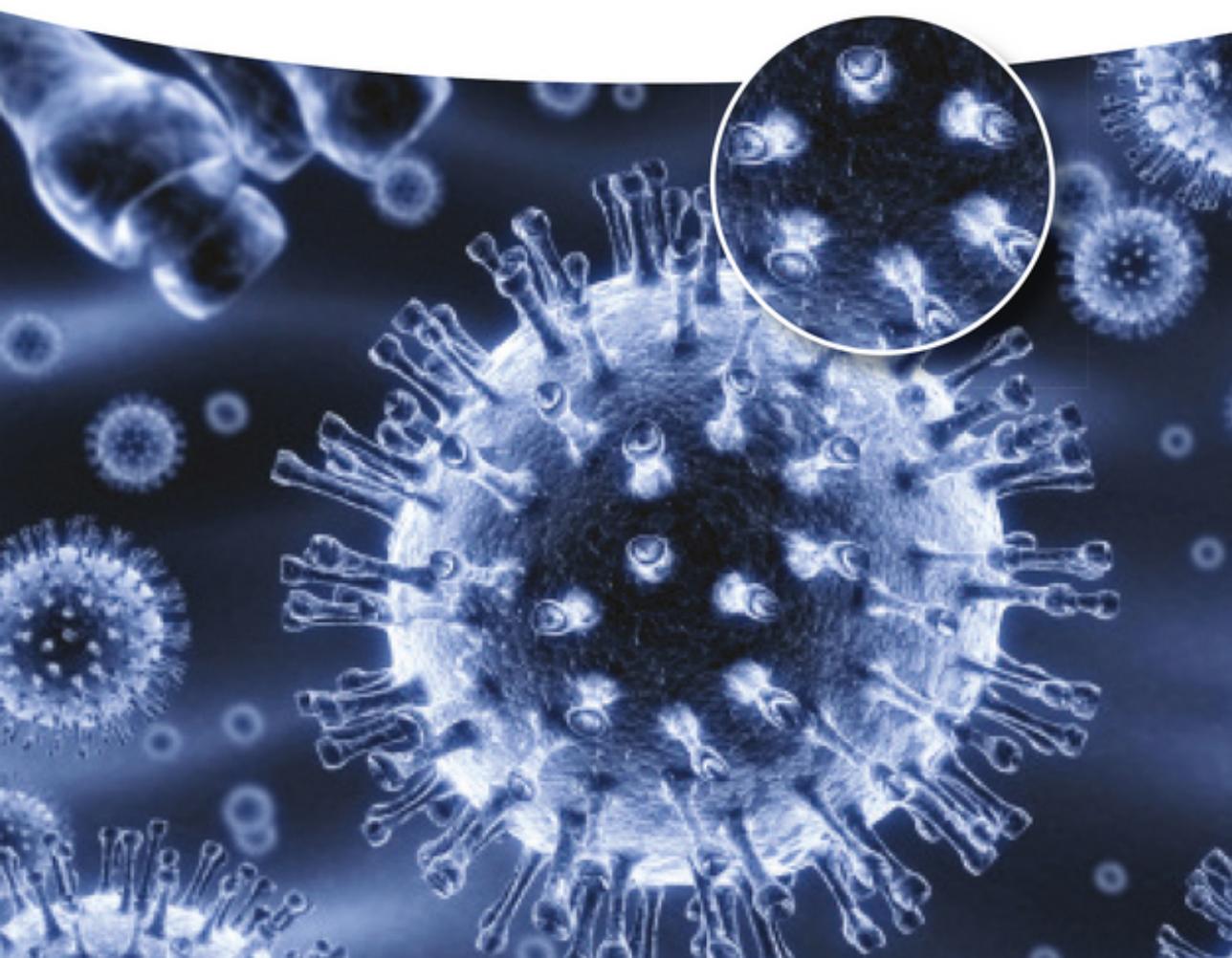


Edited by Yuliang Zhao and Youqing Shen

# Biomedical Nanomaterials





*Edited by*  
*Yuliang Zhao and Youqing Shen*

**Biomedical Nanomaterials**



*Edited by Yuliang Zhao and Youqing Shen*

## **Biomedical Nanomaterials**

**WILEY-VCH**  
Verlag GmbH & Co. KGaA

## Editors

### *Prof. Yuliang Zhao*

Chinese Academy of Sciences  
Center for Nanosciences and Technology  
19B Yuquan Road  
Beijing 100049  
China

### *Prof. Youqing Shen*

Zhejiang University  
Center for Bionanoengineering  
College of Chemical and  
Biological Engineering  
Hangzhou 310027  
PR China

All books published by **Wiley-VCH** are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

**Library of Congress Card No.:** applied for

### **British Library Cataloguing-in-Publication Data**

A catalogue record for this book is available from the British Library.

### **Bibliographic information published by the Deutsche Nationalbibliothek**

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <<http://dnb.d-nb.de>>.

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Boschstr. 12, 69469 Weinheim, Germany

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

**Print ISBN:** 978-3-527-33798-9

**ePDF ISBN:** 978-3-527-69443-3

**ePub ISBN:** 978-3-527-69441-9

**Mobi ISBN:** 978-3-527-69442-6

**oBook ISBN:** 978-3-527-69439-6

**Typesetting** SPi Global, Chennai, India  
**Printing and Binding**

Printed on acid-free paper

## Contents

### List of Contributors XV

<b>1</b>	<b>Pharmacokinetics and Pharmacodynamics (PK/PD) of Bionanomaterials</b>	<b>1</b>
	<i>Ergang Liu, Meng Zhang, and Yongzhuo Huang</i>	
1.1	Introduction	1
1.2	Commonly Utilized NMs in Pharmaceutical Research	2
1.2.1	Natural NMs	2
1.2.1.1	Lipid-Based NMs	2
1.2.1.2	Protein-Based NMs	3
1.2.1.3	Polysaccharide-Based NMs	3
1.2.2	Synthetic NMs	3
1.2.2.1	Diversity of Synthetic NMs in Forms	4
1.2.2.2	Drug Release Behaviors	4
1.2.3	Inorganic NMs	5
1.2.4	Other NMs	6
1.3	<i>In vivo</i> Biodistribution and the Evolving Targeting Principles for NMs	6
1.3.1	Organ Distribution versus Cell-Specific Targeting	6
1.3.2	Targeting Delivery Strategies	7
1.4	Processing NMs by the Biological Systems	9
1.4.1	Anatomic Basis of NMs' <i>in vivo</i> Biodistribution Behavior	10
1.4.2	Factors Affecting <i>in vivo</i> Biodistribution of NMs	11
1.4.2.1	Size	11
1.4.2.2	Zeta Potential	12
1.4.2.3	Shape and Deformability	12
1.4.2.4	Hydrophilicity and Hydrophobicity	13
1.4.3	Metabolism and Elimination of NMs	13
1.4.3.1	Common Metabolism	13
1.4.3.2	Degradable versus Nondegradable NMs	13
1.4.3.3	Free Drug versus Drug Encapsulated by NMs	13
1.5	Rational Design of Long-Circulating NMs	13
1.5.1	NMs with Optimal Physicochemical Characters	14

1.5.2	Surface Modification to Improve the Intrinsic Features of NMs	14
1.6	Mathematic Simulation of NM-Mediated Cancer Drug Delivery	15
1.6.1	Progress: From Experiment to Simulation	15
1.6.2	Compartment Models for PK Assessment of NMs	15
1.6.3	Physiologically Based Compartment Models	20
1.6.3.1	Protocols of Building a PBPK Model for NMs	21
1.6.3.2	Examples	21
1.6.4	Brief Summary	24
1.7	Experimental PK Data of the Applied NMs	25
1.7.1	PK Data of NMs Without Drugs	33
1.7.2	PK Differences Between Drugs Encapsulated by Different NMs	34
1.7.3	Reciprocal Blood and Tissue PK	40
1.7.4	PK Differences Between Different Components of the Drug-NM System	40
1.7.5	PK Variations Among Different Routes of Administration	40
1.8	Perspectives	50
1.8.1	Development of NMs	50
1.8.2	Pharmacokinetic Study and Model Development	50
	References	50
<b>2</b>	<b>Targeted Dendrimers for Cancer Diagnosis and Therapy</b>	<b>61</b>
	<i>Jingjing Hu, Ke Hu, and Yiyun Cheng</i>	
2.1	Introduction	61
2.2	Targeted Dendrimers for Cancer Therapy	63
2.2.1	Low Molecular Weight Ligand-Modified Dendrimers	63
2.2.1.1	Folic Acid-Modified Dendrimers	63
2.2.1.2	Carbohydrate-Modified Dendrimers	65
2.2.1.3	Biotin-Modified Dendrimers	66
2.2.1.4	Riboflavin-Modified Dendrimers	66
2.2.1.5	Estrogen-Modified Dendrimers	67
2.2.2	Macromolecular Ligand-Modified Dendrimers	68
2.2.2.1	Antibody-Modified Dendrimers	68
2.2.2.2	Transferrin (Tf)- and Lactoferrin (Lf)-Modified Dendrimers	69
2.2.2.3	EGF- and Fibroblast Growth Factor (FGF)-Modified Dendrimers	69
2.2.2.4	Peptide-Modified Dendrimers	70
2.2.2.5	Aptamer-Modified Dendrimers	71
2.2.2.6	Hyaluronic Acid (HA)-Modified Dendrimers	72
2.2.3	Dual-Targeting Ligand-Modified Dendrimers	72
2.3	Targeted Dendrimers for Cancer Diagnosis	73
2.3.1	Targeted Dendrimers in CT	73
2.3.2	Targeted Dendrimers in SPECT	74
2.3.3	Targeted Dendrimers in MRI	74
2.3.4	Targeted Dendrimers in NIR Fluorescence Imaging	75
2.3.5	Targeted Dendrimers in Multimodal Imaging	75

2.3.6	Targeted Dendrimers for <i>In Vitro</i> Cancer Diagnosis	77
2.4	Conclusions	77
	References	78
<b>3</b>	<b>Polymeric Micelles for Drug Delivery</b>	<b>87</b>
	<i>Wei Wu and Xiqun Jiang</i>	
3.1	Introduction	87
3.2	Amphiphilic Copolymers for Micelle Preparation	88
3.2.1	Amphiphilic Copolymers with PEG as Hydrophilic Blocks	89
3.2.2	Amphiphilic Copolymers with Poly( <i>N</i> -vinylpyrrolidone) (PVP) as Hydrophilic Blocks	90
3.2.3	Amphiphilic Copolymers with Polybetaine as Hydrophilic Blocks	91
3.3	Stability of Polymeric Micelles	91
3.4	Drug Incorporation of Polymeric Micelles	92
3.5	Functionalization of Polymeric Micelles	93
3.6	Conclusions	93
	References	94
<b>4</b>	<b>Polymeric Micelle-Based Nanomedicine</b>	<b>99</b>
	<i>Bin He</i>	
4.1	Introduction to Chemotherapy	99
4.2	Polymeric Micelle-Based Nanomedicine	100
4.2.1	Formulation of Polymeric Micelle-Based Nanomedicine	100
4.2.1.1	Size and Size Distribution	100
4.2.1.2	Surface Properties	101
4.2.1.3	Drug Loading	101
4.2.1.4	Drug Release Profiles	102
4.2.2	Interactions in Polymeric Micelle-Based Nanomedicine	102
4.2.2.1	Hydrophobic Interaction	102
4.2.2.2	Electrostatic Interaction	103
4.2.2.3	Hydrogen Bond	103
4.2.2.4	Host–Guest Interaction	103
4.2.2.5	$\pi$ – $\pi$ Stacking Interaction	103
4.2.2.6	Crystallization and Stereocomplex	104
4.2.3	Smart Drug Delivery	105
4.2.3.1	pH-Sensitive Micelles	105
4.2.4	Targeted Drug Delivery	108
4.3	Perspective	109
	References	110
<b>5</b>	<b>Microfluidics Applications in Cancer Drug Delivery</b>	<b>117</b>
	<i>Hao Zhang and Youqing Shen</i>	
5.1	Introduction	117
5.2	Basic Principles of Micellar Drug Carriers and Microfluidics	118

5.2.1	Use of Polymeric Micelles for Drug Delivery	118
5.2.2	Microfluidics as a New Solution	120
5.3	Microfluidic Fabrication of Polymer Micelles	121
5.3.1	Use of Diffusive Microfluidic Mixer to Fabricate Micelles	122
5.3.2	Use of Microarchitecture-Induced Mixing to Fabricate Micelles	126
5.3.3	Use of Droplet-Based Chaotic Mixing to Fabricate Micelles	127
5.4	On-Chip Characterization of Micelle Formation	128
5.4.1	Investigation of Self-Assembly Kinetics with High Temporal Resolution	128
5.4.2	Integrated Microfluidic Systems for High-Throughput Screening (HTS) of Copolymer Self-Assembly	131
5.4.3	Microfluidic Study of Micelle Kinetic Stability	132
5.5	Microfluidic Replications of Physiological Barriers During Delivery of Drug to Tumor	133
5.5.1	Microfluidic Models for Drug Testing	133
5.5.2	Transport Barriers of Nanomedicine to Tumors	134
5.5.3	Study of Microfluidic Micelle/Nanoparticle Vascular Transportation	135
5.5.4	Study of Microfluidic Micelle/Nanoparticle Transvascular Transportation	137
5.5.5	Use of Microfluidic Models to Investigate Tumor Interstitial Transportation	139
5.6	Conclusion and Implications for Future Research	141
	Acknowledgment	141
	References	142
<b>6</b>	<b>Antibody–Drug Conjugates</b>	<b>149</b>
	<i>Xinyu Liu and Weiping Gao</i>	
6.1	Introduction	149
6.2	History of ADCs	151
6.2.1	Concept of ADCs	151
6.2.2	First-Generation ADCs	151
6.2.3	Second-Generation ADCs	152
6.3	Components of ADCs	155
6.3.1	Drug	155
6.3.2	Antibody	158
6.3.3	Linker	161
6.3.3.1	pH-Responsive Linker	161
6.3.4	Redox-Responsive Linker	161
6.3.4.1	Enzyme-Responsive Linker	162
6.3.4.2	Noncleavable Linker	164
6.3.5	Design Strategy	165
6.4	Future Directions	167
6.4.1	Site-Specific Conjugation	167
6.4.2	Pharmacokinetics	169

6.4.3	New Paradigm Development	169
	References	170
<b>7</b>	<b>Nano-Photosensitizer for Imaging-Guided Tumor Phototherapy</b>	<b>177</b>
	<i>Zonghai Sheng, Mingbin Zheng, and Lintao Cai</i>	
7.1	Introduction for Tumor Phototherapy	177
7.1.1	PDT	177
7.1.2	PIT	178
7.1.3	PTT	178
7.2	Functionalized Nano-Photosensitizer for Tumor Targeting	178
7.2.1	PS Conjugated with Antibody	179
7.2.2	PS-Loaded Organic Nanoparticles	179
7.2.2.1	PS-Loaded Polymeric Nanomicelles	180
7.2.2.2	PS-Loaded Protein Nanoparticles	181
7.3	Nano-photosensitizer for Photodynamic Therapy	182
7.3.1	PS Conjugated Antibody for Photodynamic Therapy	183
7.3.2	PS-Loaded Nanoparticles for Photodynamic Therapy	183
7.4	Nano-Photosensitizer for Photothermal Therapy	184
7.4.1	Organic Photosensitizer for PTT	184
7.4.2	Carbon Photosensitizer for PTT	186
7.4.3	Gold Nanostructures for PTT	188
7.4.4	Other Inorganic Nanoparticles for PTT	190
7.5	Nano-Photosensitizer for Combination Therapy	191
7.5.1	Combined Photo/Chemotherapy	192
7.5.2	Combined PTT/PDT	195
7.6	Perspective and Application	197
	References	200
<b>8</b>	<b>Quantum Dots for Cancer Diagnosis</b>	<b>207</b>
	<i>Min Fang, Dai-Wen Pang, and Yan Li</i>	
8.1	Introduction	207
8.2	Detection of Solid Tumor Based on QDs	209
8.2.1	Breast Cancer (BC)	209
8.2.2	Prostate Cancer (PC)	212
8.2.3	Ovarian Cancer	212
8.2.4	Pancreatic Cancer	212
8.2.5	Liver Cancer	213
8.2.6	Lung Cancer	213
8.2.7	Other Tumors	215
8.3	SLN Mapping	215
8.4	Detection of Tumor-Associated Proteins in Blood	216
8.5	Detection of CTCs	217
8.6	Tumor Microenvironment for Invasion and Metastasis	217
8.7	Challenges of QDs into Clinical Practice Application	220

8.7.1	Biosafety	220
8.7.2	Stability and Reproducibility, Concordance, and Standard	221
8.8	Summary	221
	References	221
<b>9</b>	<b>Luminescent Gold Nanoclusters for Biomedical Diagnosis</b>	<b>227</b>
	<i>Hui Jiang and Xuemei Wang</i>	
9.1	Gold Nanostructures in Biomedical Diagnosis	227
9.2	Luminescent Au NCs for Biosensing	227
9.2.1	Detection of Reactive Oxygen Species (ROS) and Antioxidants	228
9.2.2	Detection of Heavy Metal Ions	228
9.2.3	Detection of Virus, Bacteria, and Cells	230
9.3	Au NCs for Cell Imaging	231
9.3.1	Thiols Stabilized Au NCs	231
9.3.2	Other Small-Molecule-Stabilized Au NCs	234
9.3.3	Protein-Stabilized Au NCs	236
9.3.4	Polymer-Coated Au NCs	240
9.4	Au NCs for <i>In Vivo</i> Imaging	241
9.5	Perspectives	245
	References	247
<b>10</b>	<b>Nanographene in Biomedical Applications</b>	<b>251</b>
	<i>Kai Yang and Zhuang Liu</i>	
10.1	Introduction	251
10.2	Nanographene for Drug Delivery	251
10.3	Nanographene for Gene Delivery	253
10.4	Graphene-Based Nanocomposite for Drug Delivery	255
10.5	Nanographene for Phototherapies of Cancer	259
10.5.1	Photothermal Therapy	259
10.5.2	Photodynamic Therapy	260
10.5.3	Combined Therapy Based on Nanographene	262
10.6	Graphene and its Nanocomposites for Biomedical Imaging and Imaging-Guided Therapy	263
10.6.1	Biomedical Imaging using Functionalized Nanographene	263
10.6.2	Graphene-Based Nanocomposites for Biomedical Imaging and Imaging-Guided Therapy	266
10.7	Toxicity of Nanographene	268
10.7.1	Cytotoxicity of Pristine Graphene and GO in Cell Culture	270
10.7.2	Cytotoxicity of Functionalized GO (Protein Coating, PEG Coating, etc.)	273
10.7.3	<i>In Vivo</i> Toxicity of GO and Functionalized GO After Intravenous Injection	273
10.7.4	Pulmonary Toxicity	276
10.8	Prospects and Challenges	276
	References	278

<b>11</b>	<b>Molecular Imprinting Technique for Biomimetic Sensing and Diagnostics</b>	<b>283</b>
	<i>Huiqi Zhang, Man Zhao, and Yaqiong Yang</i>	
11.1	Introduction	283
11.2	Molecularly Imprinted Polymers (MIPs)	283
11.3	MIPs for Biomimetic Sensing and Diagnostics	286
11.3.1	MIP-Based Electrochemical Sensors	287
11.3.2	MIP-Based Fluorescent Sensors	292
11.3.2.1	MIP-Based Fluorescent Sensors by Using Organic Fluorophores	293
11.3.2.2	MIP-Based Fluorescent Sensors by Using Quantum Dots (QDs)	297
11.3.3	MIP-Based SPR Sensors	300
11.3.4	MIP-Based QCM Sensors	305
11.4	Conclusions and Outlook	309
	Acknowledgments	311
	References	311
<b>12</b>	<b>Magnetic Nanostructures for MRI-Based Cancer Detection</b>	<b>327</b>
	<i>Yanglong Hou and Jing Yu</i>	
12.1	Introduction	327
12.2	Chemical Synthesis of Magnetic Nanostructures	328
12.2.1	Metal Nanoparticles	328
12.2.1.1	Iron Nanoparticles	328
12.2.1.2	Cobalt and Nickel Nanoparticles	332
12.2.2	Alloys	333
12.2.3	Metal Oxides	335
12.2.4	Metal Carbides	340
12.3	Magnetic Nanostructures for MRI-Based Cancer Detection	344
12.3.1	$T_2$ -Weighted MRI Contrast Agents	344
12.3.2	$T_1$ -Weighted MRI Contrast Agents	350
12.4	Conclusions and Perspective	354
	Acknowledgments	355
	References	355
<b>13</b>	<b>Magnetic Iron Oxide Nanoparticles: Bioapplications and Potential Toxicity</b>	<b>361</b>
	<i>Hongying Su, Yun Zeng, Chengchao Chu, and Gang Liu</i>	
13.1	Introduction	361
13.2	Bioapplications of Magnetic Iron Oxide Nanoparticles	362
13.2.1	MRI Contrast Agent	362
13.2.2	Drug Delivery	364
13.2.3	Gene Delivery	366
13.2.4	Cell Labeling and Tracking	367
13.2.5	Hyperthermia	368

13.3	Potential Toxicity of Magnetic Iron Oxide Nanoparticles	369
13.3.1	Metabolism of Magnetic Iron Oxide Nanoparticles	369
13.3.2	Mechanism of Nanotoxicity	370
13.3.3	Parameters Affecting Toxicity of Nanoparticles	371
13.3.3.1	Effect of Dose	372
13.3.3.2	Effect of Particle Size	372
13.3.3.3	Effect of Surface Charge	373
13.3.3.4	Effect of Surface Coating	374
13.3.4	Protocols for Nanotoxicity Assessment	375
13.3.4.1	<i>In Vitro</i> Cytotoxicity Test	375
13.3.4.2	<i>In Vivo</i> Toxicity Test	376
13.4	Surface Engineering for Bioapplications	377
13.5	Conclusion	379
	Acknowledgments	379
	References	379
<b>14</b>	<b>Nanostructured Hydrogels for Diabetic Management</b>	<b>387</b>
	<i>Ying Guan and Yongjun Zhang</i>	
14.1	Introduction	387
14.2	Nanostructured Hydrogels for Insulin Releasing	388
14.2.1	Glucose-Sensitive Microgels	390
14.2.2	Glucose-Sensitive Layer-by-Layer Assembled Hydrogel Films	392
14.3	Nanostructured Hydrogels for Glucose Sensing	396
14.4	Nanostructured Hydrogels in Artificial Pancreas	403
14.4.1	Hydrogels for the Generation of $\beta$ -Cell Spheroids	403
14.4.2	Hydrogels for Microencapsulation of Islets	404
14.4.3	LBL Hydrogel Films for Conformal Coating of Islets	407
14.5	Conclusions and Outlook	411
	References	412
<b>15</b>	<b>Inorganic Nanomaterials for Bone Tissue Engineering</b>	<b>421</b>
	<i>Yongxiang Luo, Chengtie Wu, and Jiang Chang</i>	
15.1	Introduction	421
15.2	Calcium Phosphate Nanomaterials for Bone Tissue Engineering	422
15.2.1	Nano-CaP Particles	422
15.2.1.1	Control Synthesis of Nano-CaP Particles	422
15.2.1.2	Interaction of CaP Nanoparticles with Bone Cells	423
15.2.2	Nano-CaP Particle/Polymer Composite	424
15.2.2.1	Preparation of Nano-CaP/Polymer Composites	424
15.2.2.2	Interaction of Nano-CaP/Polymer Composites with Bone Cells	426
15.2.2.3	<i>In Vivo</i> Study of Nano-CaP/Polymer Composites	426
15.3	CaP Blocks and Scaffolds with Surface Nanostructure	427
15.3.1	Preparation of CaP Blocks and Scaffolds with Surface Nanostructures	427

15.3.2	Interaction of Nanostructured Surface of CaP Blocks and Scaffolds with Bone Cells	428
15.3.3	<i>In Vivo</i> Study of Surface Nanostructured CaP Block and Scaffolds	429
15.4	Mesoporous Bioactive Glasses for Bone Tissue Engineering	430
15.5	Conclusions	431
	Acknowledgments	432
	References	432
<b>16</b>	<b>Nanotechnology in Coronary Artery Stent Coating</b>	<b>437</b>
	<i>Tao Liu and Junying Chen</i>	
16.1	Introduction	437
16.2	Biodegradable Polymer Coating	438
16.3	Nanocomposite Stent Coating	440
16.3.1	Carbon-Based Nanocomposites	440
16.3.2	Titanium Oxide Nanocomposites	442
16.3.3	POSS-Based Nanocomposite	443
16.4	Nanostructure in Stent Coating	443
16.4.1	Nanoporous and Nanotube	443
16.4.2	Nanoparticles	446
16.5	Bioactive Nanocoating	449
16.5.1	Extracellular Matrix Protein Coating	449
16.5.2	Cell Capture Nanocoating	451
16.5.3	Biological Induction Nanocoating	452
16.6	Summary and Future Outlook	453
	References	455
	<b>Index</b>	<b>465</b>



## List of Contributors

### *Lintao Cai*

Shenzhen Institutes of Advanced  
Technology  
Chinese Academy of  
Sciences (CAS)  
1068 Xueyuan Avenue  
Shenzhen University Town  
Shenzhen 518055  
PR China

### *Jiang Chang*

Shanghai Institute of Ceramics  
State Key Laboratory of High  
Performance Ceramics and  
Superfine Microstructure  
Chinese Academy of Sciences  
1295 Dingxi Road  
Shanghai 200050  
PR China

### *Junying Chen*

Southwest Jiaotong University  
School of Materials Science and  
Engineering  
Key Laboratory of Advanced  
Technology for Materials of  
Chinese Education Ministry  
No. 111 of North Second Ring  
Road  
Chengdu 610031  
PR China

### *Yiyun Cheng*

East China Normal University  
School of Life Sciences  
Shanghai Key Laboratory of  
Regulatory Biology  
No. 500 Dongchuan Road  
Shanghai 200241  
PR China

### *Chengchao Chu*

Xiamen University  
Center for Molecular Imaging  
and Translational Medicine  
School of Public Health  
State Key Laboratory of  
Molecular Vaccinology and  
Molecular Diagnostics  
Xiamen 361102  
PR China

***Min Fang***

Zhejiang Cancer Hospital  
Department of Radiation  
Therapy  
Zhejiang Key Laboratory of  
Radiation Oncology  
Guangji Road 38  
Hangzhou 310022  
PR China

*and*

Zhongnan Hospital of Wuhan  
University  
Hubei Cancer Clinical Study  
Center  
Department of Oncology  
Hubei Key Laboratory of Tumor  
Biological Behaviors  
Donghu Road 185  
Wuhan 430071  
PR China

***Weiping Gao***

Tsinghua University  
School of Medicine  
Department of Biomedical  
Engineering  
No. 1 Qinghuayuan  
Haidian District  
Beijing 100084  
PR China

***Ying Guan***

Nankai University  
Collaborative Innovation Center  
of Chemical Science and  
Engineering  
Institute of Polymer Chemistry  
College of Chemistry  
Key Laboratory of Functional  
Polymer Materials  
State Key Laboratory of  
Medicinal Chemical Biology  
No. 94 Weijin Road (Tianjin)  
Tianjin 300071  
PR China

***Bin He***

Sichuan University  
National Engineering Research  
Center for Biomaterials  
Chengdu 610065  
PR China

***Yanglong Hou***

Peking University  
College of Engineering  
Department of Materials Science  
and Engineering  
No. 5 Yiheyuan Road  
Haidian District  
Beijing 100871  
PR China

***Jingjing Hu***

East China Normal University  
School of Life Sciences  
Shanghai Key Laboratory of  
Regulatory Biology  
No. 500 Dongchuan Road  
Shanghai 200241  
PR China

**Ke Hu**

Renji Hospital  
 School of Medicine  
 Department of Gynecology and  
 Obstetrics  
 Shanghai Key Laboratory of  
 Gynecologic Oncology  
 No. 1630 Dongfang Road  
 Shanghai 200127  
 PR China

**Yongzhuo Huang**

Shanghai Institute of Materia  
 Medica  
 Chinese Academy of Sciences  
 501 Hai-Ke Road  
 Shanghai 201203  
 PR China

**Hui Jiang**

Southeast University  
 School of Biological Science and  
 Medical Engineering  
 State Key Laboratory of  
 Bioelectronics  
 2 Sipailou  
 Nanjing 210096  
 PR China

**Xiqun Jiang**

Nanjing University  
 College of Chemistry and  
 Chemical Engineering  
 Department of Polymer Science  
 and Engineering  
 No. 163 Xianlin Boulevard  
 Nanjing 210023  
 PR China

**Yan Li**

Capital Medical University  
 Cancer Center of Beijing Shijitan  
 Hospital  
 Department of Peritoneal Cancer  
 Surgery  
 Tieyi Road 10  
 Beijing 100038  
 PR China

*and*

Zhongnan Hospital of Wuhan  
 University  
 Hubei Cancer Clinical Study  
 Center  
 Department of Oncology  
 Hubei Key Laboratory of Tumor  
 Biological Behaviors  
 Donghu Road 185  
 Wuhan 430071  
 PR China

**Ergang Liu**

Shanghai Institute of Materia  
 Medica  
 Chinese Academy of Sciences  
 501 Hai-Ke Road  
 Shanghai 201203  
 PR China

*and*

Tianjin University  
 School of Chemical Engineering  
 and Technology  
 State Key Laboratory of  
 Chemical Engineering  
 92 Wei-jin Road  
 Tianjin 300072  
 PR China

***Gang Liu***

Xiamen University  
Center for Molecular Imaging  
and Translational Medicine  
School of Public Health  
State Key Laboratory of  
Molecular Vaccinology and  
Molecular Diagnostics  
Xiamen 361102  
PR China

***Tao Liu***

Huaiyin Institute of Technology  
School of Mechanical and  
Material Engineering  
Jiangsu Provincial Key  
Laboratory for Interventional  
Medical Devices  
No. 1 of Meicheng East Road  
Huai'an 223003  
PR China

***Xinyu Liu***

Tsinghua University  
School of Medicine  
Department of Biomedical  
Engineering  
No. 1 Qinghuayuan  
Haidian District  
Beijing 100084  
PR China

***Zhuang Liu***

Soochow University  
Institute of Functional Nano and  
Soft Materials (FUNSOM)  
199 Ren-ai Road  
Suzhou Industrial Park  
Suzhou 215123  
PR China

***Yongxiang Luo***

Shanghai Institute of Ceramics  
State Key Laboratory of High  
Performance Ceramics and  
Superfine Microstructure  
Chinese Academy of Sciences  
1295 Dingxi Road  
Shanghai 200050  
PR China

***Dai-Wen Pang***

Wuhan University  
College of Chemistry and  
Molecular Sciences  
Key Laboratory of Analytical  
Chemistry for Biology and  
Medicine (Ministry of Education)  
State Key Laboratory of Virology  
Bayi Road 299  
Wuhan 430072  
PR China

***Youqing Shen***

Zhejiang University  
Center for Bionanoengineering  
College of Chemical and  
Biological Engineering  
Hangzhou 310027  
PR China

***Zonghai Sheng***

Shenzhen Institutes of Advanced  
Technology  
Chinese Academy of  
Sciences (CAS)  
1068 Xueyuan Avenue  
Shenzhen University Town  
Shenzhen 518055  
PR China

**Hongying Su**

Kunming University of Science  
and Technology  
Faculty of Chemical Engineering  
No. 727 South Jingming Road  
Kunming 650500  
PR China

*and*

Xiamen University  
Center for Molecular Imaging  
and Translational Medicine  
School of Public Health  
State Key Laboratory of  
Molecular Vaccinology and  
Molecular Diagnostics  
Xiamen 361102  
PR China

**Xuemei Wang**

Southeast University  
School of Biological Science and  
Medical Engineering  
State Key Laboratory of  
Bioelectronics  
2 Sipailou  
Nanjing 210096  
PR China

**Chengtie Wu**

Shanghai Institute of Ceramics  
State Key Laboratory of High  
Performance Ceramics and  
Superfine Microstructure  
Chinese Academy of Sciences  
1295 Dingxi Road  
Shanghai 200050  
PR China

**Wei Wu**

Nanjing University  
College of Chemistry and  
Chemical Engineering  
Department of Polymer Science  
and Engineering  
No. 163 Xianlin Boulevard  
Nanjing 210023  
PR China

**Kai Yang**

Medical College of Soochow  
University  
School of Radiation Medicine  
and Protection  
School for Radiological and  
Interdisciplinary Sciences  
(RAD-X)  
199 Ren-ai Road  
Suzhou Industrial Park  
Jiangsu Suzhou 215123  
PR China

**Yaqiong Yang**

Nankai University  
Collaborative Innovation Center  
of Chemical Science and  
Engineering (Tianjin)  
College of Chemistry  
Key Laboratory of Functional  
Polymer Materials (Ministry of  
Education)  
State Key Laboratory of  
Medicinal Chemical Biology  
No. 94 Weijin Road  
Tianjin 300071  
PR China

**Jing Yu**

Peking University  
Department of Materials Science  
and Engineering  
College of Engineering  
No. 5 Yiheyuan Road  
Haidian District  
Beijing 100871  
PR China

**Yun Zeng**

Xiamen University  
Center for Molecular Imaging  
and Translational Medicine  
School of Public Health  
State Key Laboratory of  
Molecular Vaccinology and  
Molecular Diagnostics  
Xiamen 361102  
PR China

*and*

Sichuan University  
West China School of Preclinical  
and Forensic Medicine  
Department of Pharmacology  
No. 17 People's South Road  
Chengdu 610041  
PR China

**Hao Zhang**

Zhejiang University of  
Technology  
College of Chemical Engineering  
Hangzhou 310014  
PR China

**Huiqi Zhang**

Nankai University  
Collaborative Innovation Center  
of Chemical Science and  
Engineering (Tianjin)  
College of Chemistry  
Key Laboratory of Functional  
Polymer Materials (Ministry of  
Education)  
State Key Laboratory of  
Medicinal Chemical Biology  
No. 94 Weijin Road  
Tianjin 300071  
PR China

**Meng Zhang**

Shanghai Institute of Materia  
Medica  
Chinese Academy of Sciences  
501 Hai-Ke Road  
Shanghai 201203  
PR China

**Yongjun Zhang**

Nankai University  
Collaborative Innovation Center  
of Chemical Science and  
Engineering  
Institute of Polymer Chemistry  
College of Chemistry  
Key Laboratory of Functional  
Polymer Materials  
State Key Laboratory of  
Medicinal Chemical Biology  
No. 94 Weijin Road  
Tianjin 300071  
PR China

**Man Zhao**

Nankai University  
Collaborative Innovation Center  
of Chemical Science and  
Engineering (Tianjin)  
College of Chemistry  
Key Laboratory of Functional  
Polymer Materials (Ministry of  
Education)  
State Key Laboratory of  
Medicinal Chemical Biology  
No. 94 Weijin Road  
Tianjin 300071  
PR China

**Mingbin Zheng**

Shenzhen Institutes of Advanced  
Technology  
Chinese Academy of  
Sciences (CAS)  
1068 Xueyuan Avenue  
Shenzhen University Town  
Shenzhen 518055  
PR China



# 1 Pharmacokinetics and Pharmacodynamics (PK/PD) of Bionanomaterials

*Ergang Liu, Meng Zhang, and Yongzhuo Huang*

## 1.1

### Introduction

Nanomaterials (NMs) refer to synthetic or naturally occurring substances with size ranging from 1 to 1000 nm. The concept of “nanomaterial” was proposed by Feynman 50 years ago in the field of physics [1], which has since unveiled an era of nanotechnology. NMs contain merely tens to thousands of atoms, and are characterized by the surface and quantum size effects that are distinct from the bulk matters, and have thus gained wide applications in various areas [2]. For example, in medical application, nanotechnology has attracted specific attention in cancer therapy and diagnosis, largely due to the proposal of enhanced permeation and retention (EPR) effect by Maeda and coworkers; they demonstrated that nano-sized macromolecules displayed a preferential retention in tumor site due to the leaky vasculatures [3, 4]. The EPR effect-associated nanomedicine composed of various natural or synthetic entities in the nanoscale, which have been developed to deliver drugs/imaging agents to the tumors based on the passive targeting effect [5]. Later, in order to further increase the transport efficiency, antibodies or targeting ligands with high binding affinity to tumor-overexpressed surface antigens or receptors have been applied to conjugate onto the surface of NMs to achieve the so-called active targeting [6].

NMs can also be applied in formulation development because of their capability to improve solubility [7], drug permeation [8], and drug stability [9]. Pharmaceutical nanotechnology may thus help improve druggability of those active molecules that are otherwise considered to be unsuitable for formulation development for clinical use due to unfavorable properties such as poor solubility and low permeation to the lipid bilayer membranes [10].

The emerging nanomedicine has greatly promoted drug development, and a good number of NM-based medicine or diagnostic agents have entered clinical trials, most in the field of cancer therapy, in which the NM-based delivery strategies are characterized by EPR effect for achieving tumor targeting. However, in spite of the enhanced permeability of the tumor vasculature, not all types of NMs

could benefit from EPR effect to achieve a substantial targeting efficiency [11]. The *in vivo* ADME (absorption, distribution, metabolism, and excretion) behaviors of NMs vary because of the difference of the surface properties, size, and charges of the NMs, as well as their compositions, often leading to inconsistent therapeutic outcomes in animal studies [12].

On this account, investigation of “what the body does to NMs” may help us with a better understanding of the *in vivo* fate. We herein present a brief introduction of the commonly utilized NMs in pharmaceutical research, the anatomic features of the body and tumor, and the physiochemical natures of NMs that affect the *in vivo* fate. The established PK/PD models for simulating the *in vivo* ADME behavior of NMs will also be introduced. We hope this summary would give a glimpse into the complicated *in vivo* processes and provide helpful information for the rational design of NM-based drug delivery systems.

## 1.2

### Commonly Utilized NMs in Pharmaceutical Research

NMs can be categorized into different groups based on certain classification. To make it simple, we use the natural/synthetic classification in this chapter because the natural/synthetic NMs are generally disposed by the body in different ways. Moreover, inorganic NMs characterized by the hard-core structure bear unique physical characteristics (magnetism, thermal response to radiation, optical features, etc.) [13, 14], and are discussed as an independent section. Other resources such as cell-based NMs (e.g., RBCs [15] and MSCs [16]) and components from microbes (e.g., inactivated virus envelope [17] and TAT [18]), are usually utilized with preservation of their original natures, which are thereby discussed as a complementary to this classification.

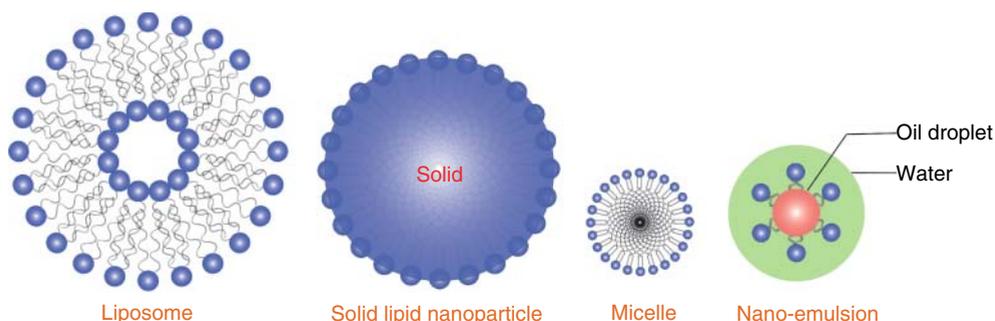
#### 1.2.1

##### Natural NMs

Natural NMs have been widely investigated because of their biodegradability and compatibility to human body. As known, lipids, proteins, carbohydrates, and nucleic acids are highly biodegradable in the body. Phospholipids are one of the most widely applied natural resources to build the nanocarriers such as liposomes and solid lipid nanoparticles (SLNs) [19]. Polysaccharides, including a variety of carbohydrates with different structures and functional groups, can be utilized to build different types of nanoparticles. Protein-based NMs (typically, serum proteins such as albumin [20], high-density lipoprotein (HDL) [21], and lactoferrin [22]) are often utilized as drug carriers.

##### 1.2.1.1 Lipid-Based NMs

Lipid-based NMs include liposomes [19], SLNs, micelles [23], and nanoemulsions [24] (Figure 1.1). The main components of liposomes are phospholipids. In aqueous solution, the phospholipids will self-assemble into a bilayer structure



**Figure 1.1** Schematic illustration of lipid-based NMs.

that functions as drug carriers with hydrophilic drugs encapsulated inside the interior, whereas hydrophobic drugs in bilayer [19]. One-tail lipids are inclined to form micelles in aqueous media [25], whereas using steric acid or oleic acid supplemented with surfactants to stabilize the solid/liquid lipids normally results in nanoparticles [26] or emulsions [27].

#### 1.2.1.2 Protein-Based NMs

Protein-based drug carriers have been widely used in pharmaceutical industry. However, owing to the concerns of protein immunogenicity (e.g., OVA, which has been utilized as adjuvant for immune activation [28]), endogenous serum-rich proteins with low immunogenicity and long half-life such as albumin, high-density lipoprotein, and lactoferrin have distinct advantages [20–22]. As a case in point, albumin-bound paclitaxel nanoparticles (Abraxane<sup>®</sup>) have attained great market success [29]. In general, proteins can either be processed to form nanoparticles [30] or directly coupled with drugs by physical adsorption or via covalent bonds [20]. In certain instances, the protein carriers are further modified with targeting ligands to achieve specific delivery [31].

#### 1.2.1.3 Polysaccharide-Based NMs

Polysaccharides originate from animal, plant, or bacterial sources. In general, the physicochemical properties of polysaccharides are governed by monosaccharide unit and the overall molecular weight [32]. The high molecular weight molecules, such as heparin and hyaluronic acid, show strong affinity to water molecules, and thus form hydrogels that have been widely applied for local administration because of their biocompatibility and sustained drug release functions [33]. The ionic polysaccharides can bind with molecules of the opposite charge, and the interaction normally leads to decreased solubility and the formation of nanoparticles [34].

### 1.2.2

#### Synthetic NMs

Although NMs based on naturally occurring materials have the advantages of biocompatibility and wide availability, structure modification is difficult

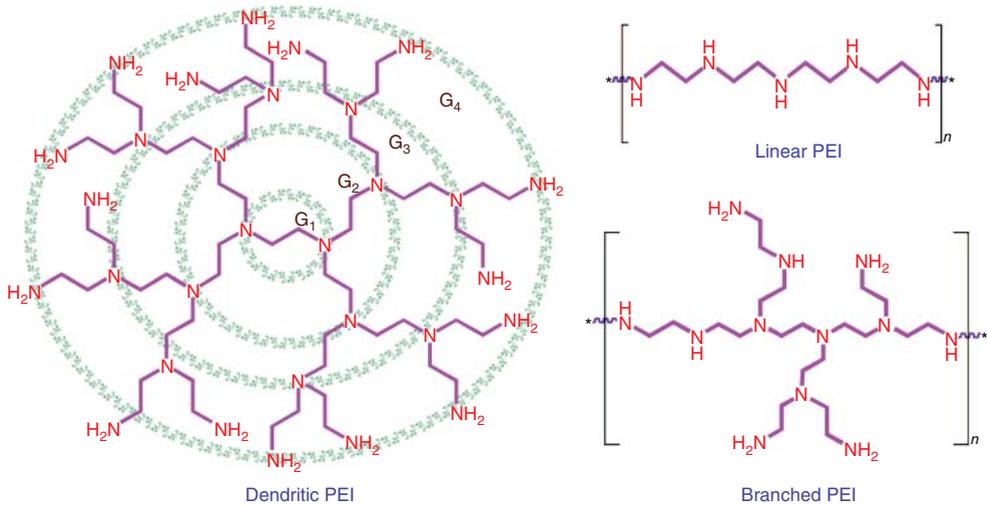


Figure 1.2 Various forms of PEI.

to process to tailor their functions to satisfy the needs from pharmaceutical application.

By contrast, synthetic polymers can be much more flexibly designed for a specific application. For example, by using the pH-sensitive synthetic materials, the NMs could release drugs in a pH-dependent manner for achieving tumor-targeting delivery [35], because the rapidly proliferic neoplastic tissues normally secrete more lactose from the hyperactive anaerobic glycolysis, leading to a decreased pH in tumor microenvironment [36].

#### 1.2.2.1 Diversity of Synthetic NMs in Forms

Synthetic NMs can be tailored for different purposes. For instance, polyethylenimine (PEI) can be synthesized in the forms of linear, branched, or dendritic structures (Figure 1.2) [37]. Synthetic polymers can be fabricated into various types of NMs such as nanoparticles, micelles, and nanocubes. For example, poly(lactic-*co*-glycolic acid) (PLGA) can be made into widely applied nanoparticles [38], microspheres [39], and micelles [40].

NMs are often used as carriers for small molecular drugs. Drugs are loaded into NMs by encapsulation or via a covalent linkage, in order to improve the PK profiles and achieve targeting delivery to a specific site.

#### 1.2.2.2 Drug Release Behaviors

Drug release from NMs is governed by the physiochemical properties of the drug and NMs. Burst release is often seen for the hydrophobic drugs in capsulation by liposomes, while sustained release for the hydrophilic drugs in liposomes, which are slow to diffuse across the lipid bilayers [41]. However, it is more complex to

investigate the drug release profile when NMs are injected to the body, and knowledge of the *in vitro in vivo* correlation (IVIVC) is still insufficient.

In order to reduce the unwanted drug exposure, a number of strategies have been developed to achieve a site-specific release of the loaded cargos.

#### **pH-sensitive NMs**

The slightly acidic tumor environment and endosome's even lower pH have attracted extensive interests in the application for designing NMs with the ability to respond to pH changes during the delivery. This strategy has been intensively explored by employing the polycationic dendrimers such as PEI and PAMAM. Acidic pH could cause the electrostatic repulsions between side chains in these polyamines because of the protonation of amino groups. As a result, the dendrimers swell in response to the acidic condition and the abrupt pH drop – the so-called “proton sponge” effect [42]. After engulfed by the cells, the swelling of NMs can lead to endosome rupture, and thus is favorable for intracellular drug release.

Another strategy is to use pH-sensitive linkage (e.g., hydrazone bonds) for cross-linking and building the NMs [43]. The NMs disassemble in a pH-dependent pattern, thus triggering drug release in acidic environments.

#### **Redox-sensitive NMs**

Besides the decreased pH, the rapidly growing tumor is also characterized by the intracellular reducibility due to the increased level of glutathione (GSH) [42]. The redox-sensitive NMs (e.g., NMs built via disulfide linkage) can display an accelerated drug release once entering the tumor cells.

#### **Enzyme-sensitive NMs**

Tumor-associated proteases have been widely investigated for their application as biomarkers in cancer diagnosis, prognosis, and therapy. Overexpression of tumor-associated proteases (e.g., MMP-2 [44], MMP-9 [45], and legumain [46]) in tumors provides ideal targets for the design of “smart” NMs with controlled release. A general strategy is to use a specific substrate peptide to modify the NMs, and the cleavage of the peptide would trigger drug release or cellular uptake.

#### **Thermo/radio wave-sensitive NMs**

Other stimulus-responsive NMs can respond to external physical stimulation (such as localized heating and electromagnetic radiation) and have been applied in drug delivery [47]. Specificity of this strategy is largely dependent on the precise control of the applied stimuli at the target sites.

### 1.2.3

#### **Inorganic NMs**

Inorganic NMs are distinguished from the organic NMs (soft matters) with the hard cores. In order to avoid aggregation in aqueous media, the inorganic cores are typically modified with surfactants or hydrophilic polymers to form a core–shell structure [48]. Of note, the *in vivo* biofate of inorganic NMs is greatly affected by the surface characteristics of the coating materials [49].

Inorganic NMs exhibit many unique physical properties – for example – fluorescence (quantum dots), superparamagnetism (iron oxide nanoparticles), photothermal effect (gold nanorods, carbon nanotubes), or special optical properties (silver nanoparticles) [13, 50]. These properties of the inorganic NMs have been utilized in cancer diagnosis and treatment applications.

#### 1.2.4

##### Other NMs

Together with the rapid development of NMs, knowledge of NMs has accumulated. Proteins, in terms of the size, can also be viewed as bionanomaterials, which are rich in the body. As a case in point, albumin (MW 67 kDa) with a diameter around 7 nm [51] can serve as a unique “protein carrier” for drugs. Another example is the red blood cells (RBCs), with diameter from several to tens of micrometers [15], which may be regarded as a type of “microliposomes” to deliver therapeutic macromolecules. Moreover, even the protein capsids of a virus (size <100 nm) can be used as a “nanocapsule” [52]. Inspired by biomimetics, these physiologically originated NMs have been explored as novel carriers for drug delivery.

Moreover, there is another form of nanodrugs – the nanocrystals of hydrophobic drugs [53]. Such nanodosage forms can solve the solubility problem, and furthermore improve the PK profiles.

### 1.3

#### *In vivo* Biodistribution and the Evolving Targeting Principles for NMs

The targeting strategies have been mostly employed in cancer therapy areas. In general, there are two types of targeting strategies: the passive targeting via EPR effect and the active targeting mediated by antibodies or ligands that can specifically bind with receptors on cancer cells. Recently, with the growing knowledge of tumor physiology, the tumor microenvironments (e.g., acidic pH and the over-expressed proteases) have been used as a target for cancer drug delivery [6]. A combination of the targeting strategies by using multifunctional NMs has also been investigated for achieving improved specific targeted delivery and controlled release [54]. The ultimate goal is to increase drug concentration in tumor while reducing its exposure to the healthy organs.

#### 1.3.1

##### Organ Distribution versus Cell-Specific Targeting

Ideally, NMs should be able to deliver the cargo drugs specifically into a target site. The *in vivo* fate of NMs is determined by a combination of multiple factors, including particle size, shape, and surface characteristics [55]. It is thus difficult to assess the overall targeting efficiency from *in vitro* data (e.g., cellular uptake by a