

## Contents

### Preface *xiii*

- 1 Micro/Nanostructured Materials from Droplet Microfluidics 1**  
*Xin Zhao, Jieshou Li, and Yuanjin Zhao*
  - 1.1 Introduction 1
  - 1.2 MMs from Droplet Microfluidics 4
    - 1.2.1 Simple Spherical Microparticles (MPs) 4
    - 1.2.2 Janus MPs 7
    - 1.2.3 Core–Shell MPs 7
    - 1.2.4 Porous MPs 9
    - 1.2.5 Other MMs 10
  - 1.3 NMs from Droplet Microfluidics 13
    - 1.3.1 Inorganic NMs 13
    - 1.3.2 Organic NMs 16
    - 1.3.3 Other NMs 16
  - 1.4 Applications of the Droplet-Derived Materials 18
    - 1.4.1 Drug Delivery 18
    - 1.4.2 Cell Microencapsulation 23
    - 1.4.3 Tissue Engineering 25
    - 1.4.4 Biosensors 29
    - 1.4.5 Barcodes 32
  - 1.5 Conclusion and Perspectives 35
  - References 36
  
- 2 Digital Microfluidics for Bioanalysis 47**  
*Qingyu Ruan, Jingjing Guo, Yang Wang, Fenxiang Zou, Xiaoye Lin, Wei Wang, and Chaoyong Yang*
  - 2.1 Introduction 47
  - 2.2 Theoretical Background 48
    - 2.2.1 Theoretical Background 48
      - 2.2.1.1 Thermodynamic Approach 49
      - 2.2.1.2 Energy Minimization Approach 50
      - 2.2.1.3 Electromechanical Approach 52
    - 2.2.2 Contact Angle Saturation 53
    - 2.2.3 Basic Microfluidic Functions by EWOD Actuation 53
  - 2.3 Device Fabrication 55

2.4	Digital Microfluidics Integrated with Other Devices	56
2.4.1	Sample Processing Systems Integrated with Digital Microfluidics	56
2.4.1.1	World-to-chip Interface	56
2.4.1.2	Magnet Separation	58
2.4.1.3	Heater Module	59
2.4.2	Detection Systems Integrated with Digital Microfluidics	59
2.4.2.1	Optical Methods	59
2.4.2.2	Electrochemical Methods	61
2.4.2.3	Other Detection Methods	62
2.5	Biological Applications on DMF	63
2.5.1	Enzyme Assays	63
2.5.2	Immunoassay	63
2.5.3	DNA-Based Applications	66
2.5.4	Cell-Based Applications	68
2.6	Conclusions and Perspectives	72
	References	73
<b>3</b>	<b>Nanotechnology and Microfluidics for Biosensing and Biophysical Property Assessment: Implications for Next-Generation <i>in Vitro</i> Diagnostics</b>	<b>83</b>
	<i>Zida Li and Ho Cheung Shum</i>	
3.1	Introduction	83
3.1.1	Nanotechnology and Microfluidics	84
3.2	Fundamentals of Nanotechnology and Microfluidics	86
3.2.1	Nanotechnology	86
3.2.2	Microfluidics	87
3.3	Biomolecule Sensing	88
3.3.1	Techniques Based on Optical Readout	89
3.3.1.1	Localized Surface Plasmon Resonance	89
3.3.1.2	Surface-Enhanced Raman Spectroscopy	90
3.3.1.3	Nanoengineered Fluorescence Probes	91
3.3.1.4	Nanotopography-Based Cell Capturing	93
3.3.2	Techniques Based on Electrical Readouts	93
3.3.2.1	Electrochemical Reactions	93
3.3.2.2	Nanotransistor-Based Assays	94
3.4	Biophysical Property Sensing	95
3.4.1	Cell Contractility Measurement	96
3.4.2	Cell Deformability	98
3.4.3	Fluid Rheology	99
3.4.4	Electrophysiology	99
3.5	Concluding Remarks	100
	Acknowledgments	100
	References	101
<b>4</b>	<b>Microfluidic Tools for the Synthesis of Bespoke Quantum Dots</b>	<b>109</b>
	<i>Shangkun Li, Jeff C. Hsiao, Philip D. Howes, and Andrew J. deMello</i>	
4.1	Introduction	109

4.1.1	Microfluidics in the Chemical and Biological Sciences	109
4.1.2	Compound Semiconductor Nanoparticles	109
4.1.3	Microfluidic Tools for Nanoparticle Synthesis	112
4.2	Design Considerations	114
4.2.1	Continuous-Flow Microfluidics	115
4.2.2	Segmented-Flow Microfluidics	115
4.3	Continuous-Flow Microfluidic Synthesis of Quantum Dots	118
4.3.1	Homogenous Core-Type Quantum Dots in Continuous Flow	118
4.3.1.1	Cadmium Sulfide (CdS)	118
4.3.1.2	Cadmium Selenide (CdSe)	119
4.3.2	Heterogenous Core/Shell Quantum Dots in Continuous Flow	121
4.3.2.1	Zinc Selenide/Zinc Sulfide (ZnSe/ZnS)	121
4.3.2.2	Cadmium Selenide/Zinc Sulfide (CdSe/ZnS) and Cadmium Telluride/Zinc Sulfide (CdTe/ZnS)	121
4.3.2.3	Copper Indium Sulfide/Zinc Sulfide (CuInS <sub>2</sub> /ZnS)	123
4.3.2.4	Indium Phosphide/Zinc Sulfide (InP/ZnS)	125
4.3.3	Heterogenous Core/Multishell Quantum Dots in Continuous Flow	125
4.3.3.1	Cadmium Selenide/Cadmium Sulfide/Zinc Sulfide (CdSe/CdS/ZnS)	126
4.3.4	Summary of QD Classes	128
4.4	Segmented-Flow Microfluidic Synthesis of Quantum Dots	128
4.4.1	Homogenous Structure Quantum Dots in Segmented Flow	129
4.4.1.1	Cadmium Sulfide (CdS)	129
4.4.1.2	Cadmium Selenide (CdSe)	130
4.4.1.3	Lead Sulfide (PbS) and Lead Selenide (PbSe)	131
4.4.1.4	Perovskite QDs	132
4.4.2	Heterogenous Core/Shell Quantum Dots in Segmented Flow	134
4.4.2.1	Copper Indium Sulfide/Zinc Sulfide (CuInS <sub>2</sub> /ZnS)	134
4.4.3	Multistep Synthesis of QDs in Segmented Flow	135
4.4.4	Nucleation and Growth Studies of Quantum Dots	138
4.5	Conclusions and Outlook	140
	References	141
<b>5</b>	<b>Microfluidics for Immuno-oncology</b>	<b>149</b>
	<i>Chao Ma, Jacob Harris, Renee-Tyler T. Morales, and Weiqiang Chen</i>	
5.1	Introduction	149
5.2	Microfluidics for Single Immune Cell Analysis	153
5.2.1	Single Immune Cells	153
5.2.1.1	T Cells	153
5.2.1.2	MΦs	156
5.2.1.3	DCs	157
5.2.1.4	B Cells	158
5.2.2	Microfluidics for Immune and Tumor Cell Interaction Analysis	159
5.2.2.1	T-cell Priming and Activation by APCs	159
5.2.2.2	Killing of Cancer Cells by Immune Effector Cells	162
5.2.2.3	Interaction Between Cancer Cells and MΦs	163
5.3	Microfluidics for Tumor Immune Microenvironment Analysis	163

5.3.1	Modeling the Tumor Immune Microenvironment	163
5.3.1.1	T-cell Trafficking and Migration	164
5.3.1.2	T-cell Priming and Activation by APCs	165
5.3.1.3	APC Processing and Presentation of TAAs	165
5.3.1.4	Interaction Between Cancer Cells and MΦs	166
5.3.2	On-chip Testing of Tumor Immunotherapy	166
5.3.2.1	TCR T Cells	167
5.3.2.2	Immune Checkpoint Blockade	167
5.4	Concluding Remarks and Future Perspectives	170
	Acknowledgments	171
	References	172
<b>6</b>	<b>Paper and Paper Hybrid Microfluidic Devices for Point-of-care Detection of Infectious Diseases</b>	<b>177</b>
	<i>Hamed Tavakoli, Wan Zhou, Lei Ma, Qunqun Guo, and XiuJun Li</i>	
6.1	Introduction	177
6.2	Fabrication of Paper-Based Microfluidic Devices	179
6.2.1	Fabrication Techniques for Paper-Based Microfluidic Platforms	179
6.2.1.1	Physical Blocking of Pores in Paper	180
6.2.1.2	Physical Deposition of Reagents on Paper Surface	181
6.2.1.3	Chemical Modification	182
6.2.1.4	Other Techniques	183
6.2.2	Fabrication of Paper Hybrid Microfluidic Devices	183
6.3	Application of Paper and Paper Hybrid Microfluidic Devices for Infectious Disease Diagnosis	184
6.3.1	Colorimetric Detection	185
6.3.2	Fluorescence Detection	187
6.3.3	Electrochemical Detection	191
6.4	Integration of Nanosensors on Paper and Paper Hybrid Microfluidic Devices for Infectious Disease Diagnosis	193
6.4.1	Carbon-Based Nanosensors	195
6.4.2	Gold-Based Nanosensors	198
6.4.3	Other Nanosensors	200
6.5	Summary and Outlook	202
	Acknowledgment	202
	References	203
<b>7</b>	<b>Biological Diagnosis Based on Microfluidics and Nanotechnology</b>	<b>211</b>
	<i>Navid Kashaninejad, Mohammad Yaghoobi, Mohammad Pourhassan-Moghaddam, Sajad R. Bazaz, Dayong Jin, and Majid E. Warkiani</i>	
7.1	Introduction	211
7.2	Quantum Dot-Based Microfluidic Biosensor for Biological Diagnosis	212
7.2.1	Qdot-Based Disease Diagnosis Using Microfluidics	213
7.3	Upconversion Nanoparticles	219

7.4	Fluorescent Biodots	221
7.5	Digital Microfluidic Systems for Diagnosis Detection	223
7.6	Paper-Based Diagnostics	226
7.6.1	Structure and Chemistry of Paper	226
7.6.2	Applications of Paper-Based Devices in the Diagnostics	227
7.6.2.1	Labeled Biosensing	228
7.6.2.2	Label-Free Biosensing	228
7.6.3	Integration of Nanoparticles with Paper-Based Microfluidic Devices	228
7.6.3.1	Gold Nanomaterials	228
7.6.3.2	Fluorescent Nanomaterials	229
7.7	Conclusion and Future Perspective	231
	Conflicts of Interest	231
	Acknowledgment	231
	References	232
<b>8</b>	<b>Recent Developments in Microfluidic-Based Point-of-care Testing (POCT) Diagnoses</b>	<b>239</b>
	<i>Dong Wang, Ho N. Chan, Zeyu Liu, Sean Micheal, Lijun Li, Dorsa B. Baniani, Ming J. A. Tan, Lu Huang, Jiantao Wang, and Hongkai Wu</i>	
8.1	Introduction	239
8.2	Cell	240
8.2.1	Blood Cell Counting	240
8.2.2	Characterization of CD64 Expression	241
8.2.3	Enumeration of CD4+ T Lymphocytes for HIV Monitoring	242
8.2.4	Circulating Tumor Cell (CTC) Isolation and Analysis	243
8.3	Nucleic Acid	245
8.3.1	Nonisothermal Amplification	245
8.3.2	Isothermal Amplification	246
8.4	Protein	253
8.4.1	Novel Chemistry and Nanomaterials	253
8.4.2	3D-Printed Microfluidic Devices	256
8.4.3	Digital and Droplet Microfluidics	259
8.5	Metabolites and Small Molecules	262
8.6	Conclusion and Outlook	271
	Acknowledgments	271
	References	271
<b>9</b>	<b>Microfluidics in Microbiome and Cancer Research</b>	<b>281</b>
	<i>Barath Udayasuryan, Daniel J. Slade, and Scott S. Verbridge</i>	
9.1	Introduction	281
9.2	What is the Microbiome?	282
9.2.1	Composition and Biogeography	282
9.2.2	The Microbiome and Cancer	285
9.2.3	<i>Helicobacter pylori</i> and Gastric Cancer	286
9.2.4	<i>Fusobacterium nucleatum</i> and CRC	287
9.2.5	Bacterial Invasion	288

9.3	Studying the Microbiome	289
9.3.1	2D Models	291
9.3.2	3D Models	291
9.3.3	Organ-on-a-Chip and the Application of Microfluidics	295
9.4	Microfluidic Intestine Chip Models	297
9.4.1	Gut-on-a-Chip Model	297
9.4.2	Co-culture of the Gut-on-a-Chip with Microbiota	298
9.4.3	The HuMiX Model	299
9.4.4	Anaerobic Human Intestine Chip	301
9.4.5	Anoxic-Oxic Interface (AOI)-on-a-Chip	303
9.4.6	Future Directions	304
9.5	Concluding Remarks and Future Perspectives	306
	Acknowledgments	308
	References	308
<b>10</b>	<b>Microfluidic Synthesis of Functional Nanoparticles</b>	<b>319</b>
	<i>Ziwei Han and Xingyu Jiang</i>	
10.1	Introduction	319
10.2	Fabrication of Microfluidic Chips	320
10.2.1	Fabrication of Microchannels: Photolithography	321
10.2.2	Fabrication of PDMS-Based Microfluidic Chips	321
10.2.3	Pressure Tolerance	321
10.3	Microfluidic Synthesis of Functional Nanoparticles	323
10.3.1	Mixing Strategy	323
10.3.1.1	Hydrodynamic Focusing	323
10.3.1.2	Microstructure to Enhance Mixing Efficiency	324
10.3.2	Bionanoparticle Interactions	325
10.3.2.1	Well-Controlled Size and Monodispersity	325
10.3.2.2	Surface Modification	326
10.3.2.3	Mechanical Properties	327
10.3.2.4	Controllable Multilayer Structure	328
10.4	Microfluidic Assembly of Nanoparticles for Biological and Medical Applications	329
10.4.1	Drug Delivery	330
10.4.1.1	pH-Sensitive Drug Release	330
10.4.1.2	Hydrophilic Drug Delivery	331
10.4.1.3	Photoresponsive Drug Release	332
10.4.1.4	Gene Delivery	332
10.4.2	Imaging	332
10.4.2.1	MRI	332
10.4.2.2	Fluorescence Imaging	333
10.4.2.3	Ultrasonic Imaging	334
10.4.3	Biosensing	334
10.4.4	Theranostics	336
10.5	Prospects of Microfluidic Synthesis	337
	Acknowledgment	338
	References	339

<b>11</b>	<b>Design Considerations for Muscle-Actuated Biohybrid Devices</b>	<b>347</b>
	<i>Yoshitake Akiyama, Sung-Jin Park, and Shuichi Takayama</i>	
11.1	Introduction	347
11.2	Characteristics and Applicability of Muscles for Biohybrid Devices	348
11.2.1	Heart Muscle (Cardiomyocytes)	348
11.2.2	Skeletal Muscle Cells	350
11.2.3	Smooth Muscle Cells	351
11.2.4	Nonmammalian Muscle Cells	352
11.3	Arrangement of Muscle Cells and Tissues on Biohybrid Devices	352
11.3.1	Interfaces Between Muscle Cells and Material	353
11.3.1.1	Interfaces in 2D Culture	353
11.3.1.2	Interfaces in 3D Culture	354
11.3.2	Mechanical Pairing of Muscles	355
11.3.3	Interface Between Medium and Air	356
11.4	Oxygen Supply in Muscle Tissue Engineering	356
11.4.1	Equation and Conditions for Numerical Simulations	357
11.4.2	Oxygen Distribution under Static Culture	357
11.4.3	Oxygen Distribution in Microfluidic Devices	359
11.4.4	Other Approaches to Improve Oxygen Supply	360
11.5	Contractile Force of Muscle Bundles and Stimulations	361
11.5.1	Tissue-Engineered Muscle Consisting of C2C12 Cells	361
11.5.2	Tissue-Engineered Muscle Consisting of Primary Myoblasts	364
11.6	Control of Muscle Contractions	366
11.6.1	Electrical Stimulation	366
11.6.2	Optical Stimulation	367
11.6.3	Others	368
11.7	Conclusions and Future Challenges	368
11.7.1	Completely 3D-Printed Biohybrid Devices	368
11.7.2	Integration with Other Tissues	369
11.7.3	Long-Term Maintenance and Self-healing	369
11.7.4	Exploring Applications	370
	Acknowledgments	370
	References	370
<b>12</b>	<b>Micro- and Nanoscale Biointerrogation and Modulation of Neural Tissue – From Fundamental to Clinical and Military Applications</b>	<b>383</b>
	<i>Jordan Moore, Diego Alzate-Correa, Devleena Dasgupta, William Lawrence, Daniel Dodd, Craig Mathews, Ian Valerio, Cameron Rink, Natalia Higueta-Castro, and Daniel Gallego-Perez</i>	
12.1	Introduction	383
12.2	General Principles	385
12.2.1	Physics of Miniaturized Systems	385
12.2.2	Material Properties	385
12.3	Areas of Study	386

12.3.1	Neurodevelopment	386
12.3.2	Neuro-oncology	388
12.3.3	Neurodegenerative Disorders	389
12.3.4	Traumatic Brain Injury	392
12.4	Applications	394
12.4.1	Neuron-Directed Cellular Reprogramming	394
12.4.2	Tissue Nanotransfection	396
12.4.3	Cancer Interrogation	398
12.4.4	FISH On-Chip for Alzheimer's Disease	401
12.4.5	On-chip Brain Injury	403
12.4.6	Military	405
12.5	Limitations and Future Outlook	406
12.6	Summary	407
	References	408
	<b>Index</b>	<b>419</b>