INTRODUCTION TO TISSUE ENGINEERING

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INTRODUCTION TO TISSUE ENGINEERING

Applications and Challenges

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IEEE Engineering in Medicine
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IEEE Press Series in Biomedical Engineering
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IEEE PRESS

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Library of Congress Cataloging-in-Publication Data:

Birla, Ravi, author.
Introduction to tissue engineering : applications and challenges / Ravi Birla.
p. ; cm. – (IEEE Press series on biomedical engineering)
ISBN 978-1-118-62864-5 (hardback)
I. Title. II. Series: IEEE Press series in biomedical engineering.
[DNLM: 1. Tissue Engineering. QT 37]
R857.T55
610.28–dc23

2013035520

Printed in the United States of America

ISBN: 9781118628645

10 9 8 7 6 5 4 3 2 1

This book is dedicated to:
My parents, Mom and Dad,
My gorgeous and supporting wife, Swati, and
My precious kids Aditya and Pooja

CONTENTS

PREFACE

This book is designed to serve as a textbook for a one-semester tissue engineering class, offered at the senior-undergraduate or first-year graduate level. The first six chapters of the book are focused on covering fundamental principles of tissue engineering and include cell sourcing, biomaterial development, tissue fabrication technology, vascularization strategies, and bioreactors for tissue engineering. These topics are at the heart of tissue engineering. The latter Chapter 3 are focused on applications of tissue engineering, which include development of 3D artificial trachea, 3D artificial bladder, and 3D artificial liver tissue.

The contents of this book are modeled after classes I teach in the Department of Biomedical Engineering at the University of Houston. I teach several classes, one of which is an introductory class in tissue engineering: BIOE 5323—Introduction to Tissue Engineering. BIOE 5323 is designed to serve as an introduction to the field of tissue engineering and is taken by senior undergraduate and first-year graduate students. When I first started teaching BIOE 5323, I put together lecture notes to provide students with a foundation in tissue engineering. Over time, these lecture notes were converted into book chapters and eventually combined into a complete textbook.

The book is designed as a textbook for use in a classroom setting. It is designed as a first text in tissue engineering and as such, does not rely on any other prerequisite classes. The book is self-contained and covers fundamental principles that are necessary to understand tissue engineering. The book is well suited for a one-semester class designed for undergraduate students at the senior level or first-year graduate students.

There is a large question bank that has been included in the book. The questions have been designed to test students' understanding of the principles of tissue

engineering and their ability to apply these principles toward the fabrication of 3D artificial tissue. Therefore, all the questions are assay-based questions which require critical thinking; many of the questions are open-ended and can have multiple correct responses. These questions are designed to probe students and test their creativity in designing processes to fabricate 3D artificial tissue.

RAVI BIRLA

ACKNOWLEDGMENTS

I would like to begin by thanking my Department Chair and mentor, Dr. Metin Akay, for his role in the preparation of this manuscript. Dr. Akay has been instrumental in this project, and without his support and encouragement, this book would never have happened. Dr. Akay was involved in this project from conception to completion and has participated in every aspect of the manuscript. Dr. Akay suggested this project to me, identified the need for this book, and shared his vision for the manuscript. Dr. Akay was enthusiastically engaged in every aspect of this project, from reviewing the preliminary proposals to suggesting ways to improve manuscript content. In addition, Dr. Akay also provided the necessary connections with important people at Wiley-IEEE Publishers, which made it all possible. Dr. Akay envisioned this project, believed in my ability to successfully undertake this task, and provided support in every way imaginable; for this, I am deeply indebted.

I would like to thank my wife, Swati, for her support during the preparation of this manuscript. My ability to complete this project in a timely manner required numerous evenings and weekends that were dedicated toward the manuscript, taking time away from personal commitments. Swati was always supportive of this project, encouraged my work throughout, and to the best of my knowledge, did not mind my absence from family commitments—I am still a married man!

I would like to thank my parents for their support and encouragement during the preparation of this book. They have taken a keen interest in this project and have been engaged in the development of the manuscript. They have also been enthusiastically waiting for the publication of this manuscript, and their eagerness to see the completed manuscript served as motivation to complete this project in a timely manner.

I would like to acknowledge the participation of my kids, Aditya and Pooja, in this project. During the writing of this manuscript, Aditya was eight years old and Pooja was six; they were both aware that I was working on this project. Every so often, Aditya and Pooja would come to me and ask "*Dad, what chapter are you on?*" I was encouraged to see the participation of Aditya and Pooja on this project. I was also reminded by my kids that I was behind schedule and needed to spend more time to catch up.

I would like to thank several people for their work in creating the illustrations that have been used in this book. I would like to thank Betsy Salazar and Kristopher Hoffman for creating all the images that have been used throughout the book. Ms. Salazar and Mr. Hoffman have devoted many hours to creating these images and their efforts have enhanced the quality of the book. These illustrations provide a valuable tool for student learning and the work by Ms. Salazar and Mr. Hoffman will go a long way in achieving this objective. I would also like to thank Mohamed A. Mohamed for creating the cover art; the cover image accurately captures the essence of the book.

I would like to thank Ms. Kelley Murfin, with the University of Houston Writing Center, for her assistance in editing and proofreading the manuscript. The time invested by Ms. Murfin in editing the manuscript has ensured accuracy of the material.

LIST OF ABBREVIATIONS

LVAD Left ventricular assist device
NSF National Science Foundation
NIH National Institute of Health
PCR Polymerase chain reaction
MHC Myosin heavy chain
MIT Massachusetts Institute of Technology
2D Two-dimensional
3D Three-dimensional
NASA The National Aeronautics and Space Administration
SERCA Sarcoplasmic endoreticulum Ca-ATPase
VEGF Vascular endothelial growth factor
HPCs Hematopoietic progenitor cells
EPCs Endothelial progenitor cells
ECM Extracellular matrix
hES Cells Human embryonic stem cells
NE Nuclear envelope
NPC Nuclear pore complex
ONM Outer nuclear membrane
INM Inner nuclear membrane
NUPs Nucleoporins
RAN Ras-related nuclear protein

GTPase Guanosine triphosphatase
RAN.GTP Ras-related nuclear protein guanosine triphosphatase
rRNA Ribosomal RNA
mRNA Messenger RNA (mRNA)
tRNA Transfer RNA
ER Endoplasmic reticulum
GAGs Glysoaminoglycans
JAMs Junctional adhesion proteins
ZO Zonula occludens
MSCs Mesenchymal stem cells
iPS induced pluripotent stem cells
HSCS Hematopoietic stem cells
MTS Mechanical testing system
PLA Polylactic acid
HA Hydroxyapatite
MAC Membrane attack complex
PGA Polyglycolic acid
PMMA Polymethyl methacrylate
EGTA Ethylene glycol tetraacetic acid
EDTA Ethylenediaminetetraacetic acid
SDS Sodium dodecyl sulfate
PEO Poly(ethyleneoxide)
PVA Poly(vinyl alcohol)
PAA Poly(acrylicacid)
P(PF-co-EG) Poly(propylene furmarate-co-ethylene glycol)
SCID Severe combined immunodeficient
PPS Poly(propylene sulfide)
MMPs Matrix metalloproteinases
PDMS Polydimethylsiloxane
PIPAAm Poly (N-isopoplyacrylaminde)
CAD Computer-aided design
CAM Computer aided machining
SFF Solid freeform fabrication
RP Rapid prototyping
TAF Tumor angiogenesis factor
EC Endothelial cells
SMCs Smooth muscle cells
MCP-1 Monocyte chemoattractant protein-1

ICAM-1 Intercellular adhesion molecule-1
VCAM-1 Vascular cell adhesion molecule-1
vWF von Willibrand factor
ADSCs Adipose-derived stromal cells
PLAGA Poly(lactide-co-glycolide)
SAWs Surface acoustic waves
IDT Interdigital transducer
Mag-TE Magnetic force-based tissue engineering
MCLs Magnetite cationic liposomes
SACs Stretch-activated channels
VSMCs Vascular smooth muscle cells
ECs Endothelial cells
VASP Vasodilator-stimulated phosphoprotein
ROCK Rho-associated coiled-coil-containing *protein*
TRPs Transient receptor potential channels
PECAM-1 Platelet endothelial cell adhesion molecule-1
NO Nitric oxide
HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
VNS Vagus nerve stimulation
TENS Transcutaneous electrical nerve stimulation
NMES Neuromuscular electrical stimulation
FES Functional electrical stimulation
PPy Polypyrrole
PANI Polyaniline
NSCs Nerve stem cells
EB Embryoid bodies
CTS Congenital tracheal stenosis
LCTS Long segment CTS
MLB Microlaryngoscopy and bronchoscopy
IC Intermittent catheterization
WDs Wolffian ducts
CND Common nephric duct
SIS Small intestinal submucosa
BAMA Bladder acellular matrix allograft
ACM Acellular Matrix
BAMGs Bladder acellular matrix grafts
ALF Acute liver failure
OLT Orthotopic liver transplantation

LDLT Living donor liver transplantation
SLT Split-liver transplantation
OPTN Organ Procurement and Transplantation Network
SRTR Scientific Registry of Transplant Recipients
HCV Hepatitis C virus
HCC Hepatocellular carcinoma
HGF Hepatocyte growth factor
TGF-β1 Transforming growth factor-β1
ADE Anterior definite endodermal

IMPORTANT TERMINOLOGY AND CONCEPTS

- **TISSUE ENGINEERING**—*Tissue engineering is a multidisciplinary field bringing together experts from engineering, life sciences and medicine, utilizing the building blocks of cells, biomaterials and bioreactors for the development of 3D artificial tissue and organs which can be used to augment, repair and/or replace damaged and/or diseased tissue.*
- **CELL-MATRIX INTERACTIONS**—*When a cell sees any given ECM protein, the cell scans the protein molecule to identify specific binding sites for which it has integrins; for example, the integrin* $\alpha 5\beta 1$ *binds to the RGD site of the fibronectin molecule. Although the fibronectin molecule is large, there is only a sequence of three amino acids that are recognized by cells having the* $\alpha 5\beta 1$ *integrin; the binding of the* $\alpha 5\beta 1$ *integrin to the RGD site on the fibronectin molecule is referred to as a specific cell-matrix interaction.*
- **CELL-CELL INTERACTIONS**—*Cells communicate with other cells via cell-cell interactions, and these are critical in maintaining cell phenotype and tissue function. There are 4 types of cell signaling, known as endocrine, paracrine, autocrine, and contact-dependent signaling. In addition, cellular junctions provide various functions at the cell-cell; gap junctions are one example. The functional coupling of cells with other cells is known as cell-cell interaction.*
- **AUTOLOGOUS CELLS**—*Autologous cells are cells that have been isolated from a tissue biopsy of the person who will also be recipient of these cells; the donor and recipient for autologous cells is the same.*

- **ALLOGENEIC CELLS**—*Allogeneic cells are isolated from a donor and then transplanted into a recipient patient, with the donor and recipient being different people*.
- **CELL TRANSPLANTATION**—*Cell transplantation has been defined as the process by which cells are delivered to the site of injury in order to improve the functional performance of injured tissue. Whole blood transfusions, packed red cell transfusions, platelet transfusions, and bone marrow transplants are examples of cell therapy*.
- **STEM CELL TRANSPLANTATION**—*Stem cell transplantation is a specialized case of cell transplantation, in which the cells being delivered are stem cells. Use of embryonic stem cells, induced pluripotent stem cells, and adult stem cells fall under the classification of stem cell transplantation*.
- **CENTRAL DOGMA OF MOLECULAR BIOLOGY**—*The central dogma of molecular biology states that DNA is transcribed to RNA, which is then translated to proteins*.
- **CHARACTERISTICS OF STEM CELLS**—*Stem cells have three important characteristics that distinguish them from other cell types: self-renewal, unspecialized function, and differentiation potential*.
- **CELL POTENCY**—*Cell potency refers to the differentiation potential of stem cells*.
- **BIOMATERIALS**—*A biomaterial is any substance that simulates the extracellular matrix by functionally interacting with isolated cells to support fabrication and maturation of 3D artificial tissue*.
- **TENSILE PROPERTIES OF BIOMATERIALS**—*The tensile properties of a material are used very frequently in engineering design as an important criterion for material selection. The tensile properties of a material provide information about the strength of the material, its ability to withstand a particular load, and information about elastic properties. All of these properties are extremely important for material selection during tissue fabrication*.
- **BIOMATERIAL DEGRADATION**—*Biomaterial degradation refers to the gradual breakdown of a biomaterial mediated in a controlled manner to support the fabrication of 3D artificial tissue*
- **BIOMATERIAL BIOCOMPATIBILITY**—*The ability of 3D artificial tissue to be accepted by host defense mechanisms upon implantation, while maintaining functional capacity, is known as biocompatibility*.
- **BIOMIMETIC BIOMATERIALS**—*A two-part definition of biomimetic biomaterials has been provided in a recent article: 1) The development of biomaterials for tissue engineering applications has recently focused on the design of biomimetic materials that are able to interact with surrounding tissues by biomolecular recognition*, 2) *The design of biomimetic materials is an attempt to make the materials such that they are capable of eliciting specific cellular responses and directing new tissue formation mediated by specific*

interactions, which can be manipulated by altering design parameters instead of by non-specifically adsorbed ECM proteins.

- **CLASSIFICATION OF BIOMATERIALS**—*Biomaterials are frequently classified based on source (natural and synthetic), based on degradation (biodegradable and non-biodegradable), and based on interatomic bonding forces (metals, polymers, and ceramics).*
- **BIOMATERIAL PLATFORMS**—*There are four platforms that have been widely used for tissue engineering applications: polymeric scaffolds, biodegradable hydrogels, decellular matrices, and self-organization strategies.*
- **DECELLULARIZED MATICES**—*This strategy is based on the utilization of naturally occurring extracellular matrix as the scaffolding material for 3D tissue formation. Tissue specimens are obtained from cadaveric or xenogeneic sources, and cells are completely removed using one of several potential strategies. Removal of cellular components from tissue specimens is known as decellularization, and the material that is obtained after removal of the cells is known as an acellular scaffold.*
- **HYRDOGELS**—*The term hydrogel is composed of "hydro" (water) and "gel," and refers to aqueous (water-containing) gels. To be more precise, it refers to polymer networks that are insoluble in water; they swell to an equilibrium volume but retain their shapes.*
- **POLYMERS**—*Polymers can be viewed as molecules of a high molecular weight that are composed of repeating monomer units.*
- **SELF-ORGANIZATION STRATEGIES**—*Self-organization is prevalent in biological systems; it involves the physical interaction of molecules in a steady-state structure. In a broad sense, self-organization can be viewed as a process that occurs in the absence of any constraining conditions, thereby providing a greater degree of freedom and flexibility.*
- **SMART MATERIALS**—*The most recent generation of biomaterials has been designed to respond to changes in the cellular environment. These materials, known as smart materials, are receptive to changes in the physiological environment and are adaptive to changes in the degree of tissue maturation.*
- **TISSUE FABRICATION TECHNOLOGIES**—*Tissue fabrication technologies can be classified into six categories, which include scaffold-free methods, cell patterning techniques, scaffold-based methods, rapid prototyping technologies, printing technology, and "organ-on-a-chip" models.*
- **SELF-ORGANIZATION TECHNOLOGY**—*Self-organization technology is based on the fabrication of extracellular matrix by cells that then use the newly formed ECM to support artificial tissue fabrication. This technology is an example of a scaffold-free tissue fabrication process and does not require external or synthetic scaffolding; rather, scaffolding is produced by cells.*

- **CELL PRINTING**—*Bioprinting process used for 2D cell patterning by depositing bio-ink on the surface of biopaper.*
- **ORGAN PRINTING**—*Bioprinting process used for fabrication of 3D tissue by depositing bio-ink on the surface of biopaper.*
- **SOLID FREEFORM FABRICATION**—*Solid freeform fabrication refers to a group of technologies that build 3D scaffolds using a layer-by-layer approach. Collectively, these technologies are known as rapid prototyping methods.*
- **SOFT LITHOGRAPHY**—*Soft lithography is a microfabrication technology used to engineer microfluidic devices, particularly microvascular networks.*
- **CELL PATTERNING**—*The process by which the spatial placement of cells is controlled to create an organized pattern of cell monolayers or 3D tissue is known as cell patterning.*
- **VASCULOGENESIS**—*Vasculogenesis refers to initial events in vascular growth in which endothelial cell precursors (angioblasts) migrate to discrete locations, differentiate in situ, and assemble into solid endothelial cords, later forming a plexus with endothelial tubes.*
- **ANGIOGENESIS**—*Angiogenesis refers to the growth, expansion, and remodeling of primitive blood vessels formed during vasculogenesis to form a mature vascular network.*
- **ARTERIOGENESIS**—*Arteriogenesis is the process by which blood vessels increase in diameter to form muscular arteries and incorporate smooth muscle cells and vaso-contraction and vaso-relaxation properties.*
- **THERAPEUTIC ANGIOGENESIS**—*Therapeutic angiogenesis refers to the stimulation of angiogenesis for therapeutic purposes.*
- **BIOLOGICALLY REPLICATED VASCULARIZATION STRATEGIES**—*Biologically replicated processes are influenced by molecular biology, with the objective being the understanding of biological phenomena and defining controlled laboratory conditions to replicate these processes. These strategies are focused on defining in vitro conditions used to drive vasculogenesis, angiogenesis, and arteriogenesis.*
- **BIOLOGICALLY MEDIATED VASCULARIZATION STRATEGIES**—*The term "biologically mediated" refers to the notion that successful implementation of these strategies requires intervention and mediation from recipient of the implanted tissue. Mediation of the vascularization process is a result of implantation of cells or artificial tissue.*
- **BIOLOGICALLY INSPIRED VASCULARIZATION STRATEGIES**—*In this case, inspiration is drawn from biological process with an objective to replicate these processes using innovative in vitro strategies. The goal is not to replicate the biological process, but replicate functionality.*

- **IN VIVO VASCULARIZATION STRATEGIES**—*The concept of in vivo vascularization revolves around culturing bioengineered tissue within specialized chambers that can be implanted to support the formation of new blood vessels within 3D artificial tissue.*
- **BIOREACTORS**—*Bioreactors are devices used extensively in tissue engineering to enable the fabrication of artificial 3D tissue and support the growth, maturation, and development of artificial tissue during controlled in vitro culture.*
- **CLASSIFICATION OF BIOREACTORS**—*Bioreactors are used for cell culture, scaffold fabrication, scaffold cellularization, and bioreactors for stretch, perfusion, and electrical stimulation.*
- **DESIGN CONSIDERATIONS FOR BIOREACTORS**—*The process flow chart for bioreactor design consists of four steps: 1) definition of stimuli, 2) control of processing variables, 3) sensor technology, and 4) stimulation protocol.*
- **BIOREACTORS FOR CELL CULTURE**—*Isolation, culture, and expansion of mammalian cells is a critical prerequisite for tissue fabrication. Automated cell culture bioreactors are designed to undertake all functions of mammalian cell culture using robotic technology.*
- **BIOREACTORS FOR SCAFFOLD FABRICATION**—*Electrospinning is one example of bioreactors that have been used for scaffold fabrication. Electrospinning is a method fabricating individual fibers of a polymer that can be combined in different configurations to promote 3D scaffold fabrication.*
- **BIOREACTORS FOR SCAFFOLD CELLULARIZATION**—*Bioreactors have been developed to aid the cellularization process, and in this section we will discuss six cellularization methods: 1) direct cell injection, 2) cell entrapment using hydrogels, 3) perfusion seeding, 4) surface acoustic waves, 5) centrifugal force, and 6) magnetic nanoparticles.*
- **PERFUSION SYSTEMS**—*In the human body, the circulatory system acts as a distribution network for the delivery of nutrients to cells and tissues while at the same time removing waste products. Perfusion systems are capable of delivering continuous fluid flow to support the metabolic activity of cells and 3D artificial tissue during controlled in vitro culture.*
- **BIOREACTORS FOR STRETCH**—*Cells have biological force sensors, which respond to changes in the force environment, embedded within the cell membrane; these biological force sensors are known as stretch-activated channels (SACs). Bioreactors have been developed to deliver controlled stretch of cells/tissue for the cardiovascular system.*
- **BIOREACTORS FOR ELECTRICAL STIMULATION**—*During normal mammalian function, changes in voltage are used as a mechanism to maintain hemostasis and as a trigger to modulate cell and tissue level*

function. Bioreactors have been developed to deliver controlled electrical stimulation to support the development and maturation of 3D artificial tissue.

- **SMALL INTESTINAL SUBMUCOSA**—*Small intestinal submucosa (SIS) has been extensively used for bladder tissue engineering. SIS is obtained from the submucosal layer of a small intestine segment that has been harvested from porcine donors. During the preparation of SIS, a segment of the small intestine layer is harvested, commonly from pigs, and all layers of the tissue, with the exception of the submucosal layer, are removed mechanically. The submucosal layer is next subjected to a decellularization protocol to remove any cells and cellular components, leaving behind an intact ECM.*
- **POLY (LACTIC-CO-GLYCOLIC ACID) (PLGA)**—*PLGA has been used extensively as a biomaterial for tissue engineering along with many other medical applications. PLGA is a degradable copolymer of lactic acid and glycolic acid; it is often described in terms of the relative percentage of these two monomers. One of the main advantages of PLGA is the nontoxicity of its degradation products; PLGA undergoes hydrolysis, and the degradation products of this reaction are the monomers lactic acid and glycolic acid, both of which are easily metabolized by the body.*