## CONTENTS

Contributors xix

Part I. Nanostructure Fabrication 1

1 Nanofabrication Techniques 3
   *Joseph W. Freeman, Lee D. Wright, Cato T. Laurencin, and Subhabrata Bhattacharyya*

   1.1 Introduction 3
   1.2 Photolithography 4
      1.2.1 Cleaning of the Substrate 4
      1.2.2 Application of the Photoresist Material 5
      1.2.3 Soft Baking 5
      1.2.4 Exposure 6
         1.2.4.1 Contact Printing 6
         1.2.4.2 Proximity Printing 6
         1.2.4.3 Projection Printing 6
      1.2.5 Developing 6
      1.2.6 Hard Baking 6
      1.2.7 Limitations of Photolithography 7
   1.3 Specialized Lithography Techniques 7
      1.3.1 Electron Beam Lithography 7
      1.3.2 Nanosphere Lithography 8
      1.3.3 Soft Lithography 8
      1.3.4 Dip Pen Lithography 8
      1.3.5 LIGA 10
         1.3.5.1 Deep X-Ray Lithography 10
         1.3.5.2 Electroplating 11
         1.3.5.3 Molding 11
   1.4 Thin Film Deposition 11
   1.5 Electrospinning 13
   1.6 Nanospheres 15
   1.7 Carbon Nanotubes 16
      1.7.1 Electric Arc Discharge 17
1.7.2 Laser Ablation 17
1.7.3 Chemical Vapor Deposition 17
1.7.4 Photolytic Laser-Assisted Chemical Vapor Deposition 18
1.7.5 Pyrolytic Laser-Assisted Chemical Vapor Deposition 19
1.7.6 Substrate-Site-Selective Growth 19
1.8 Self-Assembled Nanostructures 20
1.9 Conclusions 21
References 22

2 Micro/Nanomachining and Fabrication of Materials for Biomedical Applications 25
Wei He, Kenneth E. Gonsalves, and Craig R. Halberstadt

2.1 Introduction 25
2.2 Overview of Ion Implantation Process 26
2.3 Micro/Nanomachining of “Soft” Polymeric Biomaterials 27
  2.3.1 Orthopedic Applications 27
  2.3.2 Blood-Contacting Devices 32
  2.3.3 Other Applications 33
2.4 Micro/Nanomachining of “Hard” Metallic Biomaterials 35
  2.4.1 Orthopedic Applications 36
  2.4.2 Dental Implants 38
  2.4.3 Blood-Contacting Devices 38
  2.4.4 Other Applications 39
2.5 Novel Biocompatible Photoresists 39
2.6 Three-Dimensional Lithography 41
2.7 Conclusions 41
References 42

3 Novel Nanostructures as Molecular Nanomotors 49
Yan Chen, Jianwei Jeff Li, Zehui Charles Cao, and Weihong Tan

3.1 Introduction 49
3.2 Multi-DNA Nanomotors 51
3.3 Single DNA Nanomotors 55
3.4 Conclusions 59
References 60

4 Bioconjugation of Soft Nanomaterials 61
Neetu Singh, William H. Blackburn, and Andrew Lyon

4.1 Introduction 61
  4.1.1 Definition of Hydrogels 62
  4.1.2 Classification of Hydrogels 62
4.1.3 Stimuli-Sensitive Polymers 62
4.1.4 Microgels and Nanogels 64
4.2 Core/Shell Structured Materials 66
4.2.1 Block Copolymer Micelles 68
4.3 Bioconjugated Hydrogel Particles in Nanotechnology 70
4.3.1 Drug/Gene Delivery 70
4.3.2 Analytical Applications 77
4.3.3 Biomaterials 80
4.4 Conclusions 83
References 84

5 Nanotechnology and Drug Delivery 93
Xiaojun Yu, Chandra M. Valmikinathan, Amanda Rogers, and Junping Wang

5.1 Introduction 93
5.2 Advantages of Nanostructured Delivery Systems 94
5.2.1 Localized and Targeted Delivery 95
5.2.2 Controlled Delivery 95
5.2.3 Enhanced Circulation Time and Biodistribution 95
5.2.4 Drug Solubility 95
5.2.5 Intracellular Drug Delivery 96
5.2.6 Ability to Cross Biological Membranes 96
5.2.7 Enhanced Surface Areas 96
5.3 Activation and Targeting of Nanotechnology-Based Drug Delivery Systems (Externally and Internally) 97
5.3.1 Activation and Targeting through PhysicoChemical Stimuli 97
5.3.1.1 pH-Sensitive Carriers 97
5.3.1.2 Thermally Responsive Carriers 99
5.3.1.3 Photochemically Controlled Delivery System 100
5.3.1.4 Magnetic Targeted Drug Delivery of Nanocarriers 101
5.3.1.5 Ultrasound-Mediated Drug Delivery and Targeting 101
5.3.2 Drug Targeting through Targeting Molecules 101
5.3.2.1 Monoclonal Antibodies 101
5.3.2.2 Folate Ligands 102
5.3.2.3 Transferrin Ligands 102
5.3.2.4 Aptamers 103
5.3.2.5 Lectins 103
5.3.2.6 Synthetically Modified and Designed Peptide Ligands 104
5.3.2.7 Other Targeting Ligands 104
5.4 Multifunctional Nanoparticle Systems 105
5.4.1 Multivalent Strategies 105
5.4.1.1 Dendrimers 105
5.4.1.2 Polymeric Nanocarriers 105
5.4.1.3 Carbone Nanotubes (CNT) 107
5.4.2 Exploiting Inherent Material Properties 107
5.4.2.1 Electrical Properties 107
5.4.2.2 Optical Properties 108
5.4.2.3 Magnetic Properties 108
5.4.2.4 Thermal Properties 108
5.4.2.5 Structural Properties 108
5.4.2.6 Polymeric Micelles as Nanoreactors 109
5.5 Conclusions 109
References 109

6 Polymeric Nanoparticles and Nanopore Membranes for Controlled Drug and Gene Delivery 115
Jingjiao Guan, Hongyan He, Bo Yu, and L. James Lee

6.1 Introduction 115
6.2 Nanoparticles for Drug/Gene Delivery 116
  6.2.1 Why is Size Important for NPs in Drug/Gene Delivery? 116
    6.2.1.1 Drug/Gene Protection 117
    6.2.1.2 Delivery of Poorly Soluble Drugs 117
    6.2.1.3 Sustained Release 117
    6.2.1.4 Extended Blood Circulation 117
    6.2.1.5 Targeted Delivery 118
    6.2.1.6 Enhanced Cellular Uptake 118
    6.2.1.7 Barrier Penetration 118
  6.2.2 NPs prepared from Water-Insoluble Polymers 119
    6.2.2.1 NPs Prepared by Precipitation of Polymers 119
    6.2.2.2 NPs Prepared by Polymerization of Monomers 120
  6.2.3 NPs Prepared from Water-Soluble Polymers 121
    6.2.3.1 NPs Prepared by Cross-Linking of Polymers 121
    6.2.3.2 NPs Prepared by Self-Assembling of Block Copolymers 124
    6.2.3.3 NPs Prepared by Polymerization of Monomers 125
  6.3 Nanopore Membranes for Drug Delivery 125
    6.3.1 Overview of Nanopore-Based Devices for Sustained Drug Delivery 125
  6.3.2 Polymeric Nanopore Membranes for Drug Delivery 126
  6.4 Electrospun Polymeric Nanofibers (EPNFs) for Drug Delivery 129
  6.5 Conclusions 130
References 131
7 Development of Nanostructures for Drug Delivery Applications 139
Nikhil Dube, Joydeep Dutta, and Dhrendra S. Katti

7.1 Introduction 139

7.2 Nanosystems for Drug Delivery 141

7.3 Polymeric Nanoparticles 142
  7.3.1 Synthesis 142
    7.3.1.1 Structure and Property 143
    7.3.1.2 Applications of Nanoparticles for Drug Delivery 143

7.4 Nanofibers 145
  7.4.1 Fabrication 146
    7.4.1.1 Electrospinning 146
    7.4.1.2 Applications of Nanofibers 147

7.5 Dendrimers 152
  7.5.1 Properties of Dendrimers 153
  7.5.2 Applications of Dendrimers in Drug Delivery 154

7.6 Liposomes and Lipid Nanoparticles 157
  7.6.1 Synthesis of Liposomes and Lipid Nanoparticles 158
    7.6.1.1 High Pressure Homogenization (HPH) 158
    7.6.1.2 Microemulsion Method 159
    7.6.1.3 High Speed Stirring and/or Ultrasonication 159
  7.6.2 Drug Delivery Applications of Liposomes 159
  7.6.3 Drug Delivery Applications of Lipid Nanoparticles 161

7.7 Nanotubes and Fullerenes 162
  7.7.1 Synthesis 162
    7.7.1.1 Chemical Vapor Deposition (CVD) 162
    7.7.1.2 Electric Arc Discharge 163
    7.7.1.3 Laser Ablation 163
  7.7.2 Purification of Carbon Nanotubes 163
  7.7.3 Toxicity of Carbon Nanotubes 164
  7.7.4 Functionalization of Carbon Nanotubes 164
  7.7.5 Biomedical Applications of Carbon Nanotubes 165
    7.7.5.1 Drug Delivery by Carbon Nanotubes 166
    7.7.5.2 Nucleic Acid Delivery by Carbon Nanotubes 166
    7.7.5.3 Protein Delivery by Carbon Nanotubes 167
    7.7.5.4 Vaccine and Peptide Delivery by Carbon Nanotubes 168
  7.7.6 Biomedical Applications of Fullerenes 168

7.8 Nanogels 170
  7.8.1 Synthesis of Nanogels 170
    7.8.1.1 Emulsion Polymerization 171
    7.8.1.2 Cross-Linking Reaction of Preformed Polymer Fragments 171
7.8.2 Nanogels for Drug Delivery 171
7.9 Viral Vectors and Virus-Like Particles (VLPs) 174
  7.9.1 Recombinant Virus Vectors 175
    7.9.1.1 Adenovirus Vectors 175
    7.9.1.2 Retrovirus Vectors 175
    7.9.1.3 Adeno Associated Virus Vectors 175
  7.9.2 Applications of Recombinant Virus Vectors 176
  7.9.3 Virus-Like Particles 177
    7.9.4 Applications of Virus-Like Particles 178
      7.9.4.1 Papillomavirus-Like Particles 178
      7.9.4.2 Polyomavirus-Like Particles 178
  7.10 Nanocrystal Technology 179
    7.10.1 Approaches for the Production of Drug Nanocrystals 180
    7.10.2 Preparation of Tablets from Nanosuspensions of Poorly Soluble Drugs 182
  7.11 Conclusions 182
  References 183

8 Bioconjugated Nanoparticles for Ultrasensitive Detection of Molecular Biomarkers and Infectious Agents 207
Amit Agrawal, May Dongmei Wang, and Shuming Nie
8.1 Introduction 207
8.2 Novel Properties of Nanoparticles 208
8.3 Single-Molecule Detection 210
  8.3.1 Instrumental Setup and Principles 210
  8.3.2 Color-Coded Nanoparticles 213
  8.3.3 Single-Molecule Imaging 215
8.4 Applications 216
  8.4.1 Detection of Single Respiratory Syncytial Virus Particles 216
  8.4.2 Single-Molecule Detection by Two-Color Imaging 218
8.5 Conclusion and Outlook 219
  References 220

Part II. Bio-Nano Interfaces 223

9 ECM Interactions with Cells from the Macro- to Nanoscale 225
Steve Mwenifumbo and Molly M. Stevens
9.1 Introduction 225
9.2 Cell Microenvironment 226
  9.2.1 ECM Compositional Diversity 226
9.2.1.1 Constituent Macromolecules 226
9.2.1.2 Developmental Diversity 227
9.2.1.3 Tissue-Specific Diversity 227
9.2.2 Nanoscaled Structures of the ECM 228
  9.2.2.1 Proteins—Collagens and Elastins 229
  9.2.2.2 GAGs 235
  9.2.2.3 Proteoglycans 236
  9.2.2.4 Glycoproteins 236
9.2.3 Putting It All Together—Hierarchical Assembly 239
9.3 Cell—ECM Interactions—The Multidimensional Map 241
  9.3.1 Signaling Gradients 242
  9.3.2 Soluble Factors 243
  9.3.3 Growth Factors 244
  9.3.4 ECM Components
    9.3.4.1 Binding Domains 245
    9.3.4.2 Cryptic Sites 246
    9.3.4.3 Underlying Surface Chemistry 247
    9.3.4.4 Topography 247
  9.3.5 Environmental Stresses—Mechanical Stresses 248
  9.3.6 Cell Surface Receptors
    9.3.6.1 Integrins 249
    9.3.6.2 Cell Adhesion—Adhesion Complexes 249
    9.3.6.3 Integrin Signaling 249
  9.3.7 Guided Activities of Cells—ECM Remodeling
    9.3.7.1 ECM Remodeling 250
9.4 Conclusions 251
Acknowledgments 252
References 252

10 Cell Behavior Toward Nanostructured Surfaces 261

Sangamesh G. Kumbar, Michelle D. Kofron, Lakshmi S. Nair, and Cato T. Laurencin

10.1 Introduction 261
10.2 Nanotopographic Surfaces: Fabrication Techniques 264
  10.2.1 Cell Behavior Toward Nanotopographic Surfaces
    Created by Electron Beam Lithography 270
  10.2.2 Cell Behavior Toward Nanotopographic Surfaces
    Created by Photolithography 271
  10.2.3 Cell Behavior Toward Nanotopographic Surfaces
    Composed of Aligned Nanofibers by Electrospinning 272
  10.2.4 Cell Behavior Toward Nanotopographic Surfaces
    Created by Nanoimprinting 274
10.2.5 Cell Behavior Toward Nanotopographic Surfaces Created by Self-Assembly 276
10.2.6 Cell Behavior Toward Nanotopographic Surfaces Created by Phase Separation 277
10.2.7 Cell Behavior Toward Nanotopographic Surfaces Created by Colloidal Lithography 278
10.2.8 Cell Behavior Toward Nanotopographic Surfaces Composed of Random Nanofibers Created by Electrospinning 279
10.2.9 Cell Behavior Toward Nanotopographic Surfaces Created by Chemical Etching 280
10.2.10 Cell Behavior Toward Nanotopographic Surfaces Created by Incorporating Carbon Nanotubes/Nanofibers 282
10.2.11 Cell Behavior Toward Nanotopographic Surfaces Created by Polymer Demixing 283

10.3 Conclusions 287
References 287

11 Cellular Behavior on Basement Membrane Inspired Topographically Patterned Synthetic Matrices 297
Joshua Z. Gasiorowski, John D. Foley, Paul Russell, Sara J. Liliensiek, Paul F. Nealey, and Christopher J. Murphy

11.1 Introduction 297
11.2 Basement Membrane 298
  11.2.1 Significance of Basement Membranes in Disease 298
  11.2.2 Biochemical Attributes 299
  11.2.3 Physical Characteristics: Compliance 300
    11.2.3.1 Physical Characteristics: Topography 301
11.3 History of Biomimetic Synthetic Matrices 303
  11.3.1 Matrigel and Randomly Ordered Arrays 304
  11.3.2 Nanogroove Synthesis 305
11.4 Cell Behavior on Manufactured Nanogroove Surfaces 307
  11.4.1 Nanoscale Topography Affects Cell Proliferation 307
  11.4.2 Cellular Adhesive Strength on Nanogrooved Surfaces 308
  11.4.3 Cellular Migration Rates on Nanogroove Surfaces 309
  11.4.4 Focal Adhesion Structure and Orientation are Dictated by Nanogroove Dimensions 309
11.5 Cell Signaling and Expression on Topographical Surfaces 311
  11.5.1 Cell Morphology Changes Induced by Topography May Influence Gene Expression 311
  11.5.2 Macrophages Are Stimulated by Nanoscale Topography 312
11.5.3 Osteoblast Expression on Nanoscale Surfaces
11.5.4 The Addition of Soluble Factors Can Change Cellular Behavior on Nanogrooves

11.6 Conclusions
References

12 Focal Adhesions: Self-Assembling Nanoscale Mechanochemical Machines that Control Cell Function
Tanmay Lele and Donald E. Ingber

12.1 Introduction
12.2 Solid-State Biochemistry in Focal Adhesions
12.3 Focal Adhesion as a Mechanotransduction Machine
12.4 Mechanical Control of Molecular Binding Interactions in Focal Adhesions
12.5 The Focal Adhesion as a Multifunctional Biomaterial
12.6 Conclusions
References

13 Controlling Cell Behavior via DNA and RNA Transfections
Jaspreet K. Vasir and Vinod Labhasetwar

13.1 Introduction
13.2 Methods of DNA/RNA Transfection
13.3 Barriers to Transfection
13.4 DNA Transfection
13.4.1 Gene Therapy
13.4.2 Tissue Engineering
13.4.3 Functional Genomics
13.5 RNA Transfection
13.5.1 As a Tool to Understand Gene Function
13.5.2 As a Therapeutic
13.5.3 RNA Transfection—Delivering siRNA Inside Cells
13.5.3.1 In vitro
13.5.3.2 In vivo
13.5.4 Issues
13.5.4.1 Specificity
13.5.4.2 Resistance
13.5.4.3 Stability
13.6 Conclusions
Acknowledgments
References
14 Multiscale Coculture Models for Orthopedic Interface Tissue Engineering

Helen H. Lu and I-Ning E. Wang

14.1 Introduction 357
14.2 Cellular Interactions and the Soft Tissue-to-Bone Interface 358
14.3 Types of Coculture Models 359
  14.3.1 Coculture System with Cell–Cell Contact 359
     14.3.1.1 Mixed Coculture 359
     14.3.1.2 Temporary Dividers 360
  14.3.2 Coculture System Without Cell–Cell Contact 360
     14.3.2.1 Segregated Coculture 360
     14.3.2.2 Porous Membrane Inserts 361
     14.3.2.3 Conditioned Media Studies 361
14.4 Coculture Models for Orthopedic Interface Tissue Engineering 362
  14.4.1 Coculture Models of Osteoblasts and Fibroblasts 362
  14.4.2 Coculture Models of Osteoblasts and Chondrocytes 363
  14.4.3 Coculture and Triculture Models of Osteoblasts, Chondrocytes, and Fibroblasts 364
14.5 Macro- and Microscale Coculture 364
14.6 Two-Dimensional (2D) and Three-Dimensional (3D) Cocultures 365
14.7 Mechanism of Cellular Interactions During Coculture 366
14.8 Conclusions 368
  Acknowledgments 368
  References 368

Part III. Clinical Applications of Nanostructures 375

15 Nanostructures for Tissue Engineering/Regenerative Medicine

Syam P. Nukavarapu, Sangamesh G. Kumbar, Lakshmi S. Nair, and Cato T. Laurencin

15.1 Introduction 377
  15.1.1 Tissue Engineering/Regenerative Medicine 377
  15.1.2 Scaffolds for Tissue Engineering 378
  15.1.3 Nanofeatures of ECM 379
15.2 Nanofibrous Scaffolds 381
  15.2.1 Electrospinning 381
  15.2.2 Phase Separation 384
  15.2.3 Molecular Self-Assembly 385
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3 Surface Patterned Scaffolds</td>
<td>386</td>
</tr>
<tr>
<td>15.3.1 Micro/Nanocontact Printing</td>
<td>387</td>
</tr>
<tr>
<td>15.3.2 Capillary Force Lithography</td>
<td>387</td>
</tr>
<tr>
<td>15.3.3 Biomolecular Patterning</td>
<td>389</td>
</tr>
<tr>
<td>15.4 Relevance of Nanostructured Scaffolds in Regenerative Medicine</td>
<td>390</td>
</tr>
<tr>
<td>15.5 Role of Nanostructured Scaffolds in Tissue Engineering</td>
<td>391</td>
</tr>
<tr>
<td>15.5.1 Bone and Cartilage Tissue Engineering</td>
<td>392</td>
</tr>
<tr>
<td>15.5.2 Vascular Tissue Engineering</td>
<td>394</td>
</tr>
<tr>
<td>15.5.3 Neural Tissue Engineering</td>
<td>397</td>
</tr>
<tr>
<td>15.5.4 Cardiac Tissue Engineering</td>
<td>399</td>
</tr>
<tr>
<td>15.6 Conclusions</td>
<td>401</td>
</tr>
<tr>
<td>References</td>
<td>401</td>
</tr>
</tbody>
</table>

## 16 Nanostructures for Cancer Diagnostics and Therapy

Kumerash S. Soppimath and Guru V. Betageri

16.1 Introduction

16.1.1 Cancer and Early Diagnosis | 409 |
16.1.2 Cancer and Chemotherapy | 411 |
16.1.3 Why Nanotechnology for Treating Cancer? | 413 |

16.2 Nanotools for Early Cancer Detection

16.2.1 Quantum Dots | 414 |
16.2.2 Nanoshells | 415 |
16.2.3 Gold Nanoparticles | 416 |
16.2.4 Paramagnetic Nanoparticles | 416 |

16.3 Nanomedicine for Cancer Treatment

16.3.1 Liposomes

16.3.1.1 Long-Circulating Liposomes | 419 |
16.3.1.2 Size and Tumor Delivery | 420 |
16.3.1.3 Doxil | 420 |
16.3.1.4 Stealth Cisplatin Liposomes | 423 |
16.3.1.5 Vincristine Sphingomyelin Liposomes | 424 |
16.3.1.6 Sustained Release Liposomes | 424 |
16.3.1.7 Liposome Vaccine | 425 |
16.3.1.8 Liposomes as Solubilizing Carrier for Water Insoluble Anticancer Drugs | 426 |

16.4 Polymeric Nanoparticles

16.4.1 Albumin Nanoparticles | 427 |
16.4.2 Micellar Nanoparticles | 428 |

16.5 Conclusions | 430 |
| References | 430 |
17 Clinical Applications of Micro- and Nanoscale Biosensors

David W.G. Morrison, Mehmet R. Dokmeci, Utkan Demirci, and Ali Khademhosseini

17.1 Introduction

17.2 Classes of Biosensors
   17.2.1 Method of Biological Signaling
   17.2.2 Method of Transduction

17.3 Types of In Vitro Diagnostics
   17.3.1 Cantilever-Based Biosensors
   17.3.2 Cell and Protein Arrays
   17.3.3 Nanoparticles

17.4 In Vivo Diagnostics
   17.4.1 Quantum Dots
   17.4.2 MRI Contrast Agents

17.5 Current and Emerging Clinical Applications of Micro- and Nanoscale Biosensors
   17.5.1 Glucose Detection In Vivo
   17.5.2 Bacterial Urinary Tract Infections
   17.5.3 Human Immunodeficiency Virus (HIV) Detection
   17.5.4 Cancer Cell Targeting

17.6 Conclusions

Acknowledgments

References

18 Nanoscale Iron Compounds Related to Neurodegenerative Disorders

Joanna F. Collingwood and Jon Dobson

18.1 Introduction

18.2 Iron in the Human Brain
   18.2.1 General Overview
   18.2.2 Iron Storage
      18.2.2.1 Ferritin
      18.2.2.2 Hemosiderin
      18.2.2.3 Magnetite
      18.2.2.4 Neuromelanin
   18.2.3 Regional Distribution of Iron Compounds
   18.2.4 Iron Transport

18.3 Iron Compounds in Neurodegenerative Disorders
   18.3.1 Overview
   18.3.2 Alzheimer’s Disease
   18.3.3 Huntington’s Disease
   18.3.4 Parkinson’s Disease