Contents

Preface xxv
Acknowledgments xxviii

1 The Need for New Perspectives in Medicine 1
1.1 Nanotechnology: Why Is Something So Small So Big? 1
  1.1.1 Definitions of Nanotechnology Based on Size 2
  1.1.2 Bottom-Up Rather Than Top-Down Approach 2
  1.1.3 Nanoscale Systems Are on the Right Scale for Nanomedicine 3
1.2 The Progression of Medicine 7
  1.2.1 Human Disease Really Happens at a Single-Cell Level 7
  1.2.2 Conventional “Modern” Medicine 7
  1.2.3 “Personalized” or “Molecular” Medicine 8
  1.2.4 Nanomedicine “Single-Cell” Medicine 9
1.3 How Conventional Medicine Works for Diagnosis of Disease 10
  1.3.1 Identification of the “Diseased State” 10
  1.3.2 Collection of Medical Data by Health Professionals 10
  1.3.3 Analysis of Initial Medical Data on Patient 10
  1.3.4 More Advanced Examinations of the Patient 10
  1.3.5 Comparison of Patient Data with “Normal” Ranges 10
  1.3.6 Molecular Tests to Determine Disease State 11
1.4 How Conventional Medicine Works for Treatment of Disease 11
  1.4.1 Stabilization of the Patient: “Heal Thyself” 11
  1.4.2 Surgical Repair of Injuries 11
  1.4.3 Treatment with Drugs Locally 11
  1.4.4 Treatment with Drugs Systemically 12
  1.4.5 Treatment with Targeted Therapies 12
1.5 Factors Limiting the Progress of Medicine 12
  1.5.1 Economics 12
  1.5.2 Politics 13
  1.5.3 Ethics 13
  1.5.4 Legalities 13
  1.5.5 Regulation 14
1.6 Some Specific Problems with Conventional Medicine 15
1.6.1 Consequences of Waiting for Patient Symptoms 15
1.6.2 Trained People and Modern Drugs Are Expensive 15
1.6.3 Diagnostic Technologies Are Still Relatively Primitive and/or Expensive 16
1.6.4 Relatively Crude Targeting of Drugs 16
1.7 Some Ways Nanotechnologies Will Impact Healthcare 16
1.7.1 Nanomedicine Will Be Proactive Rather Than Reactive Medicine 16
1.7.2 Possibility of “Regenerative Medicine” 17
1.7.3 Blurring of Distinctions between Prevention and Treatment 17
1.7.4 Ophthalmology Has Embraced a Nanomedicine Approach 18
1.8 “Nano Hype” versus Reality 18
1.9 Why Nanomedicine Will Happen: The Perfect Storm 18
Chapter 1 Study Questions 19

2 Nanomedicine: Single-Cell Medicine 21
2.1 Features of Nanomedicine 21
2.1.1 Bottom-Up Rather Than Top-Down Approach to Medicine 21
2.1.2 Nanotools on the Scale of Molecules 22
2.1.3 Cell-by-Cell Repair Approach: Regenerative Medicine 22
2.1.4 Feedback Control System to Control Drug Dosing 22
2.2 Elements of Good Engineering Design 22
2.2.1 Begin with the End in Mind 22
2.2.2 Use a Previously Tested General Design, If Possible 23
2.2.3 Use Biomimicry Whenever Possible 23
2.2.4 Use Multiple Specific Molecules to Do Multistep Tasks 23
2.2.5 Control the Order of Molecular Assembly to Control the Order of Events 24
2.2.6 Perform the Molecular Assembly in Reverse Order to the Desired Order of Events 24
2.3 Building a Nanomedical Device 25
2.3.1 Choice of Core Materials 26
2.3.2 Add Drug or Therapeutic Gene 26
2.3.3 Add Molecular Biosensors to Control Drug/Gene Delivery 26
2.3.4 Add Intracellular Targeting Molecules 26
2.3.5 Result: A Multicomponent, Multifunctional Nanomedical Device 26
2.3.6 For Use, Design to Delayer, One Layer at a Time 27
2.3.7 The Multistep Drug/Gene Delivery Process in Nanomedical Systems 27
2.4 The Challenge of Drug/Gene Dosing to Single Cells 27
2.4.1 Precise Targeting of Drug Delivery System Only to Diseased Cells 28
Table of Contents

2.4.2 How to Minimize Mistargeting 28
2.4.3 How to Deliver the Right Dose per Cell 28
2.4.4 One Possible Solution: In Situ Manufacture of Therapeutic Genes 28

2.5 Bridging the Gap between Diagnostics and Therapeutics 29
2.5.1 How Conventional Medicine Is Practiced in Terms of Diagnostics and Therapeutics 29
2.5.2 The Consequences of Separating Diagnostics and Therapeutics 30
2.5.3 A New Approach: “Theragnostics” (or “Theranostics”) 30

2.6 How Theragnostics Relates to Molecular Imaging 31
2.6.1 Conventional Imaging Is Not Very Specific 31
2.6.2 Types of In Vivo Imaging 31

2.7 Engineering Nanomedical Systems for Simultaneous Molecular Imaging 35
2.7.1 Using Cell-Specific Probes for Molecular Imaging of Nanomedical Devices 35
2.7.2 Breaking the “Diffraction Limit”: Nano-Level Imaging 35

2.8 Theragnostic Nanomedical Devices 37
2.8.1 Using Nanomedical Devices to Guide Separate Therapeutic Devices 37
2.8.2 When Might We Want to Combine Diagnostics and Therapeutics? 37

Chapter 2 Study Questions 38

3 Targeted Drug Delivery 40
3.1 Overview: Targeting Nanosystems to Cells 40
3.1.1 Antibodies as Targeting Molecules 40
3.1.2 Structure of Antibodies 40
3.1.3 Labeling of Antibodies with Fluorescent Probes 41
3.1.4 Lock–Key Models of Antibody Shape Recognition of Antigens 42
3.1.5 Peptide Targeting 44
3.1.6 Aptamer Targeting 44

3.2 Antibodies: Polyclonal, Monoclonal, and “Monoclonal Cocktails” 45
3.2.1 Where Do Antibodies Come From in Nature? 45
3.2.2 How Do We Make Them in the Laboratory? 46
3.2.3 Monoclonal Antibodies 46
3.2.4 Therapy Problems with Mouse Monoclonal Antibodies 47
3.2.5 “Humanizing” Monoclonal Antibodies to Reduce Host Immune Reactions 47
3.2.6 Why Antibodies May Not Be a Good Choice for Targeting Nanosystems to Cells 47
<table>
<thead>
<tr>
<th>3.3 Peptide Targeting</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.1 How Does a Peptide Target?</td>
<td>48</td>
</tr>
<tr>
<td>3.3.2 Examples of Peptide Targeting</td>
<td>48</td>
</tr>
<tr>
<td>3.3.3 Advantages and Disadvantages of Peptide Targeting</td>
<td>48</td>
</tr>
<tr>
<td>3.4 Aptamer Targeting</td>
<td>49</td>
</tr>
<tr>
<td>3.4.1 What Are Aptamers and How Do They Target?</td>
<td>49</td>
</tr>
<tr>
<td>3.4.2 Some Different Types of Aptamers</td>
<td>49</td>
</tr>
<tr>
<td>3.4.3 How Do You Make Aptamers?</td>
<td>49</td>
</tr>
<tr>
<td>3.4.4 How Do You Screen for Useful Aptamers?</td>
<td>49</td>
</tr>
<tr>
<td>3.4.5 How Do You Find Known Aptamers and Their Targets?</td>
<td>51</td>
</tr>
<tr>
<td>3.4.6 Ligand and Small Molecule Targeting</td>
<td>51</td>
</tr>
<tr>
<td>3.5 Assessing Targeting</td>
<td>52</td>
</tr>
<tr>
<td>3.5.1 The Importance of Quantitatively Assessing Cell Targeting</td>
<td>53</td>
</tr>
<tr>
<td>3.5.2 Rare-Event Targeting of Cells In Vitro and In Vivo</td>
<td>53</td>
</tr>
<tr>
<td>3.6 Assessing Nanomedical System (NMS) Targeting at the Single-Cell Level</td>
<td>53</td>
</tr>
<tr>
<td>3.6.1 Fluorescent Labeling of NMSs</td>
<td>54</td>
</tr>
<tr>
<td>3.6.2 First Estimates of NMS Binding by Fluorescence Microscopy</td>
<td>54</td>
</tr>
<tr>
<td>3.6.3 Estimates of Cell Surface or Internal Binding of Nanoparticles by Confocal Microscopy</td>
<td>54</td>
</tr>
<tr>
<td>3.6.4 Flow Cytometric Quantitation of NMS Binding to Specific Cell Types</td>
<td>55</td>
</tr>
<tr>
<td>3.7 Image Analysis of NMS Binding to Single Cells</td>
<td>55</td>
</tr>
<tr>
<td>3.7.1 Ability to Scan/Locate Cells of Interest</td>
<td>55</td>
</tr>
<tr>
<td>3.7.2 Photobleaching Challenges</td>
<td>55</td>
</tr>
<tr>
<td>3.7.3 Detection of Nanoparticles by Super-Resolution Microscopy</td>
<td>56</td>
</tr>
<tr>
<td>3.8 A Quick Overview of Single-Cell Targeting Assessment by Flow Cytometry</td>
<td>56</td>
</tr>
<tr>
<td>3.8.1 Basic Principles of Flow Cytometry</td>
<td>56</td>
</tr>
<tr>
<td>3.8.2 Use of Flow Cytometry for Assessing Specificity and Sensitivity</td>
<td>59</td>
</tr>
<tr>
<td>3.9 Rare-Event Analysis of NMS Targeting to Desired Cells</td>
<td>59</td>
</tr>
<tr>
<td>3.9.1 Strategies for Rare-Cell Detection</td>
<td>59</td>
</tr>
<tr>
<td>3.9.2 More Advanced Flow Cytometry for Ultra-Rare-Cell Detection</td>
<td>59</td>
</tr>
<tr>
<td>3.9.3 Examples of Nanoparticle Targeting to Rare Cells</td>
<td>60</td>
</tr>
<tr>
<td>3.9.4 Rare-Cell Sampling Statistics</td>
<td>61</td>
</tr>
<tr>
<td>Chapter 3 Study Questions</td>
<td>61</td>
</tr>
</tbody>
</table>

4 Drug Delivery Cell Entry Mechanisms | 64 |
4.1 Introduction | 64 |
| 4.1.1 General Problem of Cell Entry | 65 |
| 4.1.2 Choosing Modes of Cell Entry | 66 |
| 4.1.3 How Does Nature Do It? (Biomimicry) | 67 |
4.2 Nonspecific Uptake Mechanisms 67
  4.2.1 Pinocytosis by All Cells 67
  4.2.2 Phagocytosis by Some Cells 68
4.3 Receptor-Mediated Uptake 68
  4.3.1 Receptor-Mediated Transport of Desired Molecules 68
  4.3.2 Example: Transferrin Receptor Transport of Iron for Metabolism 68
4.4 Nanoparticle Uptake 68
  4.4.1 Surface Coatings on Nanoparticles 69
  4.4.2 Size and Shape Matter 69
  4.4.3 Inhibitors Help Explore the Mechanisms of Nanoparticle Uptake 70
  4.4.4 Agglomeration Reduces Uptake 70
  4.4.5 Nanoparticles Tend to Agglomerate Inside Cells as They Are Taken Up 71
  4.4.6 Agglomerated Qdots Inside Cells 71
4.5 Drug Delivery by “Shedding” 73
  4.5.1 Extracellular Drug Delivery by Shedding 73
  4.5.2 Intracellular Drug Release by Shedding 73
4.6 Technologies for Measuring Nanomedical System Interaction with Cells 73
  4.6.1 Single-Cell Measurements to Detect the Location of Nanomedical Systems 73
  4.6.2 Below “Optical Limit” Imaging 74
  4.6.3 Nanomedical Systems with X-Ray Dense, Fluorescent, Metallic, or Magnetic Cores 74
  4.6.4 Study Live Cells with Minimally Invasive In Vivo Imaging? 74
4.7 Nanomedical Systems Evaluation Technologies 75
  4.7.1 Flow Cytometry: A Zeroth-Order Imaging Device 75
  4.7.2 Scanning and Transmission Electron Microscopy 76
  4.7.3 One- and Two-Photon Confocal Microscopy 76
  4.7.4 Hyperspectral Imaging 78
  4.7.5 Surface Plasmon Resonance Imaging 80
  4.7.6 Atomic Force Microscopy 80
4.8 Some Ways to Enhance Imaging 81
  4.8.1 Electron-Dense Contrast Agents to Enhance Scanning and TEM 81
  4.8.2 MRI Contrast Agents for In Vivo Imaging 81
Chapter 4 Study Questions 83

5 Nanomaterial Cores for Noninvasive Imaging 84
5.1 Introduction 84
  5.1.1 Core Building Blocks 84
  5.1.2 Functional Cores 84
  5.1.3 Functionalizing the Core Surface 85
# Table of Contents

5.2 Ferric Oxide Cores 85  
5.2.1 Paramagnetic Cores 86  
5.2.2 Superparamagnetic Cores 86  
5.2.3 Ferric Oxide Nanorods 86  
5.2.4 Overall Advantages and Disadvantages of Each Form of Magnetism and Geometry 87  
5.3 C60, Carbon Nanotubes, and Graphene 87  
5.3.1 Size and Structure of C60 87  
5.3.2 Elongation of C60 into Carbon Nanotubes 87  
5.3.3 Graphene 88  
5.3.4 Advantages and Disadvantages 88  
5.4 Gold and Silver Cores 89  
5.4.1 Gold Cores 89  
5.4.2 SERS Molecular Imaging and Flow Cytometry 89  
5.4.3 Spherical Gold Nanoparticles 90  
5.4.4 Gold Nanorods 90  
5.4.5 Gold Nanostars 90  
5.4.6 Gold Nanoshells 91  
5.5 Silver Nanoparticles 92  
5.6 Quantum Dot Nanoparticles 93  
5.6.1 Size Determines Spectral Qualities 95  
5.6.2 Quantum Dots Might Be Cytotoxic 95  
5.6.3 Next-Generation Quantum Dots May Be Better! 95  
5.7 Silica Cores 95  
5.7.1 Silica Nanoparticles 97  
5.7.2 Advantages and Disadvantages 97  
5.8 Hybrid Materials 100  
5.8.1 Multifunctional Hybrid Nanoparticles 100  
5.8.2 Gold–Ferric Oxide Nanoparticles and Nanorods 101  
Chapter 5 Study Questions 101

6 Attaching Biomolecules to Nanoparticles 103  
6.1 Attaching Nanomedical Structures to the Core 103  
6.1.1 Attachment Strategies Typically Depend on Core Composition 103  
6.1.2 Attachment Strategy Should Not Drive the Core Choice 103  
6.1.3 Choice of Core Depends on the Desired Overall “Multifunctional” Nanomedical Device 104  
6.2 “Surface Chemistry” Strategies for Attachment of Biomolecules to the Core 104  
6.2.1 Hydrophobic versus Hydrophilic Core Materials 105  
6.2.2 Addition of Biomolecules for Biocompatibility 105  
6.2.3 Monofunctional versus Bifunctional Surface Chemistry Strategies 106
6.2.4 Pay Attention to Overall Zeta Potential during the Surface Chemistry Process

6.3 Two Main Attachment Strategies
   6.3.1 Noncovalent (Primarily Electrostatic) Bonding Strategies
   6.3.2 Covalent Bonding Strategies

6.4 Attaching Targeting and Therapeutic Molecules
   6.4.1 Preparing the Nanoparticle for Addition of Targeting, Biosensing, and Therapeutic Molecules
   6.4.2 What Are the Special Requirements, If Any, for These Molecules?
   6.4.3 Testing for Targeting at the Single-Cell Level after Bioconjugation
   6.4.4 Testing for Therapeutic Efficacy at the Single-Cell Level after Bioconjugation

6.5 Attaching Different Types of Targeting Molecules
   6.5.1 Antibodies
   6.5.2 Peptides
   6.5.3 Aptamers
   6.5.4 Small-Molecule Ligands

6.6 Testing the Nanoparticle-Targeting Complex
   6.6.1 Ways of Detecting This Complex
   6.6.2 Ways of Assessing Targeting/Mistargeting Efficiency and Costs of Mistargeting
   6.6.3 Is the Nanoparticle Still Attached to the Targeting Molecule?

6.7 Attaching/Tethering Different Types of Therapeutic Molecules
   6.7.1 Antibodies as Therapeutic Molecules
   6.7.2 Peptides as Therapeutic Molecules
   6.7.3 Therapeutic Aptamers
   6.7.4 Transcribable Sequences
   6.7.5 Small Drugs

6.8 Testing the Nanoparticle–Therapeutic Molecule Complex for Efficacy
   6.8.1 Direct and Indirect Ways of Detecting the Therapeutic Molecules
   6.8.2 Ways of Assessing Therapeutic Efficacy at the Single-Cell Level
   6.8.3 Is the Nanoparticle Still Attached to the Therapeutic Molecule?

6.9 Nanomedical Pharmacodynamics: The Great Unknown
   6.9.1 Little Is Known about Complex Nanoparticle Pharmacodynamics
   6.9.2 Obtaining Quantitative Biodistribution Data In Vivo Is Extremely Difficult!
6.10 The Importance of “Green Chemistry”  
6.10.1 What Exactly Is “Green Chemistry”?  
6.10.2 Examples of Green Chemistry in the Synthesis of Nanoparticles and Bioconjugations

Chapter 6 Study Questions

7 Characterizing Nanoparticles

7.1 Size Characterizations of Nanoparticles  
7.1.1 “Size” Depends on How You Measure It!  
7.1.2 Shape May Also Be Important  
7.1.3 Measures of Nanoparticle Size

7.2 The Importance of the Zeta Potential  
7.2.1 Nanoparticle–Cell Interactions  
7.2.2 Zeta Potential Is Part of the Initial Nanomedical System–Cell Targeting Process  
7.2.3 Nanoparticle–Nanoparticle Interactions  
7.2.4 Agglomeration of Magnetic Nanoparticles  
7.2.5 Low Zeta Potential Leads to Low Serum Protein Binding and Longer Circulation

7.3 Zeta Potential Basics  
7.3.1 What Is the Zeta Potential?  
7.3.2 How Is Zeta Potential Measured?

7.4 Zetasizers

7.5 Some Factors Affecting the Zeta Potential  
7.5.1 pH  
7.5.2 Ionic Strength  
7.5.3 Optimal Zeta Potentials for Nanomedical Systems

Chapter 7 Study Questions

8 Nanomedicine Drug Dosing

8.1 Overview of Drug-Dosing Problem  
8.1.1 Are Animals Good Models for Humans in Terms of Nanomedical Applications?  
8.1.2 Problems of Scaling Up Doses from Animal Systems  
8.1.3 Basing Dosing on Size, Area, and Weight of Recipient  
8.1.4 Vast Differences between Adults in Terms of Genetics and Metabolism  
8.1.5 Dosing in Children: Children Are Not Smaller Adults!  
8.1.6 Pharmacokinetics: Drug Distribution, Metabolism, Excretion, and Breakdown  
8.1.7 Conventional Dosing Assumes Drug Goes Everywhere in the Body  
8.1.8 Targeted Therapies: A Model for Future Nanomedical Systems?
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>From Animal Dosing to Human Clinical Trials</td>
<td>148</td>
</tr>
<tr>
<td>8.2.1</td>
<td>Importance of Picking an Appropriate Animal Model System</td>
<td>148</td>
</tr>
<tr>
<td>8.2.2</td>
<td>Does Drug Dosing Really Scale?</td>
<td>148</td>
</tr>
<tr>
<td>8.2.3</td>
<td>The “Human Guinea Pig” in Clinical Trials and Beyond</td>
<td>149</td>
</tr>
<tr>
<td>8.3</td>
<td>Some Drug-Dosing Methods</td>
<td>149</td>
</tr>
<tr>
<td>8.3.1</td>
<td>Attempts to Scale Up Based on Area</td>
<td>149</td>
</tr>
<tr>
<td>8.3.2</td>
<td>Attempts to Scale Up Based on Weight/Volume</td>
<td>150</td>
</tr>
<tr>
<td>8.3.3</td>
<td>Attempts to Use Control Engineering Principles</td>
<td>150</td>
</tr>
<tr>
<td>8.4</td>
<td>Genetic Responses to Drug Dosing</td>
<td>150</td>
</tr>
<tr>
<td>8.4.1</td>
<td>All Humans Are Not Genomically Equivalent!</td>
<td>151</td>
</tr>
<tr>
<td>8.4.2</td>
<td>Predicting on the Basis of Family Tree Responses</td>
<td>151</td>
</tr>
<tr>
<td>8.4.3</td>
<td>SNPs, Chips, and Beyond: Predicting Individual Drug Response</td>
<td>151</td>
</tr>
<tr>
<td>8.4.4</td>
<td>Cost/Benefit of Individual Genome Sequencing</td>
<td>151</td>
</tr>
<tr>
<td>8.5</td>
<td>Dosing in the Era of Directed Therapies: A Future Model for Nanomedical Systems?</td>
<td>152</td>
</tr>
<tr>
<td>8.5.1</td>
<td>How Directed Therapies Change the Dosing</td>
<td>152</td>
</tr>
<tr>
<td>8.5.2</td>
<td>Current Generation of Directed Antibody Therapies Dosing</td>
<td>152</td>
</tr>
<tr>
<td>8.5.3</td>
<td>Some Typical Side Effects of Directed Therapies</td>
<td>153</td>
</tr>
<tr>
<td>8.5.4</td>
<td>Nanomedical Systems Are the Next Generation of Directed Therapies</td>
<td>153</td>
</tr>
<tr>
<td>8.6</td>
<td>Most Directed Therapies Are Nonlinear Processes</td>
<td>153</td>
</tr>
<tr>
<td>8.6.1</td>
<td>Current and Pending FDA-Approved Monoclonal Antibody Therapies</td>
<td>153</td>
</tr>
<tr>
<td>8.6.2</td>
<td>WHO Attempts to Standardize Therapeutic Antibody Names Worldwide</td>
<td>154</td>
</tr>
<tr>
<td>8.7</td>
<td>Other Ways of Controlling Dose Locally</td>
<td>155</td>
</tr>
<tr>
<td>8.7.1</td>
<td>Magnetic Field Release of Drugs</td>
<td>155</td>
</tr>
<tr>
<td>8.7.2</td>
<td>Light-Triggered Release of Drugs</td>
<td>156</td>
</tr>
<tr>
<td>Chapter 8 Study Questions</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Nanodelivery of Therapeutic Genes</td>
<td>159</td>
</tr>
<tr>
<td>9.1</td>
<td>Introduction and Overview</td>
<td>159</td>
</tr>
<tr>
<td>9.1.1</td>
<td>A Multistep Gene Delivery Process</td>
<td>160</td>
</tr>
<tr>
<td>9.1.2</td>
<td>Some of the Advantages of Therapeutic Genes</td>
<td>160</td>
</tr>
<tr>
<td>9.1.3</td>
<td>Concept of “Nanofactories” Manufacturing Therapeutic Products Inside a Living Cell</td>
<td>161</td>
</tr>
<tr>
<td>9.1.4</td>
<td>Some of the Advantages of Molecular Biosensor Feedback Control Systems</td>
<td>162</td>
</tr>
<tr>
<td>9.1.5</td>
<td>Why a Nanodelivery Approach to Gene Therapy Is Appropriate</td>
<td>162</td>
</tr>
</tbody>
</table>
## Contents

9.2 The Therapeutic Gene Approach 163
  9.2.1 What Constitutes a “Therapeutic Gene” at the Single-Cell Level? 163
  9.2.2 Transient versus Stable Expression Modes 164

9.3 Molecular Feedback Control Systems 164
  9.3.1 Drug Delivery Has Traditionally Not Used Feedback Controls 167
  9.3.2 Why Feedback Control Might Be a Very Good Idea! 167
  9.3.3 Positive or Negative Feedback? 167

9.4 Molecular Biosensors as a Component of a Nanomedicine Feedback Control System 168
  9.4.1 What Is a Molecular Biosensor? 168
  9.4.2 How a Molecular Biosensor Functions as a Therapeutic Gene Switch 168

9.5 Building Integrated Molecular Biosensor/Gene Delivery Systems 168
  9.5.1 Example of a Ribozyme/Antivirus System 169
  9.5.2 Example of an ARE Biosensor/DNA Repair System for Radiation-Damaged Cells 171
  9.5.3 Nanomedicine Approaches to Eye Diseases 178

9.6 The Promise of Regenerative Medicine 178
  9.6.1 Repairing Aberrant Gene Expression through siRNA Techniques 180
  9.6.2 Using Gene-Editing Techniques for Regenerative Medicine 180

9.7 The Promise of CRISPR Gene Editing 181
  9.7.1 What Is CRISPR Gene Editing, and How Does It Work? 181
  9.7.2 How Will CRISPR Technology Impact the Field of Nanomedicine? 182
  9.7.3 Ethical Issues about Application of Gene-Editing Technologies to Minorities and Ethnic Groups 184

Chapter 9 Study Questions 184

10 Assessing Nanomedical Therapies at the Single-Cell Level 186

10.1 Introduction and Overview 186
  10.1.1 Nanomedical Treatment at the Single-Cell Level Requires Evaluation at the Single-Cell Level 187
  10.1.2 Does Structure Reveal Function? 187
  10.1.3 The Difficulty of Anything but Simple Functional Assays 187
  10.1.4 The Need for Assays to Show Correlation to Functional Activity 187

10.2 Quantitative Single-Cell Measurements of One or More Proteins per Cell 187
  10.2.1 The Power of Multiple Correlated Measurements per Cell 188
  10.2.2 Why Single-Cell Measurements by Flow Cytometry Are Superior to Bulk Cell Measurements 189
10.2.3 Use of Cell Sorting to Purify Cell Subpopulations 189
10.2.4 Cell Surface or Intracellular Measures of Protein Expression on Live, Single Cells 191
10.2.5 High-Throughput Flow Cytometric Screening of Bioactive Compounds 192
10.2.6 Challenges of Measuring Protein Expression Inside Fixed, Single Cells 192
10.2.7 High-Resolution 3D Imaging Spatial Location of Proteins Inside Cells If Location Is Important 193
10.3 Quantitative Multiparameter Phospho-Specific Flow Cytometry 193
10.3.1 Attempts to Measure Functional Proteins by Detecting Phosphorylation in Single Cells 193
10.3.2 Examples of Phospho-Specific Antibody Staining by Multiparameter Flow Cytometry 194
10.3.3 Measuring Single-Cell Gene Silencing 194
10.4 Quantitative Measures of Gene Expression: The Promises and the Realities 194
10.4.1 Is Measuring Gene Expression at the Single-Cell Level Really Possible? 198
10.4.2 Gene Arrays of Purified Cell Subpopulations 200
10.4.3 Is It Even Possible, or Useful, to Try to Measure a Single Gene’s Changes Inside a Single Cell? 201
10.4.4 RNA Amplification Techniques to Attempt to Perform Single-Cell Gene Arrays 201
Chapter 10 Study Questions 202
11 Nanotoxicity at the Single-Cell Level 203
11.1 Toxicity of Nanomaterials 203
11.1.1 Nanomaterials Are Potentially a Nanotoxicity Problem due to Large Surface-to-Volume Ratios 203
11.1.2 Nanomaterial Toxicity Coatings Can Mask True Toxicity 203
11.1.3 Size, Shape, and Electrical Charge Matter 204
11.1.4 Some Nanomaterials Contain Very Toxic Elemental Components 204
11.1.5 Measuring Biodistribution of Nanomaterials Can Be Challenging 205
11.1.6 Measuring in Single Cells, Rather than in Organs, Is Important but Not Sufficient 205
11.2 Concept of Single-Cell Nanotoxicity 206
11.2.1 There Is More Than One Way for a Cell to Die! 206
11.2.2 Necrosis Is Unplanned Cell Injury 207
11.2.3 Apoptosis Is Planned Programmed Cell Death 208
11.2.4 Other Forms of Toxicity besides Necrosis and Apoptosis 208
11.2.5 Some Other Challenges in Measuring Toxicity of Nanomaterials on Specific Cell Subpopulations

11.3 Single-Cell Measures of Toxicity
11.3.1 Why Are Single-Cell Measures Important? 209
11.3.2 Measuring Cell Death at the Single-Cell Level 211
11.3.3 Are There Any Simple Measures of Cell Death? 211
11.3.4 More Sophisticated Measures of Cell Death through Apoptosis 213

11.4 Necrosis versus Apoptosis Assays 213
11.4.1 Annexin-V Assays for Early Apoptosis 214
11.4.2 DNA Ladders and the Development of Single-Cell TUNEL Assays for Late Apoptosis 214
11.4.3 More Subtle Changes Not Resulting in Direct Cell Death 216

11.5 Measuring Changes in Cell Proliferation and Cell Cycle 216
11.5.1 Measuring Cell Cycle at the Single-Cell Level 218
11.5.2 Measuring DNA per Cell Using Fluorescent DNA-Specific Dyes 218
11.5.3 Disturbances in Cyclin Checkpoint Expression Patterns at the Single-Cell Level 220
11.5.4 Changes in Cell Differentiation 221
11.5.5 Changes in Cell Gene Expression 221
11.5.6 Comet Assays for DNA Damage and Repair 222
11.5.7 A Simple Test for DNA Damage due to Oxidative Stress in Single Cells 222
11.5.8 Light Scatter Assays 224

11.6 Measuring Nanotoxicity for Cell Systems In Vitro 225
11.6.1 In Vitro Measures of Nanotoxicity 225
11.6.2 Cell Lines 225
11.6.3 Primary Cell Strains 225
11.6.4 2D versus 3D Cultures: A Need for 3D Tissue Engineering? 225

11.7 High-Throughput Methods for Measuring Nanotoxicity 226
11.7.1 Flow Cytometry 226
11.7.2 Scanning Image Cytometry 226
11.7.3 3D Confocal Scanning: Can It Be Made High Speed? 227

11.8 Animal Models for Measures of Nanotoxicity 227
11.8.1 What Are Good Animal Models for Human Disease? 227
11.8.2 Some Common Animal Model Systems 227
11.8.3 Nude Mice: Halfway between In Vitro and In Vivo 228
11.8.4 Immune-Competent Mice Add the Effects of the Immune System 228

11.9 Nanotoxicity In Vivo: Some Additional Challenges to Using Animal Models 228
11.9.1 Accumulations of Nanoparticles Can Change Toxicity Locally to Tissues and Organs 228
## Contents

- 11.9.2 Filtration Issues of Nanoparticles 229
- 11.9.3 Removal of Nanoparticles by the Immune System 229
- 11.9.4 Toxicity of PET Probes in Nanomedical Systems for Noninvasive Imaging 229
- 11.10 “Organ-on-a-Chip” Approaches to Human Disease Modeling 230

Chapter 11 Study Questions 230

### 12 Designing Nanodelivery Systems for In Vivo Use 232

#### 12.1 Overview: The In Vitro to Ex Vivo to In Vivo Paradigm 232
- 12.1.1 In Vitro Assays: Importance of Choosing Suitable Cell Lines 233
- 12.1.2 Ex Vivo Assays: Adding Complexity of In Vivo Background While Keeping the Simplicity of In Vitro 234
- 12.1.3 In Vivo Assays: All the Complexity of Ex Vivo Plus the “Active” Components of a Real Animal 234

#### 12.2 In Vivo Systems Are Open, “Active” Systems with Multiple Layers of Complexity 235
- 12.2.1 In Vitro and Ex Vivo Are Mostly “Closed” Systems, but Not Completely 235
- 12.2.2 What Is an “Open” System? 235

#### 12.3 Attempts to Reduce the Complexity of In Vivo Systems 235
- 12.3.1 Human Cells in Nude Mice: A Mixture of In Vitro and In Vivo 236
- 12.3.2 “Model” Small Animal Systems 236
- 12.3.3 Nude Mice 236
- 12.3.4 Larger Animal Model Systems 236
- 12.3.5 Using Animal Data to Estimate Safe Doses of a Drug in Humans 237
- 12.3.6 Size and Shape of Nanomedical Systems Affect the “Safe Dose” Estimate 237
- 12.3.7 Stealth Factors Have a Major Impact on Drug Circulation Times 238
- 12.3.8 Importance of Delivering a “Therapeutic Dose” 239

#### 12.4 Ex Vivo Analyses 239
- 12.4.1 Urine Samples 239
- 12.4.2 Feces Samples 239
- 12.4.3 Tissue Biopsies 240
- 12.4.4 Blood Samples 240
- 12.4.5 Excess Biopsy Materials from Patients after Surgery 240

#### 12.5 Noninvasive In Vivo Imaging Techniques 240
- 12.5.1 X-Rays and CT Scans 240
- 12.5.2 NIRF Optical Imaging 241
- 12.5.3 Magnetic Resonance Imaging 241
- 12.5.4 PET Imaging 241
Contents

12.6 Role of Animal Models in Translational Cancer Research 244
  12.6.1 Steps from the “Bench” to the “Bedside” 244
  12.6.2 The In Vivo Environment: 3D, Blood Supply, Microenvironment, and Immune System 244
  12.6.3 Studies Performed in Animal Models: Biodistribution, Pharmacokinetics, Toxicity, and Efficacy 245
  12.6.4 Expertise of the Team Needed to Take New Approaches from the Bench to the Bedside 245

12.7 Types of Animal Models Available for Translational Cancer Research 245
  12.7.1 Tumors Induced by Chemicals, Irritants, and Light/Radiation 246
  12.7.2 Syngeneic Models 246
  12.7.3 Immunocompromised Animals and “Foreign” Xenografts 246
  12.7.4 Transgenic Animals 246
  12.7.5 Naturally Occurring Animal Models of Cancer 246

12.8 Naturally Occurring Cancers in Dogs as Models for Human Bladder Cancer 247
  12.8.1 Nanomedicine Approaches in Dogs 247

12.9 Organ-on-a-Chip and Human-on-a-Chip Technologies as a New Paradigm in Medicine 247
  12.9.1 What Is Organ-on-a-Chip Technology? 247
  12.9.2 Concept of Disease-on-a-Chip 249
  12.9.3 What Are Some of the Challenges in Developing and Using These New Technologies? 250
  12.9.4 What Are the Advantages and Disadvantages of Using Organ-on-a-Chip Technologies? 250
  12.9.5 What Is Human-on-a-Chip Technology, and Why Does It Represent an Important Advance? 250
  12.9.6 Sophisticated Lung Organ-on-a-Chip That Simulates Breathing 251
  12.9.7 How Organ-on-a-Chip Is Accelerating Human Drug Studies 251
  12.9.8 Will Organ-on-a-Chip Drug Research Supplant Use of Animals? 251

Chapter 12 Study Questions 254

13 Designing and Testing Nanomedical Devices 255
  13.1 Introduction to Integrated Designs 255
    13.1.1 “Total Design,” but There Is Some Order in the Design Process 255
    13.1.2 A Brief Outline of the Total Design Process 257
  13.2 Choose Autonomous or Nonautonomous Design 257
    13.2.1 If Autonomous, Will There Be Error Checking to Correct Mistargeting? 258
13.2.2 If Autonomous, Can the NMS Perform All of the Multistep Process? 258
13.2.3 If Nonautonomous, What Form of External Modulation Will Be Used In Vivo? 259
13.2.4 If Nonautonomous, Can the External Interaction Adequately Control the Entire Process? 259
13.2.5 Evaluate Reaction of NMS to External Intervention 260
13.2.6 Compare Actions of NMS with and without External Intervention 260
13.2.7 Are the Actions of the NMS Linear or Nonlinear? 260

13.3 Choose Core Material, Size, and Shape 260
13.3.1 How Will the Core Be Used for Diagnosis? 261
13.3.2 What Is the Therapeutic Radius? 261
13.3.3 Does This Dictate the Core Material Size? 261
13.3.4 Does Shape Alter Circulation Time and Target Cell Penetration Efficiency? 262
13.3.5 Evaluate Size and Shape of the NMS Core by Electron, Atomic Force, or Super-Resolution Microscopy 262
13.3.6 Evaluate Size of the Complete NMS 262
13.3.7 Evaluate Materials Present at Each Layer of Construction 263

13.4 Design NMS Targeting and Evaluate Its Effectiveness 263
13.4.1 Choose Cell Surface Biomarkers 263
13.4.2 Choose Targeting Molecule Type 264
13.4.3 Use Flow or Image Cytometry to Evaluate Correctness of Targeting to Single Diseased Cells 265
13.4.4 Finding Diseased Cells in Human Blood 266
13.4.5 Not All Monoclonal Antibodies Are the Same, and Some Are Unsuitable for Flow Cytometry 266
13.4.6 How Much Mistargeting Is Anticipated? 267
13.4.7 Determine Degree of Mistargeting and Consider the Costs of Misclassification 267
13.4.8 Based on Costs of Misclassification, Reconsider Diseased Cell Biomarkers? 268

13.5 The Need to Evaluate Intracellular Targeting 268
13.5.1 Evaluate Intracellular Targeting by TEM If the NMS Is Not Fluorescent 268
13.5.2 Evaluate Intracellular Targeting by 3D Confocal Fluorescence Microscopy 269
13.5.3 Evaluate Intracellular Targeting by 2D Microscopy If Confocal Microscopy Is Unavailable 269
13.5.4 Evaluate Intracellular Targeting by Super-Resolution Microscopy 269

13.6 Choose Zeta Potential 269
13.6.1 Determine Required Zeta Potential for Outer/Inner Layers 270
## Contents

13.6.2 Determine pH of Encountered Microenvironments 270  
13.6.3 Determine Ionic Strength of Encountered Microenvironments 271  
13.6.4 Evaluate Suitability of Zeta Potential 271  
13.6.5 If Signs of Agglomeration, Modify Zeta Potential of NMS 271  
13.6.6 Are the NMS Sticking to Any Surfaces or Cell Types? 271  
13.6.7 Are the NMS Being Rapidly Filtered by the Kidneys In Vivo? 271  
13.6.8 Are the NMS Becoming Concentrated in the Liver over Time? 272  
13.7 Choose Stealth Molecule and Test in Different Environments In Vitro 272  
13.7.1 Determine Required Time of Circulation 272  
13.7.2 How Can We Determine Circulation Time in Peripheral Blood? 273  
13.7.3 Evaluate Effectiveness of Stealth Molecules 273  
13.7.4 Do the NMS Show Signs of Protein Deposition In Vitro or In Vivo? 273  
13.7.5 Are the Circulation Times of the NMS Adequate to Target the Diseased Cells In Vivo? 273  
13.8 Choose Type and Intracellular Target of Therapy 273  
13.8.1 Eliminate or Fix the Diseased Cells? 274  
13.8.2 If the Choice Is Elimination, Choose Appropriate Therapeutic Molecule 275  
13.8.3 How to Control a Therapeutic Molecule That “Fixes” a Cell 275  
13.8.4 Choose Molecular Measure of Effectiveness of Therapy 275  
13.8.5 Use Flow Cytometry for Molecular Measurement of Cells in Suspension 275  
13.8.6 Use Scanning Image Cytometry to Measure Attached Cells 276  
13.9 A Few Final Words on Design of Integrated NMS 276  
13.9.1 We Are Still in the Early Days of Designing NMS 277  
13.9.2 How Good Must Our Understanding Be to Make a Major Improvement in Disease Treatment? 277  

Chapter 13 Study Questions 278

14 Quality Assurance and Regulatory Issues of Nanomedicine for the Pharmaceutical Industry 279  
14.1 Nanotechnology Task Force 279  
14.1.1 Findings and Recommendations of the Nanotechnology Task Force 279  
14.1.2 The Need to Reexamine the Existing Regulations to Check Their Relevance 280  
14.1.3 The Transition from the Lab to the Workplace and Environment 281
14.1.4 The Need for Some Regulation Even during the Learning Process 281
14.1.5 The Need to Determine Some Parameters for Nanomanufacturing 281
14.2 GMP-Level Manufacturing Compared to GLP 282
14.2.1 Predictable Methods Lead to Predictable Products 282
14.2.2 The Code of Federal Regulations Sections on GMPs 283
14.2.3 What Is Covered under cGMP? 283
14.2.4 Enforcement 284
14.2.5 What Can Be Learned from the Semiconductor Industry Clean Room and Manufacturing? 284
14.2.6 Why Not Just Sample the Product in “Lots” and Analyze Statistically? 284
14.2.7 Why SOPs Are So Important 285
14.3 Bionanomanufacturing 285
14.3.1 What Is So Special about Bionanomanufacturing? 286
14.3.2 Nano-Clean Water Necessary for Nanopharmaceuticals 286
14.3.3 Contaminants at the Nanolevel 287
14.3.4 Can You Scale Up the Process? 287
14.4 Some Quality Control Issues: How to Test 287
14.4.1 Correctness of Size: Size Matters! 288
14.4.2 Composition: Atomic-Level Analyses 288
14.4.3 Monodispersity versus Agglomeration 288
14.4.4 Order of Layers and Correctness of Layers 288
14.4.5 Correctness of Zeta Potentials 289
14.4.6 Does the Nanomedical System Contain the Correct Payload? 289
14.4.7 Targeting (and Mistargeting) Specificity and Sensitivity 289
14.5 Role of US Agencies Involved in the Regulation of Nanotechnology 289
14.6 FDA and Nanomedicine Regulations 290
14.6.1 How Does the FDA Think about Nanomedical Systems? 291
14.6.2 CDER and CBER Centers within the FDA 292
14.6.3 Where the FDA May Need to Meet the EPA on Nanoscale Materials 292
14.6.4 Will the FDA Revisit GRAS Products Containing Nanomaterials? 293
14.7 How Does the FDA Consider Nanomedical Systems? 293
14.7.1 Nanomedical Systems Are Integrated Nanoscale Drug and Drug Delivery Devices 294
14.7.2 Either a Drug or a Device? How about a “Combination Product”? 294
14.7.3 Drug-Biologic Combination Products 294
14.8 Types of Human Clinical Trials 294
14.8.1 IND 295
14.8.2 Phase 0 297
## Contents

14.8.3 Phase 1 ........................................ 297
14.8.4 Phase 2 ........................................ 298
14.8.5 Phase 3 ........................................ 298
14.8.6 Phase 4 ........................................ 299

14.9 EPA and Other Regulatory Agency Issues .................. 300
14.9.1 Concept of Life-Cycle Assessment ..................... 300
14.9.2 Assessing the Environmental Impact of Emergent Nanotechnologies ......................... 301
14.9.3 Toxicity of Nanomaterials .......................... 301
14.9.4 Use of Green Chemistry to Lower Toxicity to the Environment .......................... 303

14.10 Nanotechnologies and the Workplace ...................... 303
14.10.1 NIOSH: Formulating Workplace Safety Standards for Nanotechnology .......................... 304
14.10.2 Protecting Workers in the Workplace .................. 305
14.10.3 Assessing Hazards in the Workplace .................. 305
14.10.4 Establishing a Nanotechnology Safety System .......... 307

14.11 The Future of Nano Healthcare ......................... 307

Chapter 14 Study Questions ................................ 308

References ............................................. 310

Index .................................................. 323