# Contents

**Preface**  
**List of Contributors**

## Part I  
**Examples of Natural and Nature-Inspired Materials**

1. **Biomaterials from Marine-Origin Biopolymers**  
   *Tiago H. Silva, Ana R.C. Duarte, Joana Moreira-Silva, João F. Mano, and Rui L. Reis*
   
   1.1 Taking Inspiration from the Sea  
   1.2 Marine-Origin Biopolymers  
      - 1.2.1 Chitosan  
      - 1.2.2 Alginate  
      - 1.2.3 Carrageenan  
      - 1.2.4 Collagen  
      - 1.2.5 Hyaluronic Acid  
      - 1.2.6 Others  
   1.3 Marine-Based Tissue Engineering Approaches  
      - 1.3.1 Membranes  
      - 1.3.2 Hydrogels  
      - 1.3.3 Tridimensional Porous Structures  
      - 1.3.4 Particles  
   1.4 Conclusions

2. **Hydrogels from Protein Engineering**  
   *Midori Greenwood-Goodwin and Sarah C. Heilshorn*
   
   2.1 Introduction  
   2.2 Principles of Protein Engineering  
      - 2.2.1 Protein Structure and Folding  
      - 2.2.2 Design of Protein-Engineered Hydrogels  
      - 2.2.3 Production of Protein-Engineered Hydrogels
2.3 Structural Diversity and Applications of Protein-Engineered Hydrogels 32
2.3.1 Self-Assembled Protein Hydrogels 32
2.3.2 Covalently Cross-Linked Protein Hydrogels 38
2.4 Development of Biomimetic Protein-Engineered Hydrogels for Tissue Engineering Applications 39
2.4.1 Mechanical Properties Mediate Cellular Response 40
2.4.2 Biodegradable Hydrogels for Cell Invasion 41
2.4.3 Diverse Biochemical Cues Regulate Complex Cell Behaviors 43
2.4.3.1 Cell–Extracellular Matrix Binding Domains 43
2.4.3.2 Nanoscale Patterning of Cell–Extracellular Matrix Binding Domains 44
2.4.3.3 Cell–Cell Binding Domains 45
2.4.3.4 Delivery of Soluble Cell Signaling Molecules 46
2.5 Conclusions and Future Perspective 48
References 49

3 Collagen-Based Biomaterials for Regenerative Medicine 55
Christophe Helary and Abhay Pandit
3.1 Introduction 55
3.2 Collagens In Vivo 56
3.2.1 Collagen Structure 56
3.2.2 Collagen Fibrillogenesis 56
3.2.3 Three-Dimensional Networks of Collagen in Connective Tissues 57
3.2.4 Interactions of Cells with Collagen 57
3.3 Collagen In Vitro 59
3.4 Collagen Hydrogels 59
3.4.1 Collagen I Hydrogels 59
3.4.1.1 Classical Hydrogels 59
3.4.1.2 Concentrated Collagen Hydrogels 61
3.4.1.3 Dense Collagen Hydrogels Obtained by Plastic Compression 61
3.4.1.4 Dense Collagen Matrices 61
3.4.2 Cross-Linked Collagen I Hydrogels 62
3.4.2.1 Chemical Cross-Linking 62
3.4.2.2 Enzymatic Cross-Linking 62
3.4.3 Collagen II Hydrogels 63
3.4.4 Aligned Hydrogels and Extruded Fibers 64
3.4.4.1 Aligned Hydrogels 64
3.4.4.2 Extruded Collagen Fibers 65
3.5 Collagen Sponges 65
3.6 Multichannel Collagen Scaffolds 66
3.6.1 Multichannel Collagen Conduits 66
3.6.2 Multi-Channeled Collagen–Calcium Phosphate Scaffolds 66
3.7 What Tissues Do Collagen Biomaterials Mimic? (see Table 3.1) 66
3.7.1 Skin 66
6 Biomimetic Molecular Recognition Elements for Chemical Sensing 117
Justyn Jaworski

6.1 Introduction 117
6.1.1 Overview 117
6.1.2 Biological Chemoreception 118
6.1.3 Host–Guest Interactions 119
6.1.3.1 Lock and Key 119
6.1.3.2 Induced Fit 120
6.1.3.3 Preexisting Equilibrium Model 121
6.1.4 Biomimetic Surfaces for Molecular Recognition 121
6.2 Theory of Molecular Recognition 123
6.2.1 Foundation of Molecular Recognition 123
6.2.2 Noncovalent Interactions 123
6.2.3 Thermodynamics of the Molecular Recognition Event 125
6.2.4 Putting a Figure of Merit on Molecular Recognition 127
6.2.5 Multiple Interactions: Avidity and Cooperativity 128
6.3 Molecularly Imprinted Polymers 129
6.3.1 A Brief History of Molecular Imprinting 129
6.3.2 Strategies for the Formation of Molecularly Imprinted Polymers 129
6.3.3 Polymer Matrix Design 130
6.3.4 Cross-Linking and Polymerization Approaches 131
6.3.5 Template Extraction 132
6.3.6 Limitations and Areas for Improvement 133
6.4 Supramolecular Chemistry 134
6.4.1 Introduction 134
6.4.2 Macrocyclic Effect 134
6.4.3 Chelate Effect 135
6.4.4 Preorganization, Rational Design, and Modeling 135
6.4.5 Templating Effect 136
6.4.6 Effective Supramolecular Receptors for Biomimetic Sensing 137
6.4.6.1 Calixarenes 137
6.4.6.2 Metalloporphyrins 138
6.4.7 Recent Improvement 139
6.5 Biomolecular Materials 140
6.5.1 Introduction 140
6.5.2 Native Biomolecules 141
6.5.2.1 Polypeptides 141
6.5.2.2 Carbohydrates 142
6.5.2.3 Oligonucleotides 143
6.5.3 Engineered Biomolecules 144
6.5.3.1  *In vitro* Selection of RNA/DNA Aptamers  144
6.5.3.2  Evolutionary Screened Peptides  146
6.5.3.3  Computational and Rational Design of Biomimetic Receptors  150
6.6  Summary and Future of Biomimetic-Sensor-Coating Materials  151
References  152

Part II  Surface Aspects  157

7  Biology Lessons for Engineering Surfaces for Controlling Cell–Material Adhesion  159
_Ted T. Lee and Andrés J. García_

7.1  Introduction  159
7.2  The Extracellular Matrix  159
7.3  Protein Structure  160
7.4  Basics of Protein Adsorption  161
7.5  Kinetics of Protein Adsorption  162
7.6  Cell Communication  164
7.6.1  Intracellular Communication  164
7.6.2  Intercellular Communication  165
7.7  Cell Adhesion Background  166
7.8  Integrins and Adhesive Force Generation Overview  167
7.9  Adhesive Interactions in Cell, and Host Responses to Biomaterials  170
7.10  Model Systems for Controlling Integrin-Mediated Cell Adhesion  170
7.11  Self-Assembling Monolayers (SAMs)  171
7.12  Real-World Materials for Medical Applications  172
7.12.1  Polymer Brush Systems  172
7.12.2  Hydrogels  173
7.14  Dynamic Biomaterials  176
7.14.1  Nonspecific “On” Switches  176
7.14.1.1  Electrochemical Desorption  176
7.14.1.2  Oxidative Release  177
7.14.2  Photobased Desorption  178
7.14.3  Integrin Specific “On” Switching  178
7.14.3.1  Photoactivation  178
7.14.4  Adhesion “Off” Switching  179
7.14.4.1  Electrochemical Off Switching  179
7.14.5  Reversible Adhesion Switches  181
7.14.5.1  Reversible Photoactive Switching  181
7.14.5.2  Reversible Temperature-Based Switching  182
7.14.6  Conclusions and Future Prospects  184
References  185
8 Fibronectin Fibrillogenesis at the Cell–Material Interface 189
Marco Cantini, Patricia Rico, and Manuel Salmerón-Sánchez

8.1 Introduction 189
8.2 Cell-Driven Fibronectin Fibrillogenesis 189
8.2.1 Fibronectin Structure 190
8.2.2 Essential Domains for FN Assembly 192
8.2.3 FN Fibrillogenesis and Regulation of Matrix Assembly 194
8.3 Cell-Free Assembly of Fibronectin Fibrils 195
8.4 Material-Driven Fibronectin Fibrillogenesis 202
8.4.1 Physiological Organization of Fibronectin at the Material Interface 203
8.4.2 Biological Activity of the Material-Driven Fibronectin Fibrillogenesis 206

References 210

9 Nanoscale Control of Cell Behavior on Biointerfaces 213
E. Ada Cavalcanti-Adam and Dimitris Missirlis

9.1 Nanoscale Cues in Cell Environment 213
9.2 Biomimetics of Cell Environment Using Interfaces 216
9.2.1 Surface Patterning Techniques at the Nanoscale 216
9.2.1.1 Surface Patterning by Nonconventional Nanolithography 216
9.2.1.2 Block Copolymer Micelle Lithography 217
9.2.2 Variation of Surface Physical Parameters at the Nanoscale 219
9.2.2.1 Surface Nanotopography 220
9.2.2.2 Interligand Spacing 221
9.2.2.3 Ligand Density 222
9.2.2.4 Substrate Mechanical Properties 223
9.2.2.5 Dimensionality 223
9.2.3 Surface Functionalization for Controlled Presentation of ECM Molecules to Cells 224
9.2.3.1 Proteins, Protein Fragments, and Peptides 224
9.2.3.2 Linking Systems 226
9.2.3.3 Modulation of Substrate Background 227
9.3 Cell Responses to Nanostructured Materials 227
9.3.1 Cell Adhesion and Migration 228
9.3.2 Cell–Cell Interactions 230
9.3.3 Cell Membrane Receptor Signaling 231
9.3.4 Applications of Nanostructures in Stem Cell Biology 232
9.4 The Road Ahead 233

References 234

10 Surfaces with Extreme Wettability Ranges for Biomedical Applications 237
Wenlong Song, Natália M. Alves, and João F. Mano

10.1 Superhydrophobic Surfaces in Nature 237
10.2 Theory of Surface Wettability 239
10.2.1 Young’s Model 239
10.2.2 Wenzel’s Model 240
10.2.3 The Cassie–Baxter Model 240
10.2.4 Transition between the Cassie–Baxter and Wenzel Models 240
10.3 Fabrication of Extreme Water-Repellent Surfaces Inspired by Nature 241
10.3.1 Superhydrophobic Surfaces Inspired by the Lotus Leaf 241
10.3.2 Superhydrophobic Surfaces Inspired by the Legs of the Water Strider 243
10.3.3 Superhydrophobic Surfaces Inspired by the Anisotropic Superhydrophobic Surfaces in Nature 244
10.3.4 Other Superhydrophobic Surfaces 245
10.4 Applications of Surfaces with Extreme Wettability Ranges in the Biomedical Field 245
10.4.1 Cell Interactions with Surfaces with Extreme Wettability Ranges 246
10.4.2 Protein Interactions with Surfaces with Extreme Wettability Ranges 249
10.4.3 Blood Interactions with Surfaces with Extreme Wettability Ranges 251
10.4.4 High-Throughput Chips Based on Surfaces with Extreme Wettability Ranges 252
10.4.5 Substrates for Preparing Hydrogel and Polymeric Particles 254
10.5 Conclusions 254
References 255

11 Bio-Inspired Reversible Adhesives for Dry and Wet Conditions 259
Aránzazu del Campo and Juan Pedro Fernández-Blázquez
11.1 Introduction 259
11.2 Gecko-Like Dry Adhesives 260
11.2.1 Fibrils with 3D Contact Shapes 262
11.2.2 Tilted Structures 263
11.2.3 Hierarchical Structures 265
11.2.4 Responsive Adhesion Patterns 265
11.3 Bioinspired Adhesives for Wet Conditions 268
11.4 The Future of Bio-Inspired Reversible Adhesives 270
Acknowledgments 270
References 270

12 Lessons from Sea Organisms to Produce New Biomedical Adhesives 273
Elise Hennebert, Pierre Becker, and Patrick Flammang
12.1 Introduction 273
12.2 Composition of Natural Adhesives 274
12.2.1 Mussels 274
12.2.2 Tube Worms 278
12.2.3 Barnacles 279
12.2.4 Brown Algae 280
12.3 Recombinant Adhesive Proteins 281
12.3.1 Production 281
12.3.2 Applications 283
12.4 Production of Bio-Inspired Synthetic Adhesive Polymers 284
12.4.1 Adhesives Based on Synthetic Peptides 285
12.4.2 Adhesives Based on Polysaccharides 285
12.4.3 Adhesives Based on Other Polymers 286
12.5 Perspectives 288
Acknowledgments 288
References 288

Part III Hard and Mineralized Systems 293

13 Interfacial Forces and Interfaces in Hard Biomaterial Mechanics 295
Devendra K. Dubey and Vikas Tomar
13.1 Introduction 295
13.2 Hard Biological Materials 298
13.2.1 Role of Interfaces in Hard Biomaterial Mechanics 299
13.2.2 Modeling of TC–HAP and Generic Polymer–Ceramic-Type Nanocomposites at Fundamental Length Scales 301
13.2.2.1 Analytical Modeling 302
13.2.2.2 Atomistic Modeling 304
13.3 Bioengineering and Biomimetics 306
13.4 Summary 308
References 309

14 Nacre-Inspired Biomaterials 313
Gisela M. Luz and João F. Mano
14.1 Introduction 313
14.2 Structure of Nacre 316
14.3 Why Is Nacre So Strong? 318
14.4 Strategies to Produce Nacre-Inspired Biomaterials 320
14.4.1 Covalent Self-Assembly or Bottom-Up Approach 320
14.4.2 Electrophoretic Deposition 322
14.4.3 Layer-by-Layer and Spin-Coating Methodologies 323
14.4.4 Template Inhibition 325
14.4.5 Freeze-Casting 326
14.4.6 Other Methodologies 326
14.5 Conclusions 328
Acknowledgements 329
References 329
## 15 Surfaces Inducing Biomineralization 333
Natália M. Alves, Isabel B. Leonor, Helena S. Azevedo, Rui. L. Reis, and João. F. Mano

15.1 Mineralized Structures in Nature: the Example of Bone 333
15.2 Learning from Nature to the Research Laboratory 336
15.2.1 Bioactive Ceramics and Their Bone-Bonding Mechanism 337
15.2.2 Is a Functional Group Enough to Render Biomaterials Self-Mineralizable? 338
15.2.2.1 How the Surface Charge of Functional Group Can Be Correlated to Apatite Formation? 338
15.2.2.2 Designing a Properly Functionalized Surface 339
15.3 Smart Mineralizing Surfaces 343
15.4 \textit{In Situ} Self-Assembly on Implant Surfaces to Direct Mineralization 345
15.5 Conclusions 348
Acknowledgments 348
References 348

## 16 Bioactive Nanocomposites Containing Silicate Phases for Bone Replacement and Regeneration 353
Melek Erol, Jasmin Hum, and Aldo R. Boccaccini

16.1 Introduction 353
16.2 Nanostructure and Nanofeatures of the Bone 354
16.2.1 The Structure of Bone as a Nanocomposite 354
16.2.2 Cell Behavior at the Nanoscale 356
16.3 Nanocomposites-Containing Silicate Nanophases 356
16.3.1 Nanoscale Bioactive Glasses 356
16.3.1.1 Synthetic Polymer/Nanoparticulate Bioactive Glass Composites 357
16.3.1.2 Natural Polymer/Bioactive Glass Nanocomposites 360
16.3.2 Nanoscaled Silica 363
16.3.2.1 Composites Containing Silica Nanoparticles 364
16.3.3 Nanoclays 365
16.3.3.1 Composites Containing Clay Nanoparticles 366
16.4 Final Considerations 372
References 375

## Part IV Systems for the Delivery of Bioactive Agents 381

## 17 Biomimetic Nanostructured Apatitic Matrices for Drug Delivery 383
Norberto Roveri and Michele Iafisco

17.1 Introduction 383
17.2 Biomimetic Apatite Nanocrystals 384
17.2.1 Properties 384
17.2.2 Synthesis 386
Contents

19.2.5 Theranostics  466
19.3 Final Remarks  467
References  467

Part V Lessons from Nature in Regenerative Medicine  471

20 Tissue Analogs by the Assembly of Engineered Hydrogel Blocks  473
Shilpa Sant, Daniela F. Coutinho, Nasser Sadr, Rui L. Reis, and Ali Khademhosseini
20.1 Introduction  473
20.2 Tissue/Organ Heterogeneity In Vivo  474
20.3 Hydrogel Engineering for Obtaining Biologically Inspired Structures  477
20.3.1 Structural Cues  477
20.3.2 Mechanical Cues  478
20.3.3 Biochemical Cues  480
20.3.4 Cell–Cell Contact  482
20.3.5 Combination of Multiple Cues  483
20.4 Assembly of Engineered Hydrogel Blocks  485
20.5 Conclusions  488
Acknowledgments  489
References  489

21 Injectable In-Situ-Forming Scaffolds for Tissue Engineering  495
Da Yeon Kim, Jae Ho Kim, Byoung Hyun Min, and Moon Suk Kim
21.1 Introduction  495
21.2 Injectable In-Situ-Forming Scaffolds Formed by Electrostatic Interactions  496
21.3 Injectable In-Situ-Forming Scaffolds Formed by Hydrophobic Interactions  497
21.4 Immune Response of Injectable In-Situ-Forming Scaffolds  500
21.5 Injectable In-Situ-Forming Scaffolds for Preclinical Regenerative Medicine  500
21.6 Conclusions and Outlook  501
References  502

22 Biomimetic Hydrogels for Regenerative Medicine  503
Iris Mironi-Harpaz, Olga Kossover, Eran Ivanir, and Dror Seliktar
22.1 Introduction  503
22.2 Natural and Synthetic Hydrogels  503
22.3 Hydrogel Properties  505
22.4 Engineering Strategies for Hydrogel Development  506
22.5 Applications in Biomedicine  508
References  511
23 Bio-inspired 3D Environments for Cartilage Engineering  515
José Luis Gómez Ribelles
23.1 Articular Cartilage Histology  515
23.2 Spontaneous and Forced Regeneration in Articular Cartilage  517
23.3 What Can Tissue Engineering Do for Articular Cartilage Regeneration?  517
23.4 Cell Sources for Cartilage Engineering  519
23.4.1 Bone Marrow Mesenchymal Cells Reaching the Cartilage Defect from Subchondral Bone  519
23.4.2 Autologous Mesenchymal Stem Cells from Different Sources  520
23.4.3 Mature Autologous Chondrocytes  521
23.5 The Role and Requirements of the Scaffolding Material  524
23.5.1 Gels Encapsulating Cells as Vehicles for Cell Transplant  524
23.5.2 Macroporous Scaffolds: Pore Architecture  524
23.5.3 Cell Adhesion Properties of the Scaffold Surfaces  525
23.5.4 Mechanical Properties  525
23.5.5 Can Scaffold Architecture Direct Tissue Organization?  526
23.5.6 Scaffold Biodegradation Rate  527
23.6 Growth Factor Delivery In Vivo  528
23.7 Conclusions  528
Acknowledgments  529
References  529

24 Soft Constructs for Skin Tissue Engineering  537
Simone S. Silva, João F. Mano, and Rui L. Reis
24.1 Introduction  537
24.2 Structure of Skin  537
24.2.1 Wound Healing  538
24.3 Current Biomaterials in Wound Healing  539
24.3.1 Alginate  539
24.3.2 Cellulose  540
24.3.3 Chitin/Chitosan  541
24.3.4 Hyaluronic Acid  543
24.3.5 Collagen and Other Proteins  544
24.3.6 Synthetic Polymers  545
24.4 Wound Dressings and Their Properties  545
24.5 Biomimetic Approaches in Skin Tissue Engineering  546
24.5.1 Commercially Available Skin Products  549
24.6 Final Remarks  549
Acknowledgments  552
List of Abbreviations  552
References  553

Index  559