

现代生物技术前沿

TISSUE ENGINEERING

(美) B. Ø. 帕尔森 著
S. N. 巴蒂亚

组织工程

(影印版)



科学出版社

www.sciencep.com



.....
TWO DEGREE

.....

组织工程

.....

.....

现代生物技术前沿

TISSUE ENGINEERING

(美) B. Ø. 帕尔森 著
S. N. 巴蒂亚

组织工程

(影印版)

科学出版社
北京

图字:01-2004-3035 号

内 容 简 介

本书是 Pearson Prentice Hall 出版社新近推出的生物工程原理与应用丛书之一,全面阐释了当前生物医学工程对重要生理组织的研究,涵盖心血管、内分泌、神经、视觉、听觉、消化和呼吸系统。主要分为四部分:定量细胞和组织生物学,包括组织块、组织动力学、形态发生、干细胞、细胞程序与调等等;细胞和组织分化,包括高通量生物学数据、细胞和组织特性、细胞和组织培养和基因转移等;工程学方法与设计,包括时间常数、缩放比例、细胞分离、生物材料成型与制作等;临床应用,包括常规方式、宿主适应和治疗性组织的生产等。

本书为读者提供了众多实用的定义、生理基础数据表格以及参考书目、索引,同时,还介绍了骨髓、骨骼肌和软骨等组织器官的组织工程。此外,还提出了组织工程研究中的一些难点和重点问题。通过阅读本书,读者会对组织工程及其细胞生物学基础有一个全面清晰的了解,也会获得更多创新的想法来进一步探索这一日益发展并且壮大的研究领域。

本书适合生物工程、生物材料、生物医学、生物化学、分子生物学以及医学等相关研究领域的高年级本科生、研究生以及教学科研人员参考使用。

English reprint edition copyright 2004 by PEARSON EDUCATION NORTH ASIA LIMITED and SCIENCE PRESS.

Original English language title: Tissue Engineering, by Bernhard Ø. Palsson and Sangeeta N. Bhatia, copyright 2004

All rights reserved

Published by arrangement with the original publisher, Pearson Education, Inc., publishing as Pearson Prentice Hall.

This edition is authorized for sale only in the People's Republic of China (excluding the Special Administrative Region of HongKong and Macau.)

本书封面贴有 Pearson Education 出版集团激光防伪标签,无标签者不得销售。

图书在版编目(CIP)数据

组织工程/(美)帕尔森(Palsson, B. Ø.)等著. —影印本. —北京:科学出版社,2004.6

(现代生物技术前沿)

ISBN 7-03-013407-9

I. 组… II. 帕… III. 人体组织学-生物技术-英文 IV. R329

中国版本图书馆 CIP 数据核字(2004)第 041647 号

责任编辑:莫结胜 / 责任印制:安春生 / 封面设计:王 浩 陈 敬

科学出版社 出版

北京东黄城根北街16号

邮政编码:100717

<http://www.sciencep.com>

西源印刷厂 印刷

科学出版社发行 各地新华书店经销

*

2004年6月第 一 版 开本:850×1168 1/16

2004年6月第一次印刷 印张:26 1/2

印数:1—3 000 字数:603 000

定价:55.00 元

(如有印装质量问题,我社负责调换〈新欣〉)

To
Bert Y.C. Fung
and
Edwin N. Lightfoot
For their inspiration and leadership

Preface

Tissue engineering holds the promise to repair or replace damaged organs. As a discipline, the field has evolved dramatically from its origins in the late 1980s. In particular, the rapid advances in stem cell biology have rekindled the enthusiasm to use cell-based approaches for the treatment of disease. For success in this area, we must learn to manipulate, produce, and deliver collections of cells as building blocks of tissues. Transplanted cells and tissue constructs are influenced by their microenvironment and can be manipulated to effectively interact with patients.

The underpinnings of tissue engineering are thus broad, and span a wide spectrum of scientific and engineering fundamentals. This list includes basic biological sciences (cell biology, physiology, embryology, and wound healing); engineering fundamentals (fluid dynamics, transport phenomena, materials science, mechanics, and chemical kinetics); many clinical aspects (surgery and transplantation, immunology, pathology, radiology, and medicine), and various relevant biotechnologies (cell culture, cell separation, and gene transfer). This long list of specialized knowledge makes it challenging to organize all the necessary background material for the student of tissue engineering in a clear and succinct manner.

In writing this book, we aimed to lay the foundation for students studying tissue engineering at both the undergraduate and the graduate level. We have attempted to provide a conceptual framework that includes exposure to all of the necessary background material. Thus, we cannot treat any particular subject in great detail but rather can provide the needed conceptual background in all areas. Instructors will find this text to be a useful framework since it is amenable to augmentation based on the instructors area of expertise and desired focus of a course in tissue engineering. The text is written primarily for senior bioengineering students or first-year graduate students and assumes a working knowledge of the engineering fundamentals. In this spirit, we have provided a series of engineering-style homework problems and solutions in order to allow students to work through the concepts presented. Nonetheless, we also hope that the text will be useful to traditional engineering students, material scientists, medical students, laypeople, and biologists.

We have chosen to present the material in four parts: quantitative cell and tissue biology, cell and tissue characterization, engineering methods and design, and clinical implementation. Throughout the text, we have emphasized relevant time and length scales of physicochemical processes in cell biology and medicine. Armed with these fundamentals, we seek to have students establish a conceptual framework within which to place further advances in the field. Many societal and technical challenges

still remain for the field to move forward, and we have highlighted these to the extent possible.

Writing a textbook like this one is a significant undertaking. There are many individuals and organizations to thank for helping us with various aspects of the writing process. The Whitaker Foundation generously provided financial support for this project through their Teaching Materials program. Their recognition that textbooks are required to build consensus and firmly establish a field will be an important part of their legacy. Two individuals were key in preparing this text. Marc Abrams tirelessly assisted in compiling all aspects of this text and prepared it using L^AT_EX, which made the production and publication an easier process. Salman Khetani made heroic efforts in reading all of the material in detail and offering perceptive criticism, suggestions, and input that significantly improved the quality of the text. He and Valerie Liu provided important feedback on their experience teaching from this text and help with preparing homework problems. Christophe Schilling, Ramprasad Ramakrishna, Jason Papin, Stephen Fong, and Timothy Allen also provided help with homework problems. Some individuals provided significant help with particular chapters; Nathan Price and Karl Francis with Chapter 6, Jason Papin with Chapter 7, and Markus Herrgard with Chapter 8. Michelle Williamson provided the original illustrations.

A number of individuals assisted in proofreading the text; including Tim Allen, Derren Barken, Markus Covert, Iman Famili, Steve Fong, Anu Raghunathan, Jennifer Reed, Sharon Wiback, Kanika Chawala, and Thuy Vo. Many of our colleagues have provided feedback, pointed us to important resources, or shared preprints and course notes. Thanks go especially to Francois Berthiaume, John Bischof, Christopher Chen, Tejal Desai, Jennifer Elisseeff, Robert Sah, Fred Schoen, Mehmet Toner, and Peter Zandstra.

Finally, we wish to thank our families for their support of this seemingly never-ending project. In particular, Sangeeta thanks her husband, Jagesh, for his insight and love and her Dad, Narain, for his encouragement and wisdom.

Bernhard Palsson has a number of people and institutions to thank. The Fulbright and Ib Henriksen Foundations provided him with fellowship support to spend a leave at the Technical University of Denmark in 1996. Jens Nielsen and John Villadsen acted as his hosts and encouraged him to prepare a series of lectures on tissue engineering during his stay in Denmark. (Those lectures nucleated this book.) The Hougén Visiting Professorship at the University of Wisconsin in 2000 provided another leave during which portions of this text were written. He is grateful for the support and understanding of his wife Mahshid and children Shireen and Sirus who helped with the preparation of the index of this book.

BERNHARD Ø. PALSSON
SANGEETA N. BHATIA

Contents

Preface xiii

1

Introduction 1

- 1.1 Cells as Therapeutic Agents 2
- 1.2 Illustrative Examples 4
 - 1.2.1 Cartilage and chondrocytes 5
 - 1.2.2 Liver 5
 - 1.2.3 Pancreas β -islet cells 7
 - 1.2.4 Skin 8
 - 1.2.5 Bone-marrow transplantation (BMT) 8
 - 1.2.6 Tissue engineer faces diverse challenges 11
- 1.3 Cell Numbers and Growth Rates 11
 - 1.3.1 Cell numbers *in vivo*: orders of magnitude 12
 - 1.3.2 What are clinically meaningful numbers of cells? 12
 - 1.3.3 What are the fundamental limitations to the production of primary cells? 12
 - 1.3.4 How rapidly do primary cells grow in culture? 14
 - 1.3.5 How are these cells currently produced? 15
 - 1.3.6 How are these cells preserved and harvested? 15
 - 1.3.7 How are cells best delivered? 15
- 1.4 Outline of Book 15
- 1.5 Summary 17
- 1.6 Further Reading 17

Part I Quantitative Cell and Tissue Biology

2

Tissue Organization 20

- 2.1 Tissue Components 20
 - 2.1.1 Extracellular matrix 20
 - 2.1.2 Cells 25
- 2.2 Tissue Types 27

- 2.2.1 Epithelial tissues 27
- 2.2.2 Connective tissue 28
- 2.2.3 Other tissue types 28
- 2.3 Functional Subunits 29
- 2.4 Problem Decomposition 30
- 2.5 Summary 33
- 2.6 Further Reading 33

3

Tissue Dynamics 34

- 3.1 Dynamic States of Tissues 35
- 3.2 Homeostasis in Highly Proliferative Tissues 35
 - 3.2.1 Bone marrow 35
 - 3.2.2 Villi in the small intestine 36
 - 3.2.3 Skin 37
- 3.3 Tissue Repair 38
 - 3.3.1 Sequence of events that underlie wound healing 38
 - 3.3.2 Engineering wound healing 40
 - 3.3.3 Fetal wound healing 42
- 3.4 Tissue Dynamics as Interacting Cellular-Fate Processes 43
- 3.5 Summary 44
- 3.6 Further Reading 45

4

Morphogenesis 46

- 4.1 Morphogenic Processes 46
 - 4.1.1 Induction 46
 - 4.1.2 Important mesenchymal–epithelial interaction 48
 - 4.1.3 Adult transdifferentiation 48
 - 4.1.4 Gastrulation 48
- 4.2 Morphogenic Dynamics 50
 - 4.2.1 Initiation of morphogenesis 51
 - 4.2.2 Spatio-temporal process 51
 - 4.2.3 Final state 52
 - 4.2.4 Some cellular processes involved in morphogenesis 52
- 4.3 Constraints on Morphogenesis 57
- 4.4 Summary 59
- 4.5 Further Reading 60

5

Stem Cells 61

- 5.1 Basic Concepts 61
 - 5.1.1 Stem-cell properties 61
 - 5.1.2 Telomeres and self-renewal 63
 - 5.1.3 Stem cells and tissue engineering 64
- 5.2 Examples of Stem-Cell Systems 65
 - 5.2.1 Mesenchymal stem cells (MSC) 65
 - 5.2.2 Liver stem cells 66
 - 5.2.3 Neuronal stem cells 67
 - 5.2.4 Embryonic stem cell: the mother of all cells 67
- 5.3 Dynamic Function of Stem-Cell Systems 69
 - 5.3.1 Conceptual models of stem-cell proliferative behavior 69
 - 5.3.2 Dynamic models of stem cell proliferative behavior 70
- 5.4 Summary 73
- 5.5 Further Reading 73

6

Cellular-Fate Processes 74

- 6.1 Cell Differentiation 74
 - 6.1.1 Differentiation as measured by changes in gene expression 74
 - 6.1.2 Differentiation as measured by changes in cell function 76
 - 6.1.3 Describing cell differentiation mathematically 79
- 6.2 Cell Migration 81
 - 6.2.1 Underlying biochemical process 81
 - 6.2.2 Describing cell migration mathematically 83
- 6.3 Cell Division 87
 - 6.3.1 Mitotic cell cycle 87
 - 6.3.2 Describing the cell cycle mathematically 89
- 6.4 Cell Death 93
 - 6.4.1 Biological description of apoptosis 93
 - 6.4.2 Describing apoptosis mathematically 95
- 6.5 Dynamics of Interacting Cellular-Fate Processes 96
 - 6.5.1 Effects of cell division on the differentiation process 96
 - 6.5.2 Dynamic interplay among differentiation, division, and apoptosis 99
- 6.6 Summary 103
- 6.7 Further Reading 104

7

Coordination of Cellular-Fate Processes 105

- 7.1 Soluble Signals 105
 - 7.1.1 Types of growth factors and chemokines 106
 - 7.1.2 Sending a paracrine signal 106
 - 7.1.3 Receiving a signal 109
 - 7.1.4 Processing a signal 111
 - 7.1.5 Integrated responses 112
 - 7.1.6 Soluble growth-factor receptors 113
 - 7.1.7 Malfunctions in soluble signaling 116
- 7.2 Cell–Extracellular Matrix Interactions 117
 - 7.2.1 Binding to the ECM 118
 - 7.2.2 Modifying the ECM 120
 - 7.2.3 Analyzing the rate of ECM modification 120
 - 7.2.4 Malfunctions in ECM signaling 122
- 7.3 Direct Cell–Cell Contact 124
 - 7.3.1 Cell junctions in tissues 124
 - 7.3.2 Malfunctions in direct cell–cell contact signaling 126
- 7.4 Response to Mechanical Stimuli 127
- 7.5 Interaction between Signaling Mechanisms 128
 - 7.5.1 Multiple-input/single-output model in fibroblast growth-factor-2 signaling 128
- 7.6 Summary 130
- 7.7 Further Reading 130

Part II Cell and Tissue Characterization

8

High-Throughput Biological Data 132

- 8.1 Basics of Molecular Biology 133
 - 8.1.1 DNA molecule 133
 - 8.1.2 Some historical milestones 134
- 8.2 Genomics 135
 - 8.2.1 Chain termination 135
 - 8.2.2 Automated sequencing 136
 - 8.2.3 Informatics challenge 138
 - 8.2.4 Sequence variation and individuality 139
 - 8.2.5 Sequence annotation 139
- 8.3 Transcriptomics 140
 - 8.3.1 Measuring how genomes are used 140

- 8.3.2 Microarray data analysis 142
- 8.3.3 Using gene-expression profiling 145
- 8.4 Proteomics 146
- 8.5 Metabolomics 148
- 8.6 Phenomics 150
- 8.7 Era of Systems Biology 151
- 8.8 Summary 152
- 8.9 Further Reading 153

9

Cell and Tissue Properties 154

- 9.1 Basic Tools 154
 - 9.1.1 Microscopy 154
 - 9.1.2 Detection of biochemical components 155
 - 9.1.3 *In vivo* imaging 156
- 9.2 Measurement of Cell Characteristics 159
 - 9.2.1 Cell morphology 159
 - 9.2.2 Cell number and viability 160
 - 9.2.3 Cell-fate processes 162
 - 9.2.4 Measuring cell motility 162
 - 9.2.5 Cell function 164
 - 9.2.6 Mechanical 165
- 9.3 Measurement of Tissue Characteristics 168
 - 9.3.1 General appearance 168
 - 9.3.2 Cellular component 168
 - 9.3.3 Extracellular matrix component 168
 - 9.3.4 Function 168
 - 9.3.5 Mechanical measurements 169
 - 9.3.6 Physical properties 170
- 9.4 Summary 170
- 9.5 Further Reading 171

10

Cell and Tissue Culture 172

- 10.1 Definition and History 172
- 10.2 Types of Tissue Culture 173
 - 10.2.1 Types of primary culture 173
 - 10.2.2 Cell lines 174
 - 10.2.3 Immortalized cell lines 175
 - 10.2.4 Variation in cell lines 176

- 10.3 Media 176
 - 10.3.1 Dissolved gases 176
 - 10.3.2 Advantages and disadvantages of serum 179
- 10.4 Culture Environment and Maintenance of Cells *In Vitro* 181
 - 10.4.1 Tissue-culture environment 181
 - 10.4.2 Kinetics of growth 183
- 10.5 Characterization of Cell Function in Tissue Culture 184
- 10.6 Cryopreservation 184
- 10.7 Contaminants 187
- 10.8 Summary 187
- 10.9 Further Reading 188

11

Gene Transfer 189

- 11.1 Gene Transfer for Gene Therapy 189
- 11.2 Gene-Transfer Methods 191
 - 11.2.1 Retrovirally mediated gene transfer 191
 - 11.2.2 Adenovirus-mediated gene transfer 193
 - 11.2.3 Nonviral methods 196
- 11.3 Retrovirally Mediated Gene-Delivery Process 198
 - 11.3.1 Finding the target cell 198
 - 11.3.2 Nonspecific virus binding to the target cell 201
 - 11.3.3 Specific virus binding to the target cell 203
 - 11.3.4 Viral entry 203
- 11.4 Gene Transfer for Modifying Cellular Functions 204
- 11.5 Summary 206
- 11.6 Further Reading 206

Part III Engineering Methods and Design

12

Time Constants 208

- 12.1 Definition of Time Constants 208
 - 12.1.1 General definitions 208
 - 12.1.2 Linear systems 209
- 12.2 Important Time Constants 211
 - 12.2.1 Diffusion 211
 - 12.2.2 Chemical reactions 214
 - 12.2.3 Fluid flow 215
 - 12.2.4 Biological time constants 216

- 12.3 Simplifying Dynamic Descriptions 217
 - 12.3.1 Basic concept 217
 - 12.3.2 Simultaneous diffusion and chemical reaction 218
- 12.4 Summary 221
- 12.5 Further Reading 222

13

Scaling up for *Ex Vivo* Cultivation 223

- 13.1 Using *in vivo* Conditions as a Guide 223
 - 13.1.1 Respiratory functions of blood 223
 - 13.1.2 Perfusion rates in human bone-marrow cultures 225
 - 13.1.3 Nutrient transport in liver reactions 226
- 13.2 Key Design Challenges 226
 - 13.2.1 Delivering oxygen 226
 - 13.2.2 Delivering and removing growth factors 229
 - 13.2.3 Delivering nutrients and removing waste products 231
- 13.3 Fluid Flow 232
 - 13.3.1 Uniformity 232
 - 13.3.2 Residence-time distributions 234
- 13.4 Cellularity 235
- 13.5 Geometry of the Microenvironment 238
- 13.6 Multivariable Optimization 239
 - 13.6.1 Controllable cell-culture variables 239
 - 13.6.2 Biological differences among individuals 241
- 13.7 Summary 241
- 13.8 Further Reading 243

14

Cell Separation 244

- 14.1 Basis for Cell Separation 244
 - 14.1.1 Physical properties 244
 - 14.1.2 Biochemical properties 245
- 14.2 Characterizing Cell Separation 245
- 14.3 Practiced Cell-Separation Methods 246
 - 14.3.1 Treating populations of cells 246
 - 14.3.2 Treating cells individually 249
- 14.4 Summary 250
- 14.5 Further Reading 251

15

Biomaterial Scaffolds 252

- 15.1 Biomaterial Properties 252
 - 15.1.1 Surface properties 252
 - 15.1.2 Bulk properties 256
 - 15.1.3 Mechanical properties 256
 - 15.1.4 Biological properties 258
- 15.2 Types of Biomaterials 261
 - 15.2.1 Biologic materials 261
 - 15.2.2 Synthetic materials 262
- 15.3 Summary 269
- 15.4 Further Reading 269

16

Tailoring Biomaterials 270

- 16.1 Tailoring Surface Chemistry and Topography 270
- 16.2 Subcellular Length Scale ($< 10 \mu\text{m}$) 272
 - 16.2.1 Surface chemistry 272
 - 16.2.2 Bulk chemistry 274
 - 16.2.3 Surface topography 275
- 16.3 Cellular Scale ($10\text{--}100 \mu\text{m}$) 277
 - 16.3.1 Surface chemistry 278
 - 16.3.2 Topography 280
- 16.4 Supracellular Scale ($100 \mu\text{m}\text{--}1 \text{cm}$) 285
 - 16.4.1 Influence of chemistry 285
 - 16.4.2 Influence of architecture 285
 - 16.4.3 Supracellular scale processing 285
- 16.5 Functions of Tailored Biomaterials 286
- 16.6 Summary 286
- 16.7 Further Reading 287

Part IV Clinical Implementation

17

Conventional Clinical Approaches to Tissue Dysfunction 290

- 17.1 Medical Therapies for Tissue Dysfunction 290
- 17.2 Surgical Therapies for Tissue Dysfunction 291
 - 17.2.1 Repair 291
 - 17.2.2 Replacement 292

- 17.2.3 Reconstruction from an alternative tissue type 295
- 17.2.4 Removal 296
- 17.3 Temporary Support Using Extracorporeal Devices 297
- 17.4 Tissue-Engineered Therapies 297
 - 17.4.1 Mesodermal tissue case study: articular cartilage 297
 - 17.4.2 Ectodermal tissue case study: skin 299
 - 17.4.3 Endodermal tissue case study: liver 301
- 17.5 Summary 302
- 17.6 Further Reading 302

18

Host Integration: Interacting Cell-Fate Processes 303

- 18.1 Wound-Healing Response 303
 - 18.1.1 Hemostasis (seconds to minutes) 304
 - 18.1.2 Inflammation (minutes to days) 304
 - 18.1.3 Proliferative phase (days to weeks) 304
 - 18.1.4 Remodeling phase (weeks to year) 305
- 18.2 Angiogenesis 305
 - 18.2.1 Basic process 306
 - 18.2.2 Modifying angiogenesis 307
- 18.3 Immune Response 308
 - 18.3.1 Basics 308
 - 18.3.2 Characteristic numbers 310
 - 18.3.3 Mechanisms of graft rejection 312
 - 18.3.4 Immune response in tissue engineering 314
 - 18.3.5 Strategies for modifying the immune response 315
- 18.4 Summary 317
- 18.5 Further Reading 319

19

Producing Tissue-Engineered Therapies 320

- 19.1 Product Characterization 320
 - 19.1.1 Components 320
 - 19.1.2 Safety 321
 - 19.1.3 Efficacy 324
- 19.2 Preservation 325
 - 19.2.1 Freezing 325
 - 19.2.2 Drying 328
- 19.3 Patent Protection 330
- 19.4 Regulation of Tissue-Engineered Products 331

19.5	Ethical Issues	333
19.6	Summary	334
19.7	Further Reading	334

A

Tissue-Engineering Study Problems 335

A.1	Part I: Quantitative Cell and Tissue Biology	335
A.2	Part II: Cell and Tissue Characterization	358
A.3	Part III: Engineering Methods and Design	362
A.4	Part IV: Clinical Implementation	371

References	375
------------	-----

Index	397
-------	-----