regeneration. Surgical repair, artificial prostheses, and mechanical devices considered "gold standard" treatments, however, fall short in total repair and long-term recovery from significant tissue/organ damage. Chapter 13 focuses on tissue devices being used today for bone, cartilage, tendon, skin, nerves, and tissue interfaces and also discusses the research being done to develop them for future applications.

Despite emerging as a strong alternative to current treatments, the complexity of tissues and organs introduce challenges for researchers and engineers to ensure that these products are safe and effective prior to clinical trials and commercialization. The Food and Drug Administration (FDA) evaluates the safety and effectiveness of medical products based on scientific and regulatory considerations assessed dependent on the product's characteristics, preclinical studies, and proposed clinical trials. Thus, product development is often a long-term, multidisciplinary effort in order to develop a proper scientific and technical database to ensure a product's complete safety and effectiveness. Several tissue-engineered medical products exist, addressing the repair and regeneration of various tissues such as cartilage (NeoCart®, CARTIPATCH®), tendon (Graftjacket®, X-Repair®), and bone (OP-1/BMP-7, IngeniOs HA®). Many more tissue-engineered medical products are under development, attempting to further expand the field to address larger, more serious, complex, and even total organ applications. This section also elaborates the health and safety implications of nanoparticles such as toxicological assessment

techniques, nanoparticle dosing, and factors that influences the toxicity of nanoparticles such as material properties and host factors.

Advanced biomaterials and nanotechnology approaches for tissue engineering has convincingly progressed to commercialization of clinical products. The nanotechnology interventions and polymeric biomaterials for soft and hard tissues discussed in this book would provide an insight to the readers about the progress made in this field and also future challenges that need to be addressed by tissue engineers. This book covers the fundamentals and recent advances benefitting both the beginners and experts working in this field.

The editors express their gratitude to all the experts for their valuable contributions and sharing their expertise in the completion of this book.

S. Swaminathan, PhD
K. Uma Maheswari, PhD
S. Anuradha, PhD

Editors

Swaminathan Sethuraman is the dean, sponsored research and director, Centre for Nanotechnology & Advanced Biomaterials (CeNTAB), School of Chemical & Biotechnology at SASTRA University, Thanjavur, Tamil Nadu, India. Dr. Sethuraman earned his PhD from the Department of Chemical Engineering at Drexel University, Philadelphia, and worked on the development of low temperature setting polymer–ceramic composite cements for bone tissue engineering. Later, he joined as a research associate in the Department of Orthopaedic Surgery at the University of Virginia, Charlottesville. He is the recipient of several awards including Innovative Young Biotechnology Award (IYBA—2006) from the Department of Biotechnology, Government of India, Material Research Society of India Medal for the year 2009 from Materials Research Society of India, and Young Career Award in Nano Science and Nanotechnology from the Department of Science and Technology, Government of India, in 2015.

Uma Maheswari Krishnan is currently the associate dean in the School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu, India, and Deakin Indo-Australian Chair professor. She earned her PhD in applied chemistry from Bharathiyar University, Tamil Nadu, India. Prior to research training from University of Arkansas, in 2005, she was a postdoctoral fellow at the University of Texas, in 2002. Dr. Krishnan's research interests are drug delivery, gene delivery, tissue engineering, and biosensors.

Anuradha Subramanian is an assistant professor—Research, at the Centre for Nanotechnology & Advanced Biomaterials (CeNTAB), School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu, India. Her PhD work was on development of novel 3D nanofibrous scaffolds for neural regeneration and earned her degree from the School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu, India. Dr. Subramanian's research interests include tissue engineering, theranostics, self-assembled nano-structures, and 3D bioprinting.

Contributors

Olajide Abiola
Institute for Regenerative Engineering
Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences
Department of Orthopaedic Surgery
The University of Connecticut Health Center
Farmington, Connecticut

Joëlle Amédée-Vilamitjana
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Nandana Bhardwaj
Life Science Division
Institute of Advanced Study in Science and Technology (IASST)
Assam, India

Bibhas Kumar Bhunia
Department of Bioscience and Bioengineering
Indian Institute of Technology Guwahati (IITG)
Assam, India

Jean-Michel Bourget
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Sylvain Catros
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Wei Chang
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Kaushik Chatterjee
Department of Materials Engineering
Indian Institute of Science
Karnataka, India

Clancy J. Clark
Department of Surgery, Wake Forest University School of Medicine
Wake Forest School of Medicine
Winston-Salem, North Carolina

Hélène Desrus
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Raphaël Devillard
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Amit Dinda
Department of Pharmacology
All India Institute of Medical Sciences
New Delhi, India

Joseph W. Freeman
Department of Biomedical Engineering
Rutgers University
Piscataway, New Jersey

Jean-Christophe Fricain
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Y. K. Gupta
Department of Pharmacology
All India Institute of Medical Sciences
New Delhi, India

Jérôme Kalisky
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Matthew Kandl
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Olivia Kérourédan
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Karen Kong
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Uma Maheswari Krishnan
Centre for Nanotechnology & Advanced Biomaterials (CeNTAB)
School of Chemical and Biotechnology (SCBT)
SASTRA University
Tamil Nadu, India

Jadi Praveen Kumar
Department of Bioscience and Bioengineering
Indian Institute of Technology Guwahati (IITG)
Assam, India

Sachin Kumar
Department of Materials Engineering
Indian Institute of Science
Karnataka, India

Sangamesh G. Kumbar
Institute for Regenerative Engineering
Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences
Department of Orthopaedic Surgery
The University of Connecticut Health Center
Farmington, Connecticut

and

Department of Biomedical Engineering
University of Connecticut
Storrs, Connecticut

Cato T. Laurencin
Institute for Regenerative Engineering
Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences
Department of Orthopaedic Surgery
and
Department of Reconstructive Sciences
The University of Connecticut Health Center
Farmington, Connecticut

and

Department of Chemical and Biomolecular Engineering
and
Department of Biomedical Engineering, Department of Materials Science and Engineering
University of Connecticut
Storrs, Connecticut

Paul Lee
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Biman B. Mandal
Department of Bioscience and Bioengineering
Indian Institute of Technology Guwahati (IITG)
Assam, India

Ohan S. Manoukian
Institute for Regenerative Engineering
Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences
Department of Orthopaedic Surgery
The University of Connecticut Health Center
Farmington, Connecticut

and

Department of Biomedical Engineering
University of Connecticut
Storrs, Connecticut

Shreya Mehrotra
Department of Bioscience and Bioengineering
Indian Institute of Technology Guwahati (IITG)
Assam, India

Sai Rama Krishna Meka
Department of Materials Engineering
Indian Institute of Science
Karnataka, India

Prabha D. Nair
Division of Tissue Engineering and Regeneration Technologies
Biomedical Technology Wing
Sree Chitra Tirunal Institute for Medical Sciences and Technology
Kerala, India

Jonathan Nip
Institute for Regenerative Engineering
Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences
Department of Orthopaedic Surgery
The University of Connecticut Health Center
Farmington, Connecticut

Emmanuel C. Opara
Wake Forest Institute for Regenerative Medicine
Virginia Tech-Wake Forest School of Biomedical Engineering & Sciences
Wake Forest School of Medicine
Winston-Salem, North Carolina

Janani Radhakrishnan
Centre for Nanotechnology & Advanced Biomaterials (CeNTAB)
School of Chemical and Biotechnology (SCBT)
SASTRA University
Tamil Nadu, India

Rahul V. G.
Division of Tissue Engineering and Regeneration Technologies
Biomedical Technology Wing
Sree Chitra Tirunal Institute for Medical Sciences and Technology
Kerala, India

Murielle Rémy
Institut National de la Sante et de la Recherche Medicale (INSERM)
Bioingénierie Tissulaire
Université Bordeaux
Bordeaux, France

Rachel Rosa
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Swaminathan Sethuraman
Centre for Nanotechnology & Advanced Biomaterials (CeNTAB)
School of Chemical and Biotechnology (SCBT)
SASTRA University
Tamil Nadu, India

Munish Shah
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Yogendra Pratap Singh
Department of Bioscience and Bioengineering
Indian Institute of Technology Guwahati (IITG)
Assam, India

Kevin J. Smith
Department of Biomedical Engineering
University of Connecticut
Storrs, Connecticut

Anuradha Subramanian
Centre for Nanotechnology & Advanced Biomaterials (CeNTAB)
School of Chemical and Biotechnology (SCBT)
SASTRA University
Tamil Nadu, India

Brittany L. Taylor
Department of Biomedical Engineering
Rutgers University
Piscataway, New Jersey

Varadraj N. Vernekar
Institute for Regenerative Engineering
Raymond and Beverly Sackler Center for Biomedical Biological, Physical and Engineering Sciences
Department of Orthopaedic Surgery
The University of Connecticut Health Center
Farmington, Connecticut

Xiaojun Yu
Department of Chemistry, Chemical Biology, and Biomedical Engineering
Stevens Institute of Technology
Hoboken, New Jersey

Section I

Introduction

1

Nanotechnology Approaches to Regenerative Engineering

Varadraj N. Vernekar, Kevin J. Smith, and Cato T. Laurencin

CONTENTS

1.1 Introduction and Background

The deficit in organ and tissue donation is a significant unmet healthcare need worldwide. In the United States itself, the organ waiting lists have swelled disproportionately in comparison to the increase in the number of transplants (U.S. Department of Health and Human Services, 2015). Due to the acute shortage of donor organs, many die while on the waiting list. Currently, typical treatments for replacement of damaged or lost tissue are the use of autografts and allografts (Dlaska et al., 2015). Whereas these two options perform fairly well, allografts carry the risk of infections, and autografts have issues with availability and donor morbidity. To supplant these approaches, the field of biomaterials has explored alternative materials and methods to repair damaged or diseased tissue (Ratner et al., 2013). Advances in chemistry have enabled the use of metals, ceramics, and polymers, which can be tailored for specific mechanical, biological, and chemical properties; however, these options still have problems associated with foreign body response and imperfect integration into the body. These challenges associated with first-generation biomaterials have led to the generation of intelligent or stealth biomaterials that are not easily detected by the bodies' immune system or isolated by fibrous capsules. Notwithstanding these advances in biomaterials, we have still not been able to provide seamless tissue and organ replacement to effectively meet the outstanding worldwide demand. Toward that end, tissue engineering started about 30 years ago as an alternative approach to achieve

replacement of damaged tissues and organs (Skalak and Fox, 1988; Nerem, 1991; Langer and Vacanti, 1993).

On the basis of the toolkits and knowledge base available from the mid-1980s, different definitions of tissue engineering have been seen. The following, perhaps, captures the main idea of tissue engineering from that time: tissue engineering is an interdisciplinary field utilizing knowledge of engineering, materials science, and the life sciences to generate structural components that can be used as biological substitutes that replace, restore, maintain, or improve damaged tissues and organs function (Langer and Vacanti, 1993). Laurencin further defined it as "the application of biological, chemical and engineering principals toward the repair, restoration, or regeneration of living tissue using biomaterials, cells, and factors alone or in combination" (Laurencin et al., 1999, p. 21). Historically, tissue engineering approaches have been categorized into three distinct classes: isolated cells, tissue-inductive materials, and cell-loaded matrices (Langer and Vacanti, 1993). The delivery of isolated cells is appealing due to its simplicity, but suffers from low cellular retention rates and is insufficient for large-scale defects. The use of tissue-inducing materials is a more promising option that encompasses aspects of controlled drug release from the biomaterial. Nevertheless, cell-loaded matrices of natural, synthetic, or composite materials are the most comprehensive and robust platform that can include the benefits of both the previous approaches and more. The objective is for the scaffold matrices to provide a physiologically relevant microenvironment to the cells seeded within them, with eventual resorption of these matrices as they get replaced by the extracellular matrix (ECM) produced by the cells. The idea underlying tissue engineering is that the scaffold-seeded cells can spontaneously reassemble into organotypic/tissue-mimetic structures, given instructive guidance and support via appropriate scaffold material, bioactive factors, and external physical stimulation, usually in some combination. Several bioresorbable materials such as poly(lactic-*co*-glycolic acid) (PLGA), spurred initially by the development of resorbable sutures, and subsequently by controlled drug release platforms have emerged as excellent candidates for this purpose, and have received FDA approval (Lee and Mooney, 2001).

Although material scientists tend to specify the 1–100 nm as the realm of nanotechnology (Nalwa, 1999; Sobolev, 2015), the size range of bioactive features that influence cellular behavior is actually in the nanometer–micrometer range. Therefore, we refer to nanotechnology as the process of manipulating components at the nanoscale in the 1 to <1000 nm (submicron range). Nanotechnology is distinguished from chemistry by its focus on nanoscale physical structures that present chemical functions rather than the chemistry itself. Nanotechnology is engaged with the processes for manufacturing and manipulating properties at the nanoscale. Nanofabrication techniques can create precise physical structures or mimic biological structures that in combination with their chemical and mechanical properties can influence specific properties of cells. Materials used to synthesize nanostructures include proteins, polymers, dendrimers, fullerenes and other carbon-based structures, lipid–water micelles, viral capsids, metals, metal oxides, and ceramics. Some target structures that nanotechnology seeks to mimic include ECM components; growth factors; signaling molecules; and subcellular organelle components such as ribosomes, proteasomes, ion channels, and transport vesicles. Using nanotechnology, biomimetic physical correlates of these entities such as monolayers, micelles, particles, fibers, and scaffolds can be fabricated with uniformity and specificity. In turn, these nanoscale features with unique and defined characteristics such as pore size, porosity, tortuosity, biocompatibility, biodegradability, and mechanical properties, can influence higher-level functions in tissue regeneration.

1.2 Tissue Engineering

Tissue engineering is a rapidly developing interdisciplinary field that seeks to repair, restore, replace, or enhance biological tissue and organs. It integrates biology, chemistry, material science, and engineering. Tissue engineering approaches are based on the principle that by incorporating biocompatible scaffolds containing specific tissue-mimetic extracellular structures, appropriate cell types, and the necessary tissue-specific signaling, trophic, and vascularization cues the cells will be maintained and regulated to spontaneously organize into higher-order functional tissues and even whole organs.

When the field of tissue engineering was emerging, the available knowledge and toolkits were not as developed and expansive as we see now. For example, material science was applied to tissue engineering, but the use of nanotechnology in tissue regeneration was not yet realized. With the insight that, beyond the microarchitecture, it is the integral nanoarchitecture and topography of the ECM that influences local cellular behavior by supporting a host of cell–ECM, cell–cell, and cell–soluble factor interactions (Taipale and Keski-Oja, 1997) there has been a consistent shift in focus from the micro- to the nanoscale. For example, with the understanding that the cellular niche is essentially a natural web of hierarchically organized nanofibers of structural proteins such as collagen and elastin, cell adhesive proteins such as laminin and fibronectin, and fillers such as the brush-like glycosaminoglycans (GAGs), the design of ECM mimicking scaffolds has shifted focus from the macro-, to the micro-, to the nanoscale, over the years.

Tissue engineering has faced the following challenges: (1) the generated scaffold platform must provide a conducive biocompatible environment for the assembly and housing of cells by promoting cell adhesion, viability, growth, proliferation, differentiation, morphogenesis, and integration; (2) engineered tissue must address wound healing; and (3) the engineered tissue is vascularized for long-term maintenance, and, if necessary, innervation. For true biomimetic tissue formation, all three of these challenges must be addressed and the new engineered tissue must fully (structurally and functionally) integrate into the body. Progress in the fulfillment of these goals has led to applications in skin (Supp and Boyce, 2005), cartilage (Freed et al., 1997), bladder (Oberpenning et al., 1999), bone (Thesleff et al., 2011), cornea (Shah et al., 2008), and blood vessels (L'heureux et al., 1998), but we have still not seen as many regulatory authority-cleared commercially available tissue-engineered products in the market. Furthermore, the third challenge is still significantly unmet, and; therefore, the early developments have been in tissues that are either poorly vascularized natively or show very high-intrinsic regenerative potential. Perhaps, the incorporation of techniques and knowledge from the rising fields of nanotechnology, stem cell science, and developmental biology need to be incorporated into the shifting paradigm of tissue regeneration to overcome the challenges that traditional tissue engineering has faced so far.

1.3 Regenerative Engineering

Advances in materials science have allowed us to harness nanotechnology as a tool for engineering tissues, which was not the case when the field of tissue engineering began roughly 30 years ago. Alongside our deepening understanding of biology, our understanding of biological chemistry has advanced to the point that in many cases we now precisely

understand the molecular level interactions governing cellular behavior. We have realized that static and dynamic cues from the extracellular space are, in fact, key to influencing cellular behavior in a spatiotemporal manner, and therefore, can assist in the larger goal of tissue regeneration. Multiple signaling pathways are transmitted from the extracellular environment at the nanoscale via different transducing agents through the cell membrane, cytoskeleton, organelles, and cytosol, to the cell nucleus, eventually resulting in the production of new proteins. In turn, these generated proteins determine cellular destiny. Likewise, with advances in chemistry and processing techniques, materials science has developed in precision from the millimeter, to the micrometer, to the nanometer range at the molecular level itself, giving birth to the field of nanotechnology. Since we now know that nanoscale cues determine the behavior of cells and thereby control their destiny, nanotechnology holds tremendous potential in the manipulation of cellular behavior for engineering tissue regeneration.

Over the last 15 years, we now have a deeper understanding of both adult and embryonic stem cells, and have even developed induced pluripotent stem cells, developing new knowledge about tissue genesis from stem cells and related effective tools in our toolkit to regenerate tissue. Furthermore, although we have barely scratched the surface, our understanding of the developmental biology of limb regeneration in the salamander and the newt has brought new insights into the process of wound repair and regeneration.

With these new developments in mind, the shifting paradigm of tissue engineering can be more accurately described as "regenerative engineering" (Laurencin and Khan, 2013). Laurencin recently defined this new field as the integration of tissue engineering with advanced material science, stem cell science, and areas of developmental biology for the regeneration of complex tissues, organs, and organ systems (Reichert et al., 2011). Whereas tissue engineering brought together the fields of engineering and science in general, regenerative engineering will specifically converge the fields of tissue engineering, advance materials, stem cell science, and developmental biology toward the goal of tissue regeneration.

1.3.1 Nanotechnology Applications in Regenerative Engineering

Nanotechnology is enabling medicine through advances in diagnostics and therapeutics, biomaterials and drug delivery, and regenerative engineering. Cellular and tissue environments usually have individual components in the five to several hundreds of nanometers in size range. It envisages that these individual nanocomponents when combined, sort of like a "lego" puzzle following specific "rules," into a superstructure along with cells will give rise to tissues with unique properties. Advances in nanotechnology have started to fill this nanoscale design need in tissue engineering by enabling the fabrication of nanoarchitectural structural mimics of the ECM, the creation of nanotopographical surfaces, and nanoencapsulated drug release systems with high spatiotemporal control (Goldberg et al., 2007). Although these embodiments are pushing the boundaries at controlling and instructing cellular behavior, the challenge still remains in augmenting the properties of these structural mimics with the essential functional complexity of the composite material that is the ECM, toward truly emulating it, and successfully regenerating tissues and organs. In the following subsections we discuss some of the applications of nanotechnology in regenerative engineering toward addressing these challenges.

1.3.1.1 Nanofabricated Topography

ECM structural features such as fibers and pores, with characteristic dimensions in the length-scale of cellular protrusions, may influence contact guidance mechanisms by which

cells migrate through three-dimensional (3D) extracellular environments (Abraham et al., 1999; Lutolf and Hubbell, 2005). As a first step to assess the influence of nanoscale environments in controlling cell function and fate, cell-contacting substrates have been engineered to present different nanoscale topographies through a combination of geometry and patterns (Shi et al., 2010). For example, applying lithographic techniques, nanopatterns such as grooves, posts, and pits can be created (Norman and Desai, 2006; Bettinger et al., 2009). Likewise, micelle lithography, anodization, and electrospinning methods can be applied to fabricate nanoscale spheres, tubes, and fibers (Xu et al., 2004; Park et al., 2007; Huang et al., 2009). Furthermore, polymer demixing, phase separation, electrospinning, colloidal lithography, chemical etching, self-assembly methods can be applied to fabricate unordered nanotopographies (Norman and Desai, 2006). Using these different nanotopographies different cellular processes such as cell morphological changes (Xu et al., 2004; Yim et al., 2007; Kim et al., 2010), alignment (Xu et al., 2004; Yim et al., 2007; Kim et al., 2010), signaling (Ranzinger et al., 2009; Kim et al., 2010), adhesion (Xu et al., 2004; Yim et al., 2007; Kim et al., 2010), migration (Xu et al., 2004), proliferation (Yim et al., 2007), and differentiation (Dalby et al., 2007; Yim et al., 2007; Oh et al., 2009) can be manipulated. Furthermore, instructive biorecognition can be provided to the different nanotopographies through the incorporation of bioactive ECM molecules such as collagen-I, III, IV, laminin, fibronectin; bioactive peptides such as RGD (oligopeptide arginine–glycine–aspartic acid), IKVAV (oligopeptide isoleucine–lysine–valine–alanine–valine), and YIGSR (oligo peptide tyrosine–isoleucine–glycine–serine–arginine); and growth factors by using various surface modification techniques (Kumar, 2005). Our emerging understanding of how cells respond to different nanofeatures in their immediate vicinity will determine future combinations of nanotopography and cell types to achieve desirable outcomes in regenerative engineering.

1.3.1.2 Nanofabricated Scaffolds

Simultaneously as our understanding of cell–nanotopography interactions has advanced, so has the development of 3D nanofeatured scaffolds for housing the cells. The basic feature of 3D nanoscaled scaffolds—the nanofiber—seeks to mimic the physical structure of protein nanofibers in the ECM. The high surface area to volume ratio, porosity, and spatial interconnectivity that all types of nanofibrous structures present can promote tissue regeneration by maximizing cell–ECM interactions; the transport of trophic factors, oxygen, nutrients, carbon dioxide, and waste; cell migration; and vascularization.

The primary methods to fabricate nanofibrous structures are electrospinning, which creates both aligned and randomly distributed fibers; self-assembly, which perhaps most closely emulates natural ECM nanofibrous assembly; and phase transition, which allows for the fabrication of sponge-like structures out of a fibrous network (Shi et al., 2010). These techniques use different types of polymeric, nanocomposite, and carbon nanotube-based materials (Murugan and Ramakrishna, 2005; Edwards et al., 2009).

Due of the close connection between the cellular cytoskeleton and the ECM via different types of junctions and ligand–receptor interactions, cells can sense and respond particularly to the mechanical properties of their environment. Consequently, beyond the biochemical properties, the biophysical properties of the ECM exert a major influence on various cellular functions such as adhesion, migration, proliferation, development, and differentiation. Therefore, control over cell-housing scaffolding structure properties is important. Of particular importance are the properties of surface chemistry, topography, and mechanical properties. Surface chemistry entails surface functionality, charge, hydrophobicity, hydrophilicity, and adhesiveness. Topography entails features size, aspect ratio,

geometry, spacing, roughness, porosity, and tortuosity. Finally, mechanical properties include properties such as elasticity, fatigue strength, etc. Nanotechnology can be used to tune all of these properties. For example, by fine tuning the elasticity of a matrix used to house cells the differentiation of stem cells to different lineages was demonstrated (Engler et al., 2006). Manipulating such properties can enable implants to be seeded with cell precursors that will naturally develop into the desired cells and tissues over time, which is of particular relevance when using stem cells in regenerative engineering.

Toward incorporating multiple desirable properties into scaffolds, Burdick and coworkers recently developed a nanofibrous hydrogel that uses spatially patterned chemical cues to create selective cell development patterns within a scaffold (Wade et al., 2015). This sort of nanoscale control is enticing because it provides a methodology to selectively influence different types of cells including stem cells within a single scaffold based on their location in the scaffold. Some of the prominent nanofabrication techniques used for regenerative engineering applications are discussed next.

1. *Self-Assembly*: Molecular self-assembly approaches the engineering of tissue scaffolding materials by emulating natural ECM both structurally and functionally and promoting cell–matrix interactions. Spontaneous self-assemblies of amphiphilic peptides results from the additive action of weak and noncovalent interactions of individual nanofibrous components to generate higher-order structures such as tubules, micelles, and gels, all of which can be used as structural components to build ECM-like scaffolds (Zhang and Zhao, 2004). These peptidal assemblies can have fiber diameters as small as 10 nm, and scaffold pore sizes between 5 and 200 nm, significantly smaller than those produced by electrospinning (Zhang, 2003). 3D interwoven nanofiber-based hydrogels formed using self-assembling peptides such as the RADA (oligo peptide with repeating sequence arginine–alanine–aspartic acid–alanine) peptides offer an elegant solution as an injectable drug delivery and regenerative engineering system (Koutsopoulos and Zhang, 2012). Other widely investigated ECM-derived self-assembling polypeptides used to synthesize injectable hydrogel scaffolds include elastin- and collagen-based proteins, as well as fibrin and silk proteins. The advantage of using the hydrogel approach is that the delivery of the cell-loaded scaffold can be done minimally invasively into irregularly shaped wound sites that they can eventually conform to and set upon phase transition. Moreover, these hydrogel scaffolds can shorten operating times, minimize postoperative pain and scar tissue, potentially reducing cost. Furthermore, these self-assembling peptides show good biocompatibility, minimal immune responses, degrade into amino acids that are readily metabolized *in vivo*, and therefore, provide a resorbable scaffold that is advantageous for both drug delivery and regenerative engineering applications (Koutsopoulos and Zhang, 2012).
2. *Electrospinning*: Although the first patent on electrospinning was granted over 80 years ago, application of this technique to regenerative engineering has increased dramatically only in the last 15 years since the pioneering work by Laurencin and coworkers (Li et al., 2002; Laurencin and Ko, 2004). Electrospinning is the extraction of polymeric nanofibers from an evaporating polymeric solution emanating from a needle jet under a high-voltage electrical field that leads to the deposition of ECM-like nanofibrous structures on a target electrode plate. Electrospinning produces nonwoven nanofibrous meshes that exhibit physical structures similar to that of the fibrous protein-based architecture in native ECM. Although the diameters of the

electrospun fibers are usually at the upper limits of the 50–500 nm range that is seen in natural ECM, by tuning the various fabrication process parameters, physical properties such as average fiber diameter, pore size, tensile strength, and drug-release characteristics of these nanofibrous meshes can be optimized for specific design goals (Goldberg et al., 2007). One of the challenges that all scaffold designs face, particularly electrospun fibrous scaffolds, is control over uniformly seeding adequate number of cells into the scaffold. Recent developments include a methodology to incorporate cells within electrospun scaffolds that typically exhibit poor cell infiltration capabilities; thus, providing a solution to this problem (Sampson et al., 2014). Likewise, polymeric electrospun nanofibers intrinsically lack biochemical recognition cues; therefore, they require the immobilization of tissue-conducive cell-responsive domains (Kim and Mooney, 1998; Lutolf and Hubbell, 2005).

3. *Layer-by-Layer (LBL) Fabrication*: LBL fabrication is a method that produces electrostatically stabilized multiple polyelectrolyte layers with nanometer scale precision. This nanofabrication method is advantageous for its tunability, drug delivery properties, and biocompatibility. The use of LBL coatings can assist in mitigating an immune response and facilitate wound healing through drug delivery (Tang et al., 2006). Self-degradable polyelectrolytes such as polyglutamic acid have been used to generate degradable LBL materials that are independent of qualifying factors such as enzymatic degradation, ionic strength, or electrical stimulation (Tang et al., 2006). Such LBL deposition methods can also be used to fine tune the surface properties of polymeric scaffolds fabricated by other methods (Hammond, 2004).
4. *Bioanalyte-Sensitive Polymeric Hydrogel Synthesis*: This method is more linker-chemistry than nanotechnology; however, we have included it as it provides nanoscale control for both regenerative engineering and drug delivery applications in regenerative engineering. A wide variety of biohybrid hydrogels has been developed for these applications. These include the incorporation into the hydrogels of highly specific and high-affinity proteins and peptides that are enzymatically cleavable, serve as cell adhesion molecules, or get released as signals to trigger a biological process, which is key for the success of the engineered scaffold tissue such as vascularization (Mann et al., 2001; Burdick and Anseth, 2002; Lutolf and Hubbell, 2005; Phelps et al., 2010). A popular base polymer used for such hydrogel synthesis, followed by specific bioanalyte "decoration," is polyethylene glycol (PEG), known for its high hydrophilicity, very low protein adsorption, and general bioinert "stealth" characteristics.

1.3.1.3 Nanoscale Drug Delivery

Localized delivery of multiple factors, controlled for parameters such as release rate, sequence, pattern, period, bioavailability, pharmacodynamics, and cell-specific targeting within 3D scaffolds is key to dynamically instructing cells toward tissue regeneration in a highly controlled manner (Richardson et al., 2001). This level of control is achieved by controlling the size and geometry of drug carriers using nanotechnology. An outstanding challenge for the long-term viability of tissue-engineered scaffolds is vascularization, which is necessary for the transport of key nutrients and oxygen and removal of wastes. It is well known that the release of angiogenic and vasculogenic factors such as vascular endothelial growth factor can promote vascularization. However, indiscriminate release of angiogenic factors is associated with multiple risks factors; therefore, the release has to be controlled,

localized, and sustained over a specified period. Likewise, specific considerations apply to biologically instructive factors other than growth factors, such as proteins, nucleic acids, deoxyribonucleic acid (DNA), small-interfering ribonucleic acid (siRNA), aptamers, etc., which need to be released into engineered scaffolds as well, depending on the application.

To achieve controlled release, several fabrication approaches have been investigated such as the encapsulation of these biological factors by physical blending or chemical conjugation offering different levels of success (Thanou and Duncan, 2003; Jiang et al., 2004). Nanofabrication provides an addition to this toolkit to fine tune drug release characteristics (Zhang and Uludağ, 2009). Polymeric nanoparticle-based drug delivery is one of the well-researched subcategories for drug delivery due to the high level of control over polymers and the ease of fabrication. Other nanosystems that can be employed for drug delivery in regenerative engineering include lipid–water micelles, dendrimer, fullerenes and other carbon-based structures, and inorganic nanomaterials. Some of these nanocarriers enable coencapsulation of multiple drugs and control individual agent release in a temporal fashion (Sengupta et al., 2005). Others enable triggering of drug release in response to environmental stimuli such as pH, temperature, light, drugs, etc. (Caldorera-Moore and Peppas, 2009); these type of "intelligent" systems can be designed to specifically sense and respond directly to pathophysiological conditions. Tuning the nanofabrication process parameters, material formulations, drug loading, biocompatibility, and degradation characteristics can help achieve the desired localized delivery of multiple factors that are controlled for key release parameters within 3D scaffolds.

More drug candidates for regenerative engineering beyond growth factors and nucleic acids include hormones, secondary messenger molecules, adhesion molecules, chemokines, cytokines, small molecule drugs, etc. The application of nanotechnology to several of these candidates has extended the drug half-life *in vivo*; improved hydrophobic drug solubility; selectively sequestered, controlled, and tuned simultaneous hydrophilic and hydrophilic drug release; and reduced potential immunogenicity, drug toxicity, administration frequency, etc. For example, biological molecules can be conjugated with PEG to form PEGylated molecules, which improve their stability and retention times *in vivo,* in addition to providing them with "stealth" properties avoiding activating the immune system (Veronese and Mero, 2008). Nanoscale drug delivery methods can be subdivided into four primary categories (Hughes, 2005).

1. *Polymeric Nanoparticle-Based*: This type of drug delivery can be conducted with a number of different polymers allowing greater control over material and release properties. Polymeric nanoparticles can be synthesized from a variety of polymers such as polyesters, polyethers, polyphosphazenes that offer excellent control of properties such as release rate, degradation rate, etc. (Ma, 2008). One such application includes the use of PLGA to form nanoparticles densely loaded with siRNA for sustained gene silencing (Woodrow et al., 2009), which has applications in the coordinated steps of cell transformation in regenerative engineering.
2. *Carbon Based*: This type of drug delivery employs higher-order structures of carbon such as carbon nanotubes and fullerenes, which are readily internalized into cells because of their very small size, and therefore, provide an efficacious platform for drug delivery. Furthermore, carbon nanotubes provide very high aspect ratio and surface area for functionalization with small molecules or proteins-based drugs. Beyond drug delivery, carbon nanotubes is an important regenerative engineering nanomaterial that can be applied to diagnostics (cell tracking and sensing microenvironments), delivering transfection agents, and creating tissue structural

scaffolding with novel properties such as electrical conductivity that may aid in directing cell outcome (Harrison and Atala, 2007). However, concerns about the long-term biocompatibility of these nonbiodegradable carbon-based nanomaterials, related to oxidative stress, inflammation, and genetic damage, have not been fully addressed in a systematic manner (Kunzmann et al., 2011; Novoselov et al., 2012).

3. *Metal Based*: This type of drug delivery is through hollow metal nanoshell particles. Such group of nanoparticles can be particularly useful if they also carry distinctive magnetic properties and other tunable characteristics. Advances in fabrication techniques of metallic nanoparticles enable the attachment of various ligands or coatings to the generated nanoparticles. These ligands or coatings can serve many purposes, such as protective coatings to prevent degradation, barrier coatings to mitigate immune response, therapeutic drugs for drug delivery, and fluorophores for ease of imaging (Sun et al., 2008).
4. *Nanofiber Based*: Drug delivery can also be achieved from drug-loaded nanofibers prepared by electrospinning or self-assembly techniques (Sun et al., 2003; Hosseinkhani et al., 2006). The subsequent drug release from nanofiber-based drug delivery scaffolds can be tuned to obtain a linear release rate, ideal for therapeutic applications, by the use of nanofibers with core–shell structures with internally core-loaded drugs (Sun et al., 2003). The drug release kinetics can be further fine tuned by changing the porosity and thickness of the nanofiber shell.

1.3.1.4 Nanodiagnostics

Nanotechnology has provided alternative approaches such as magnetic nanoparticles (Riehemann et al., 2009), quantum dots (Dubertret et al., 2002), gold nanoparticles (Chanda et al., 2010), and carbon nanotubes (De La Zerda et al., 2008) to track cell fate in engineered scaffolds and monitor the progress of tissue formation in a noninvasive manner *in vivo*. Cell tracking *in vivo* is important; for example, in the delivery of stem cells through an engineered scaffold it is important to verify that the stem cells target the desired area and verify the therapeutic effects attributed to the stem cells.

By using internalized superparamagnetic iron oxide (SPIO) nanoparticles, researchers can track the commonly used mesenchymal stem cells (MSCs). The magnetic nanoparticles are then imaged using magnetic resonance imaging (MRI), providing information about the placement of individual cells. This is useful for determining migration of cells, penetration into a scaffold, and other vital outcomes in tissue-engineered solutions.

A second stem cell labeling method that utilizes nanotechnology is quantum dot labeling. Quantum dots are nanocrystals with tunable excitation and emission properties. Quantum dots are internalized in stem cells through peptides, such as RGD, TAT (oligo peptide glycine–arginine–lysine–lysine–arginine–arginine–glutamine–arginine–arginine–arginine–proline–glutamine), or cholera toxin (Chen et al., 2014). The use of quantum dots is advantageous because the optical imaging that is used for detection is both more widespread and less expensive than MRI imaging used for the SPIO nanoparticles (Engler et al., 2006).

Furthermore, nanotechnology applications in diagnostics can be combined with therapeutic drug delivery in a site-specific fashion; this has led to the emerging field of theranostics (Debbage and Jaschke, 2008). This technology enables the examination of the site of drug delivery to inform the commensurate and simultaneous release of drugs, and can

help in ensuring effective coordination of the various engineered regenerative steps in a well-orchestrated manner.

1.4 Concluding Remarks

Regenerative engineering aims at true recreation of biological tissues. Nanotechnology is the process of manipulating components at the nanoscale. The potential for the use of nanotechnology in regenerative engineering and related drug delivery and diagnostics applications is currently under extensive investigation. There are several exciting possibilities such as the combination of nanotechnologies with other technologies to potentiate outcomes that cannot be accomplished by any individual technology alone. For example, the incorporation of nanostructures into microfabricated engineered scaffolds could enable better control of cell function via cell–nanotopography interactions (Dvir et al., 2011).

Yet, the unique structural organization, biochemical composition, and viscoelastic characteristics of different tissue-types present nontrivial challenges for their regeneration using a "one-design-fits-all" approach. Furthermore, there will be hurdles to cross before clinical translation of these emerging complex scaffolds technologies; for example, long-term biocompatibility and biodegradation, industrialized fabrication and processing, sterilization procedures without affecting the nanomicrostructures and compromising the activity of the proteins therein, controlled and uniform cell seeding within these scaffolds and subsequent bioreactor processing, and the entire clinical logistics involved would need to be figured out on a case-by-case basis. The complexity of the task to accurately recapitulate the spatial and temporal components of the extracellular environment, from the micro- and nanoscale structure of ECM to the presentation of cell adhesion molecules, growth factors, and cytokines, is a major challenge faced by regenerative engineering.

As our understanding of the nanoscale structural, compositional, and mechanical rules of hierarchical organization of tissues and organs and the cell–material interface from the molecular to the macroscale advances, advancing nanotechnology will push the field of biomaterials toward the rational design and development of complex and smart materials that will interact with cells in unprecedented ways and be instructive in directing tissue regeneration. For example, nanotechnological tools for guiding cells in a controlled manner to desired locations within advanced engineered scaffolds will be useful for engineering complex multicellular tissues.

Many other interesting areas also stand to benefit from these development at the intersection of nanotechnology and regenerative engineering such as biomaterials- (Bae et al. 2014), organ-on-a-chip- (Huh et al., 2010), cell sheet- (Elloumi-Hannachi et al., 2010), and stem cell- (Liu et al., 2015) engineering. The cumulative achievement of these endeavors will impact not only regenerative medicine, but other fields in medicine and beyond as well.

References

Abraham, V.C., Krishnamurthi, V., Taylor, D.L., Lanni, F. 1999. The actin-based nanomachine at the leading edge of migrating cells. *Biophys J.* 77: 1721–1732.

Bae, H., Chu, H., Edalat, F. et al. 2014. Development of functional biomaterials with micro- and nanoscale technologies for tissue engineering and drug delivery applications. *J Tissue Eng Regen Med*. 8: 1–14.

Bettinger, C.J., Langer, R., Borenstein, J.T. 2009. Engineering substrate topography at the micro- and nanoscale to control cell function. *Angew Chem Int Ed Engl.* 48: 5406–5415.

Burdick, J.A., Anseth, K.S. 2002. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. *Biomaterials.* 23: 4315–4323.

Caldorera-Moore, M., Peppas, N.A. 2009. Micro- and nanotechnologies for intelligent and responsive biomaterial-based medical systems. *Adv Drug Deliv Rev.* 61: 1391–1401.

Chanda, N., Kattumuri, V., Shukla, R. et al. 2010. Bombesin functionalized gold nanoparticles show *in vitro* and *in vivo* cancer receptor specificity. *Proc Natl Acad Sci USA.* 107: 8760–8765.

Chen, G., Tian, F., Zhang, Y., Zhang, Y., Li, C., Wang, Q. 2014. Tracking of transplanted human mesenchymal stem cells in living mice using near-infrared Ag_2S quantum dots. *Adv Funct Mater.* 24: 2481–2488.

Dalby, M.J., Gadegaard, N., Tare, R. et al. 2007. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat Mater.* 6: 997–1003.

Debbage, P., Jaschke, W. 2008. Molecular imaging with nanoparticles: Giant roles for dwarf actors. *Histochem Cell Biol.* 130: 845–875.

De la Zerda, A., Zavaleta, C., Keren, S. et al. 2008. Carbon nanotubes as photoacoustic molecular imaging agents in living mice. *Nat Nanotechnol.* 3: 557–562.

Dlaska, C.E., Andersson, G., Brittberg, M., Suedkamp, N.P., Raschke, M.J., Schuetz, M.A. 2015. Clinical translation in tissue engineering—The surgeon's view. *Curr Mol Bio Rep.* 1: 61–70.

Dubertret, B., Skourides, P., Norris, D.J., Noireaux, V., Brivanlou, A.H., Libchaber, A. 2002. *In vivo* imaging of quantum dots encapsulated in phospholipid micelles. *Science.* 298: 1759–1762.

Dvir, T., Timko, B.P., Kohane, D.S., Langer, R. 2011. Nanotechnological strategies for engineering complex tissues. *Nat Nanotechnol.* 6: 13–22.

Edwards, S.L., Werkmeister, J.A., Ramshaw, J.A. 2009. Carbon nanotubes in scaffolds for tissue engineering. *Expert Rev Med Devices.* 6: 499–505.

Elloumi-Hannachi, I., Yamato, M., Okano, T. 2010. Cell sheet engineering: A unique nanotechnology for scaffold-free tissue reconstruction with clinical applications in regenerative medicine. *J Intern Med.* 267: 54–70.

Engler, A.J., Sen, S., Sweeney, H.L., Discher, D.E. 2006. Matrix elasticity directs stem cell lineage specification. *Cell.* 126: 677–689.

Freed, L.E., Langer, R., Martin, I., Pellis, N.R., Vunjak-Novakovic, G. 1997. Tissue engineering of cartilage in space. *Proc Natl Acad Sci USA.* 94: 13885–13890.

Goldberg, M., Langer, R., Jia, X. 2007. Nanostructured materials for applications in drug delivery and tissue engineering. *J Biomater Sci.* 8: 241–268.

Hammond, P.T. 2004. Form and function in multilayer assembly: New applications at the nanoscale. *Adv Mater.* 16: 1271–1293.

Harrison, B.S., Atala, A. 2007. Carbon nanotube applications for tissue engineering. *Biomaterials.* 28: 344–353.

Hosseinkhani, H., Hosseinkhani, M., Khademhosseini, A., Kobayashi, H., Tabata, Y. 2006. Enhanced angiogenesis through controlled release of basic fibroblast growth factor from peptide amphiphile for tissue regeneration. *Biomaterials.* 27: 5836–5844.

Huang, J., Grater, S.V., Corbellini, F. et al. 2009. Impact of order and disorder in RGD nanopatterns on cell adhesion. *Nano Lett.* 9: 1111–1116.

Hughes, G.A. 2005. Nanostructure-mediated drug delivery. *Nanomedicine.* 1: 22–30.

Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., Ingber, D.E. 2010. Reconstituting organ-level lung functions on a chip. *Science.* 328: 1662–1668.

Jiang, H., Fang, D., Hsiao, B., Chu, B., Chen, W. 2004. Preparation and characterization of ibuprofen-loaded poly (lactide-co-glycolide)/poly (ethylene glycol)-g-chitosan electrospun membranes. *J Biomater Sci.* 15: 279–296.

Kim, B.S., Mooney, D.J. 1998. Development of biocompatible synthetic extracellular matrices for tissue engineering. *Trends Biotechnol.* 16: 224–230.

Kim, D.H., Lipke, E.A., Kim, P. et al. 2010. Nanoscale cues regulate the structure and function of macroscopic cardiac tissue constructs. *Proc Natl Acad Sci.* 107: 565–570.

Koutsopoulos, S., Zhang, S. 2012. Two-layered injectable self-assembling peptide scaffold hydrogels for long-term sustained release of human antibodies. *J Control Release.* 160: 451–458.

Kumar, C.S.S.R. 2005. *Biofunctionalization of Nanomaterials*. Weinheim: Wiley-VCH.

Kunzmann, A., Andersson, B., Thurnherr, T., Krug, H., Scheynius, A., Fadeel, B. 2011. Toxicology of engineered nanomaterials: Focus on biocompatibility, biodistribution and biodegradation. *Biochim Biophys Acta.* 1810: 361–373.

Langer, R., Vacanti, J.P. 1993. Tissue engineering. *Science.* 260: 920–926.

Laurencin, C.T., Ambrosio, A.M., Borden, M.D., Cooper J.A. Jr. 1999. Tissue engineering: Orthopedic applications. *Ann Rev Biomed Eng.* 1: 19–46.

Laurencin, C.T., Khan, Y. 2013. *Regenerative Engineering.* Boca Raton: CRC Press.

Laurencin, C.T., Ko, F.K. 2004. Hybrid nanofibril matrices for use as tissue engineering devices. Google Patents. US 20030050711 A1.

Lee, K.Y., Mooney, D.J. 2001. Hydrogels for tissue engineering. *Chem Rev.* 101: 1869–1880.

L'Heureux, N., Pâquet, S., Labbé, R., Germain, L., Auger, F.A. 1998. A completely biological tissue-engineered human blood vessel. *FASEB J.* 12: 47–56.

Li, W.J., Laurencin, C.T., Caterson, E.J., Tuan, R.S., Ko, F.K. 2002. Electrospun nanofibrous structure: A novel scaffold for tissue engineering. *J Biomed Mater Res.* 60: 613–621.

Liu, H., Zhang, Z., Seong Toh, W., Ng, K.W., Sant, S., Salgado, A. 2015. Stem cells: Microenvironment, micro/nanotechnology, and application. *Stem Cells Int.* 2015: 398510.

Lutolf, M.P., Hubbell, J.A. 2005. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotech.* 23 (1): 47–55.

Ma, P.X. 2008. Biomimetic materials for tissue engineering. *Adv Drug Deliv Rev.* 60: 184–198.

Mann, B.K., Gobin, A.S., Tsai, A.T., Schmedlen, R.H., West, J.L. 2001. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: Synthetic ECM analogs for tissue engineering. *Biomaterials.* 22: 3045–3051.

Murugan, R., Ramakrishna, S. 2005. Development of nanocomposites for bone grafting. *Compos Sci Technol.* 65: 2385–2406.

Nalwa, H.S. 1999. *Handbook of Nanostructured Materials and Nanotechnology.* Vol. 3. Cambridge: Academic Press.

Nerem, R.M. 1991. Cellular engineering. *Ann Biomed Eng.* 19: 529–545.

Norman, J.J., Desai, T.A. 2006. Methods for fabrication of nanoscale topography for tissue engineering scaffolds. *Ann Biomed Eng.* 34: 89–101.

Novoselov, K.S., Fal'Ko, V.I., Colombo, L., Gellert, P.R., Schwab, M.G., Kim, K. 2012. A roadmap for grapheme. *Nature.* 490: 192–200.

Oberpenning, F., Meng, J., Yoo, J.J., Atala, A. 1999. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat Biotechnol.* 17: 149–155.

Oh, S., Brammer, K.S., Li, Y.S. et al. 2009. Stem cell fate dictated solely by altered nanotube dimension. *Proc Natl Acad Sci USA.* 106: 2130–2135.

Park, J., Bauer, S., von der Mark, K., Schmuki, P. 2007. Nanosize and vitality: TiO_2 nanotube diameter directs cell fate. *Nano Lett.* 7: 1686–1691.

Phelps, E.A., Landázuri, N., Thulé, P.M., Taylor, W.R., García, A.J. 2010. Bioartificial matrices for therapeutic vascularization. *Proc Natl Acad Sci USA.* 107: 3323–3328.

Ranzinger, J., Krippner-Heidenreich, A., Haraszti, T. et al. 2009. Nanoscale arrangement of apoptotic ligands reveals a demand for a minimal lateral distance for efficient death receptor activation. *Nano Lett.* 9: 4240–4245.

Ratner, B.D, Hoffman, A.S., Schoen, F.J., Lemons, J.E. 2013. *Introduction-Biomaterials Science: An Evolving, Multidisciplinary Endeavor*. Academic Press Elsevier Inc.: Waltham, MA, USA.

Reichert, W.M., Ratner, B.D., Anderson, J. et al. 2011. 2010 Panel on the biomaterials grand challenges. *J Biomed Mater Res A.* 96: 275–1287.

Richardson, T.P., Peters, M.C., Ennett, A.B., Mooney, D.J. 2001. Polymeric system for dual growth factor delivery. *Nat Biotechnol.* 19: 1029–1034.

Riehemann, K., Schneider, S.W., Luger, T.A., Godin, B., Ferrari, M., Fuchs, H. 2009. Nanomedicine—Challenge and perspectives. *Angew Chem Int Ed Engl.* 48: 872–897.

Sampson, S.L., Saraiva, L., Gustafsson, K., Jayasinghe, S.N., Robertson, B.D. 2014. Cell electrospinning: An *in vitro* and *in vivo* study. *Small.* 10: 78–82.

Sengupta, S., Eavarone, D., Capila, I. et al. 2005. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature.* 436: 568–572.

Shah, A., Brugnano, J., Sun, S., Vase, A., Orwin, E. 2008. The development of a tissue-engineered cornea: Biomaterials and culture methods. *Pediatr Res*. 63: 535–544.

Shi, J., Votruba, A.R., Farokhzad, O.C., Langer, R. 2010. Nanotechnology in drug delivery and tissue engineering: From discovery to applications. *Nano Lett.* 10: 3223–3230.

Skalak, R., Fox, C.F. (ed.) 1988. Tissue engineering. In *Proceedings of a workshop*, Granlibakken, Lake Tahoe, California.

Sobolev, K. 2015. Nanotechnology and nanoengineering of construction materials. In *Nanotechnology in Construction*. New York City: Springer, 3–13.

Sun, C., Lee, J.S., Zhang, M. 2008. Magnetic nanoparticles in MR imaging and drug delivery. *Adv Drug Deliv Rev.* 60: 1252–1265.

Sun, Z., Zussman, E., Yarin, A.L., Wendorff, J.H., Greiner, A. 2003. Compound core–shell polymer nanofibers by co-electrospinning. *Adv Mater.* 15: 1929–1932.

Supp, D.M., Boyce, S.T. 2005. Engineered skin substitutes: Practices and potentials. *Clin Dermatol.* 23: 403–412.

Taipale, J., Keski-Oja, J. 1997. Growth factors in the extracellular matrix. *FASEB J.* 11: 51–59.

Tang, Z., Wang, Y., Podsiadlo, P., Kotov, N.A. 2006. Biomedical applications of layer-by-layer assembly: From biomimetics to tissue engineering. *Adv Mater.* 18: 3203.

Thanou, M., Duncan, R. 2003. Polymer–protein and polymer–drug conjugates in cancer therapy. *Curr Opin Investig Drugs*. 4: 701–709.

Thesleff, T., Lehtimäki, K., Niskakangas, T. et al. 2011. Cranioplasty with adipose-derived stem cells and biomaterial: A novel method for cranial reconstruction. *Neurosurgery*. 68: 1535–1540.

U.S. Department of Health and Human Services, OPTN/SRTR. 2013. Annual data report: Introduction. 2015. *Am J Transplant*. 15: 8–10.

Veronese, F.M., Mero, A. 2008. The impact of PEGylation on biological therapies. *BioDrugs*. 22: 315–329.

Wade, R.J., Bassin, E.J., Gramlich, W.M., Burdick, J.A. 2015. Nanofibrous hydrogels with spatially patterned biochemical signals to control cell behavior. *Adv Mater.* 27: 1356–1362.

Woodrow, K.A., Cu, Y., Booth, C.J., Saucier-Sawyer, J.K., Wood, M.J., Saltzman, W.M. 2009. Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. *Nat Mater.* 8: 526–533.

Xu, C.Y., Inai, R., Kotaki, M., Ramakrishna, S. 2004. Aligned biodegradable nanofibrous structure: A potential scaffold for blood vessel engineering. *Biomaterials.* 25: 877–886.

Yim, E.K., Pang, S.W., Leong, K.W. 2007. Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Exp Cell Res.* 313: 1820–1829.

Zhang, S. 2003. Fabrication of novel biomaterials through molecular self-assembly. *Nat Biotechnol.* 21: 1171–1178.

Zhang, S., Uludağ, H. 2009. Nanoparticulate systems for growth factor delivery. *Pharm Res.* 26: 1561–1580.

Zhang, S., Zhao, X. 2004. Design of molecular biological materials using peptide motifs. *J Mater Chem.* 14: 2082–2086.

2

Nanofibers Design for Guided Cellular Behavior

Anuradha Subramanian and Swaminathan Sethuraman

CONTENTS

2.1 Introduction

Native tissue has hierarchically ordered dynamic nanostructured extracellular matrix (ECM) components that regulate cellular behavior such as polarity, adhesion, proliferation, migration, orientation, and differentiation (Kozel et al., 2006). ECM has been proposed to be an excellent cellular glue comprising a complex and dynamic network of fibrous proteins, majorly collagen and elastin in the viscous microenvironment of glycoaminoglycans, glycoproteins, proteoglycans, and several soluble growth factors. Fibrous collagen and elastin in the ECM provides tensile strength and extensibility to the tissues thereby enabling them to resist the plastic deformation and rupture, and endure mechanical loading. In addition to scaffolding, ECM also coordinates cellular function through physical and mechanical stimulus. This chapter explains the role of fibrous ECM components on the regulation of cellular fate in tissues to understand the rationale of nanofiber geometry on control of cell behavior and how the nanofiber–ECM analog helps to organize the cellular function and tissue progression for tissue engineering applications.

2.2 Fibrous ECM Components

Majority of the ECM components such as collagen, elastin, fibronectin, and laminin are fibrous in nature contributing to structural and adhesive support for tissue progression

(Muiznieks and Keeley, 2013). This section outlines the role of supramolecular assembly of ECM proteins toward the mechanical and biological properties of the tissues. Collagen is the chief fibrous protein present in the ECM with a broad range of functions such as structural support, adhesive function, cell migration, angiogenesis, tissue morphogenesis, and organogenesis (Gosline et al., 2002). Collagens are classified based on the function and domain homology, which includes fibril-forming collagen, fibril-associated collagen, network-forming collagen, transmembrane collagens, endostatin-producing collagen, anchoring fibrils, and beaded filament-forming collagen (Kadler et al., 2007). Of the various types of collagens, fibrillar collagen (type I, II, III, V, and XI) is the principal form assembled into collagen fibrils of 10–300 nm in diameter (Kadler et al., 2007). Fibril-associated collagens such as type IX and XII helps the collagen fibrils to link one another and also to other ECM components (Mayne and Burgeson, 1987). In network-forming collagens (type IV and VII), type VII tends to assemble into specialized structures called anchoring fibrils, which helps to attach the basal lamina of epithelial to connective tissue (Than et al., 2002). Mature basal laminae are mainly made up of type IV, organized into mesh-like sheets contributing to the barrier function and mechanical support for adjacent cells (Alberts et al., 1994). Establishment of covalent cross-links between the lysine residues of collagen molecules determines the tensile strength of fibrils (Kadler et al., 1996).

Elastic fibers are another class of fibrous proteins present in the ECM providing mechanical strength especially elasticity to the tissues. Tissues such as blood vessels, skin, lungs, and other dynamic connective tissues require resilience that helps to recoil at the end of the transient stretch (Sage, 1982). Elastic fibers comprise inner core cross-linked elastin surrounded by microfibrillar layer (Faury, 2001). Microfibrils consist of many proteins such as fibrillin, fibulin, and microfibril-associated glycoproteins and provides a structural and organizational support for the assembly of elastins (Midwood and Schwarzbauer, 2002). In addition, the stretching ability of elastin is mainly controlled by the tight association of collagen fibrils.

Fibronectin protein exists in fibrous form possessing binding site for collagen in its N-terminal end and two other binding sites in C-terminal region for both glycosaminoglycans and integrins (Tarone et al., 1982). This fibronectin fibrils offer elasticity and contribute to the major adhesive function of ECM apart from maintaining the hemostasis and tissue organization (Abu-Lail et al., 2006). Further, fibronectin binds to cell-surface integrins such as integrin $\alpha5\beta1$, $\alpha4\beta1$, and $\alpha v\beta3$, which are connected to the cytoskeleton and maintain cell phenotype through the organization of intracellular actin filaments (Singh and Schwarzbauer, 2012). Laminin is one of the ubiquitous ECM fibrous proteins present in the basement membrane assisting cell adhesion, migration, proliferation, and differentiation in many tissues. Cell-surface receptors such as integrins, dystroglycan, and syndecan promote the self-association of laminin into the independent polymeric fibrous networks (Neal et al., 2009).

Thus, the fibrous architecture of major ECM proteins demonstrate the scaffolding function by providing excellent mechanical strength at the tissue level. Apart from the presence of biological recognition motif, this geometry also promotes physical cell–ECM communication through integrin–actin networks, thereby controlling the cell fate based on the extracellular environment. Hence, biomaterial scaffolds with nanofiber geometry receives considerable attention in tissue engineering applications.

2.3 Nanofiber Geometry as ECM Analog

Cells can control growth and differentiation in response to external stimuli based on their ability to sense the nano- and microgeometries from the environment. Nanofiber

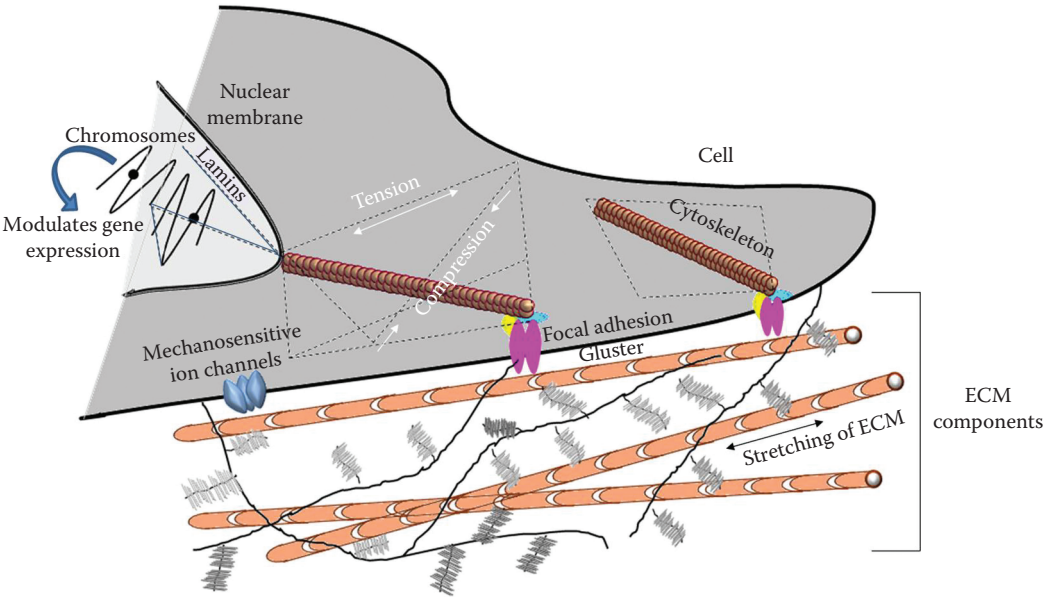

FIGURE 2.1
Major events in the focal adhesion-mediated signal transduction.

geometry mediates cell adhesion through the spatial distribution of focal contacts (Singhvi et al., 1994; Curtis and Wilkinson, 1997). This integrin-mediated cell–substrate adhesion has been found to control cell morphology, polarization, viability, proliferation, and differentiation through the regulation of intracellular signals named focal adhesion-mediated signal transduction (Figure 2.1). Factors such as fiber morphology, diameter, orientation, and spacing between the fibers play a vital role in the guidance of cellular responses (Wang and Nain, 2014). This section describes the influence of fibrous architecture toward cell fate.

2.4 Role of Nanofibers on the Guidance of Cell Behavior

2.4.1 Cell Polarity

Cell polarization is a key process in maintaining the specific cellular shapes and structures and in mediating the specialized cellular functions. Biomaterial topography has altered the polarization of embryonic hippocampal neurons, thereby supporting the axonal outgrowth. It was observed that the biomaterial surface features smaller than the soma had shown twofold increase in the polarization of neurons than on the smoother substrate (Gomez et al., 2007). Similarly, electrospun poly(lactic-*co*-glycolic acid) (PLGA) substrate improved the neuronal polarization up to 30%–50% than the casted films, thus, confirming the influence of subcellular features on neurons (Lee et al., 2010). Variation in polarization may be due to the topography-enabled reorganization of focal adhesion clusters, which may directly alter both cytoskeleton proteins as well as gene expressions. Dorsal root ganglia (DRG) neurons cultured on the Poly L-lactic acid (PLLA) fibers enhanced the neurite