Contents

List of Contributors XIXPreface XXV

1	Synthesis, Functionalization, and Characterization 1
	Jianxun Xu, Xing Lu, and Baowen Li
1.1	Introduction 1
1.2	Fullerenes and Metallofullerenes 1
1.2.1	Synthesis and Purification 2
1.2.1.1	Synthesis 2
1.2.1.2	Purification 2
1.2.2	Chemical Functionalization 3
1.2.2.1	Carbene Reaction 3
1.2.2.2	Bingel – Hirsch Reaction 4
1.2.2.3	Prato Reaction 5
1.2.2.4	Bis-Silylation 5
1.2.2.5	Diels – Alder Reaction and Benzyne Reaction 5
1.2.2.6	Singly Bonded Addition 6
1.2.2.7	Supramolecular Complexes of EMFs 6
1.2.3	Characterization 6
1.2.3.1	Synchrotron Radiation Powder Diffraction
	(SRPD)/Rietveld/MEM 6
1.2.3.2	Nuclear Magnetic Resonance (NMR) Spectroscopy
1.2.3.3	Theoretical Calculation 7
1.2.3.4	Single-Crystal X-ray Diffraction Crystallography 7
1.2.3.5	Others 8
1.2.4	Questions and Future Directions 8
1.3	Carbon Nanotubes 8
1.3.1	Synthesis 9
1.3.1.1	Arc Discharge Method 9
1.3.1.2	Laser Ablation Method 10
1.3.1.3	CVD Method 10
1.3.1.4	Synthesis of CNTs with a Defined Structure 10
1.3.2	Functionalization 11



VIII	Contents	
	1.3.2.1	Covalent Chemical Reactions 11
	1.3.2.2	Noncovalent Modifications 11
	1.3.3	Characterization 12
	1.3.3.1	Microscopic Characterizations 12
	1.3.3.2	Spectroscopic Characterizations 13
	1.3.4	Questions and Future Directions 13
	1.4	Graphene 14
	1.4.1	Synthesis and Characterization 14
	1.4.2	Functionalization of Graphene and Graphene Oxide 17
	1.4.3	Prospects and Challenges 18
	1.5	Summary and Outlook 20
		References 21
	2	Identification and Detection of Carbon Nanomaterials in Biological Systems 29
		Haifang Wang, Zheng-Mei Song, Yi-Fan Yang, Aoneng Cao, and Yuanfang Liu
	2.1	Introduction 29
	2.2	Available Techniques for Qualitative and Quantitative
	2.2	Determination 30
	2.2.1	Optical Microscopic Observation 30
	2.2.2	Electron Microscopic (EM) Observation 31
	2.2.3	Raman Spectroscopic Measurement 33
	2.2.4	Fluorescence Analysis 36
	2.2.4.1	Intrinsic Fluorescence Analysis 36
	2.2.4.2	Labeled Fluorescence Analysis 39
	2.2.5	Isotope Labeling Method 39
	2.2.5.1	Radioisotope Labeling 40
	2.2.5.2	Stable Isotope Labeling 43
	2.2.5.3	Tips for Isotopic Labeling 43
	2.2.6	Chromatographic Technique 45
	2.2.7	Flow Cytometry Method 45
	2.2.8	Other Methods 46
	2.3	Summary and Outlook 47
		Acknowledgments 48
		References 48
	3	Biodistribution and Pharmacokinetics of Carbon Nanomaterials
		In Vivo 55
		Sheng-Tao Yang, Xiaoyang Liu, and Jingru Xie
	3.1	Introduction 55
	3.2	Amorphous Carbon Nanoparticles 55
	3.2.1	Ultrafine Carbon Particles 56
	3.2.2	Carbon Nanoparticles 58
	3.2.3	Carbon Dots 59
	3.3	sp ² Carbon Nanomaterials 62

3.3.1 3.3.2 3.3.3 3.3.4 3.3.5 3.4 3.5	Fullerene 62 Carbon Nanotubes 69 Carbon Nanohorns 77 Graphene 80 Graphene Quantum Dots 85 Nanodiamonds 87 Summary and Outlook 89 Acknowledgments 90 References 90
4	Interaction of Carbon Nanomaterials and Components in Biological Systems 97
4.1	Jian Tian and Cuicui Ge
4.1 4.2	Introduction 97
4.2.1	Factors Affecting Interaction 99 Characteristics of Carbon Nanomaterials 99
4.2.1.1	Size and Layer 99
4.2.1.1	Surface Modification and Functionalization 100
4.2.1.2	Biological Microenvironment 102
4.2.2.1	pH 103
4.2.2.2	Ionic Strength 104
4.2.2.3	Weak Interactions 104
4.2.2.4	Cell Selectivity 105
4.3	Interaction of Carbon Nanomaterials with Various Components in
1.0	Biological Systems 107
4.3.1	Characterization and Methodology of Interaction of Carbon
	Nanomaterials with Components in the Biological System 107
4.3.2	Carbon Nanomaterial – Phospholipid Interaction 108
4.3.3	Carbon Nanomaterial – Protein Interaction 111
4.3.4	Carbon Nanomaterial – DNA Interaction 115
4.3.5	Carbon Nanomaterial – Cell Interaction 119
4.4	Conclusion and Perspectives 120
	References 122
5	Biomedical Applications of Carbon Nanomaterials 131
r 1	Liangzhu Feng and Zhuang Liu Introduction 131
5.1	
5.2 5.2.1	Biomedical Applications of Fullerenes 132 Fullerenes as Antioxidants and Neuroprotective Agents 132
5.2.2	Fullerenes as Antitumor Agents 134
5.2.3	Metallofullerenes as MRI Contrast Agent 136
5.2.4	Fullerenes for Other Applications 136
5.2.4	Biomedical Applications of Carbon Nanotubes 137
5.3.1	Carbon Nanotubes for Drug Delivery 138
5.3.1.1	Carbon Nanotubes for the Delivery of Small Drug Molecules 139
0.0.1.1	Carbon Handlabes for the Delivery of Small Drug Molectures 139

x	Contents	
	5.3.1.2	Carbon Nanotubes for the Delivery of Biomacromolecules 141
	5.3.2	Carbon Nanotubes for Photothermal and Combined Therapies of Tumors 142
	5.3.2.1	Carbon Nanotubes for Photothermal Therapy of Tumors 142
	5.3.2.2	Carbon Nanotubes for Combined Therapies of Tumors 143
	5.3.3	Carbon Nanotubes for Bioimaging 144
	5.3.3.1	Carbon Nanotubes for Fluorescence Imaging 144
	5.3.3.2	Carbon Nanotubes for Raman Imaging 145
	5.3.3.3	Carbon Nanotubes for Photoacoustic Imaging 145
	5.3.3.4	Carbon Nanotubes for Other Bioimaging Modalities 145
	5.3.4	Carbon Nanotubes for Other Biomedical Applications 146
	5.4	Biomedical Applications of Graphene 146
	5.4.1	Graphene for Drug Delivery 147
	5.4.1.1	Graphene for the Delivery of Small Drug Molecules 148
	5.4.1.2	Graphene for the Delivery of Biomacromolecules 148
	5.4.2	Graphene for Photothermal and Combined Therapies of
		Tumors 151
	5.4.3	Graphene for Bioimaging 152
	5.4.4	Graphene for Other Biomedical Applications 153
	5.5	Conclusion and Perspectives 153
		Acknowledgments 154
		References 155
	6	Pulmonary Effects of Carbon Nanomaterials 163
		Liying Wang, Donna C. Davidson, Vincent Castranova, and Yon Rojanasakul
	6.1	Introduction 163
	6.2	Physicochemical Properties of Carbon Nanomaterials 164
	6.2.1	Types of Carbon Nanomaterials 165
	6.2.2	Effects of Size 165
	6.2.3	Effects of Agglomeration State 166
	6.2.4	Aspect Ratio Considerations 168
	6.2.5	Surface Modifications 168
	6.3	Fate of Pulmonary Exposed Carbon Nanoparticles (Deposition,
		Distribution, Translocation, and Clearance) 169
	6.3.1	Deposition and Distribution of Carbon Nanoparticles in the
	600	Lung 169
	6.3.2	Translocation of Carbon Nanoparticles 172
	6.3.3	Clearance of Carbon Nanomaterials from the Lungs 175
	6.4	Carbon Nanomaterial – Induced Lung Responses 176
	6.4.1	Key/Specific Target Lung Cell Types of Pulmonary-Exposed Carbon
	6.4.2	Nanoparticles 176
	6.4.2	Lung Inflammation 178
	6.4.3	Immune Response 179
	6.4.4	Fibrosis 180
	6.4.5	Genotoxicity 181

6.4.6	Cancer 182
6.4.7	Cardiovascular Effects Following Pulmonary Exposure of Carbon Nanomaterials 184
6.5	Summary 184
0.0	Disclaimer 184
	References 189
	*
7	Cardiovascular and Hemostatic Effects of Carbon Nanomaterials 195 Xiaoyong Deng, Cheng Li, Jiajun Wang, and Pan Chen
7.1	Background 195
7.2	Carbon Nanotubes 195
7.2.1	Hemotoxicity of CNTs 196
7.2.1.1	What Is Hemotoxicity 196
7.2.1.2	Complement System 197
7.2.1.2	Red Blood Cells 199
7.2.1.4	Hemostatic System and Coagulation/Thrombosis/Atheroma 200
7.2.1.4	Effects on Cardiovascular System 201
7.2.2	Fullerenes 203
7.3.1	Fullerenes' Escape from Lungs into Circulation 203
7.3.1	Toxicity of Fullerenes on the Cardiovascular System 204
7.3.2	Graphene-Related Nanomaterials 205
7. 3 7.5	Conclusions and Outlook 208
7.5	Acknowledgments 208
	References 208
	References 200
8	Modulation of the Immune System by Fullerene and Graphene
	Derivatives 213
	Ligeng Xu and Chunying Chen
8.1	Introduction 213
8.2	The Immunological Effects of Fullerene and Its Derivatives 213
8.2.1	Fullerene Derivatives Can Inhibit Inflammation via Blocking ROS
	Generation 213
8.2.2	Fullerene Derivatives Promote Immune Responses via Modulating
	Macrophages and/or Antigen Presenting Cells (APCs) 215
8.3	Immunological Effects of Graphene and Its Derivatives 222
8.3.1	Immunological Effect of Pristine Graphene 225
8.3.2	Immunological Effects of Graphene Oxide and Its Derivatives 227
8.4	Perspectives and Outlook 231
	References 234
9	Neuro-, Hepato-, and Nephrotoxicity of Carbon-based
	Nanomaterials 239
	Jia Yao and Yongbin Zhang
9.1	Carbon-based Nanomaterials: Introduction 239
9.2	Neurotoxicity of Carbon-based Nanomaterials 240

ХII	Contents	
'	9.2.1	Blood – Brain Barrier and BBB Penetration by Carbon-based Nanomaterials 240
	9.2.2	Neurotoxicity of Carbon Nanotubes 241
	9.2.3	Strategies to Reduce Neurotoxicity of Carbon Nanotubes 243
	9.2.4	Neurotoxicity of Other Carbon-based Nanomaterials 244
	9.3	Hepato and Nephrotoxicity of Carbon-based Nanomaterials 245
	9.3.1	Carbon Nanotube Biodistribution in the Liver and Kidney 245
	9.3.2	Biodistribution of Other Carbon Nanomaterials 248
	9.3.3	Hepatotoxicity of Carbon Nanotubes 251
	9.3.4	Carbon Nanotube Nephrotoxicity/Renal Toxicity 254
	9.3.5	Hepatotoxicity and Nephrotoxicity of Other Types of Carbon-based
		Nanomaterials 254
	9.4	Points of Consideration for Toxicity Evaluation of Carbon-based
		Nanomaterials 257
	9.5	Summary 259
		Acknowledgments 259
		References 259
	10	Genotoxicity and Carcinogenic Potential of Carbon
		Nanomaterials 267
		Todd A. Stueckle, Linda Sargent, Yon Rojanasakul, and Liying Wang
	10.1	Introduction 267
	10.1.1	Engineered Nanomaterials and Long-Term Disease Risk: An
		Introduction 269
	10.1.2	Carcinogenesis: A Multistep Process 270
	10.1.2.1	Genotoxicity and Initiation 271
	10.1.2.2	Promotion 272
	10.1.2.3	Progression 274
	10.1.3	Current Knowledge and Challenges in Carcinogenesis Studies 274
	10.2	Carbon Nanomaterials: Genotoxicity and Carcinogenic
		Potential 275
	10.2.1	Physicochemical Properties of ECNMs 275
	10.2.2	Ultrafine Carbon Black 276
	10.2.2.1	In Vivo Studies 277
	10.2.2.2	In Vitro Studies 278
	10.2.3	Carbon Nanotubes 278
	10.2.3.1	In Vivo Studies 279
	10.2.3.2	In Vitro Studies 291
	10.2.4	Fullerenes and Derivatives 296
	10.2.4.1	In Vivo Studies 297
	10.2.4.2	In Vitro Studies 299

Graphene and Graphene Oxide 300

Carbon Nanofibers and Other Particles 307

In Vivo Studies 302

In Vitro Studies 304

10.2.5

10.2.5.1

10.2.5.2

10.2.6

10.2.6.1	In Vivo Studies 307
10.2.6.2	<i>In Vitro</i> Studies 308
10.3	Future Challenges in Carbon Nanomaterial Carcinogenesis Risk
	Assessment 308
10.3.1	Exposure Characterization and Fate 308
10.3.2	Dosimetry 309
10.3.3	Model Choice 310
10.3.4	Systematic Evaluation of Genotoxicity 311
10.3.5	Role of ROS and Inflammation 311
10.4	Assessment of ECNM-Induced Genotoxicity and
	Carcinogenesis 312
10.4.1	Recommendations for Screening ENMs for Carcinogenic Potential 312
10.4.2	Systematic Screening Paradigm and Workflow for ENM
	Carcinogenicity Risk Assessment 314
10.5	Concluding Remarks 316
	Acknowledgments 316
	Disclaimer 316
	References 317
11	Effect on Reproductive System of Carbon Nanomaterials 333
	Ying Liu and Chunying Chen
11.1	Introduction 333
11.2	Effects of Carbon Nanomaterials on the Reproductive System 334
11.2.1	Carbon Nanotubes 335
11.2.2	Fullerene Derivatives 340
11.2.3	Carbon Black Nanoparticles 340
11.3	Insights into the Molecular Mechanisms 342
11.3.1	Potential Toxicity to the Female Reproductive System 342
11.3.2	Potential Toxicity to Male Reproduction of Carbon
	Nanomaterials 343
11.3.3	Potential Toxicity to Offspring of Carbon Nanomaterials 345
11.3.4	Impact on the Endocrine Organs and Hormone
	Biosynthesis/Metabolism 346
11.3.5	Others 348
11.4	Conclusion and Perspectives 348
	Acknowledgments 352
	References 352
12	Immunological Responses Induced by Carbon Nanotubes Exposed to
	Skin and Gastric and Intestinal System 357
	Haiyan Xu, Jie Meng, Qiang Ma, and Xiaojin Li
12.1	Introduction 357
12.2	Biological Effects of CNTs by Dermal Exposure 358
12.2.1	In Vitro Assessment in Dermal-Related Cell Lines 358

XIV	Contents	
-	12.2.2	<i>In Vivo</i> Studies on the Responses Elicited by Skin Exposed with CNTs 361
	12.3	Immunological Reactions Elicited by Subcutaneous Administration of MWCNTs 362
	12.3.1	Preparation and Characterization of Multiwalled Carbon Nanotubes for Uses in Studies 362
	12.3.2	Distribution of Subcutaneously Injected Carbon Nanotubes 363
	12.3.3	Immunological Responses Induced by Subcutaneously Injected MWCNTs 369
	12.3.3.1	Macrophages Responses Exerted by MWCNTs 370
	12.3.3.2	MWCNTs Attract Naïve Monocyte Macrophages Through
	10000	Activating Macrophages in the Subcutis 373
	12.3.3.3	Subcutaneously Injected MWCNTs Induce Complement Activation 375
	12.3.3.4	Subcutaneously Injected MWCNTs Elevate Pro-inflammatory Cytokines in the Blood 376
	12.4	Immunological Responses Induced by Subcutaneous Administration
	12.1	of MWCNTs in Tumor-Bearing Mice 377
	12.4.1	MWCNTs Induce Systematic Immune Responses in Tumor-Bearing
	12.1.1	Mice 378
	12.4.2	MWCNTs Upregulate Multiple Pro-inflammatory Cytokines in the
	12.1.2	Blood 378
	12.4.3	MWCMTs Mediate Cytotoxicity of Lymphocytes 379
	12.4.4	MWCNTs Induce Complement Activation 380
	12.4.5	MWCNTs Attract Monocyte-Macrophages to Affect the
		Microenvironment of Tumor Mass 380
	12.5	CNTs as Antigen Delivery System to Enhance Immune Responses
		Against Tumors 383
	12.6	Immunological Responses of Gastric and Intestinal Systems Exposed
		to Carbon Nanotubes 386
		References 389
	13	Modulation of Immune System by Carbon Nanotubes 397
		Marit Ilves and Harri Alenius
	13.1	Immune System 397
	13.1.1	Innate Immunity Cells and Their Main Functions 398
	13.1.2	Adaptive Immunity Cells and Their Main Functions 399
	13.2	Carbon Nanotubes (CNTs) and Innate Immunity 400
	13.2.1	Complement Activation 401
	13.2.2	Macrophages 402
	13.2.3	Activation of Inflammasome Complex and IL-1β Secretion 405
	13.2.4	Neutrophils 406
	13.2.5	Innate Lymphoid Cells (ILCs) 408
	1326	Dendritic Cells 408

CNTs and Adaptive Immunity 409

13.3

13.3.1	The Effects of CNTs on Vaccine Delivery and Immunotherapy 409
13.3.2	Utilization of CNT Scaffolds in the Expanding and Modulation of
	Immune Cells 411
13.3.3	Immunosuppressive Effects of CNTs 413
13.4	The Effect of CNTs in Allergy and Asthma 414
13.4.1	Allergic Reactions and Their Immunological Mechanisms 414
13.4.2	Asthma 415
13.4.3	Allergic Pulmonary Inflammation Induced by Airway Exposure to CNTs 417
13.4.4	Modulation of Allergen-Induced Airway Inflammation by Exposure to CNTs 418
13.4.5	CNT in the Context of Mast Cells and Eosinophils 420
13.4.6	Role of IL-33 Pathway in CNT-Induced Allergic Responses 420
13.5	Conclusions and Future Prospects 422 References 424
14	Carbon Dots: Synthesis, Bioimaging, and Biosafety Assessment 429 Jie Wang and Yao He
1.4.1	Introduction 429
14.1 14.1.1	Synthesis and Fabrication of C-dots 429
14.1.2	Bioimaging of C-dots 431
14.1.2	Biosafety Assessment of C-dots 432
14.1.5	Synthetic Strategies 433
14.2.1	Microwave-Assisted Methods 433
14.2.1	Hydrothermal Carbonization 434
14.2.2	Electrochemical Synthesis 437
14.2.4	Chemical Oxidation 439
14.2.5	Ultrosonication 442
14.2.6	Plasma Treatment 444
14.2.7	Laser Ablation Methods 445
14.2.8	Supported Methods 446
14.2.9	Thermal Routes 448
14.3	C-Dots-based Fluorescent Probes for Bioimaging Applications 450
14.3.1	Fluorescent Probes for Bioimaging Applications 450
14.3.2	In Vitro Imaging 451
14.3.3	In Vivo Imaging 456
14.3.4	Conclusion 462
14.4	Toxicity Assessment 462
14.4.1	In Vitro Toxicity Assessment 463
14.4.2	In Vivo Toxicity Assessment 469
14.4.3	Conclusion 475
14.5	Perspectives 477
14.5.1	Unequivocal PL Mechanism 477
14.5.2	Expanding the Spectral Coverage 478
14.5.3	QY Improvement 478
± 1.0.0	21 mprovement 170

xvı	Contents	
	14.5.4	Bioimaging 478
	14.5.5	Toxicity Assessment 479
		References 479
	15	Transport in the Environment and Ecotoxicity of Carbon Nanomaterials 487
	15.1	Yingying Xu and Chunying Chen
	15.1	Introduction 487
	15.2 15.2.1	Transport of Carbon Nanomaterials in the Environment 488 Entry of Carbon Nanomaterials into the Environment 488
	15.2.1	Entry of Carbon Nanomaterials into the Environment 488 Fate and Transformation in the Environment 488
	15.2.2.1	Oxidation 488
	15.2.2.1	Photochemical Transformation 490
	15.2.2.3	Dissolution and Precipitation 491
	15.2.2.4	Adsorption 492
	15.2.2.4	Biodegradation 493
	15.3	Ecotoxicity of Fullerene 494
	15.3.1	Effect of Fullerene on Microorganisms 494
	15.3.2	Effect of Fullerene on Animals 495
	15.3.2.1	Effect of Fullerene on Invertebrates 495
	15.3.2.2	Effect of Fullerene on Vertebrates 496
	15.3.3	Effect of Fullerene on Plants 496
	15.3.3.1	Effect of Fullerene on Algae 496
	15.3.3.2	Effect of Fullerene on Higher Plants 497
	15.4	Ecotoxicity of Carbon Nanotubes (CNTs) 498
	15.4.1	Effect of CNTs on Microorganisms 498
	15.4.2	Effect of CNTs on Animals 499
	15.4.2.1	Effect of CNTs on Invertebrates 499
	15.4.2.2	Effect of CNTs on Vertebrates 501
	15.4.3	Effect of CNTs on Plants 502
	15.4.3.1	Effect of CNTs on Algae 502
	15.4.3.2	Effect of CNTs on Higher Plants 503
	15.5	Ecotoxicity of Graphene 504
	15.6	Conclusion and Perspectives 506
		Acknowledgments 506
		References 506
	16	Exposure Scenarios in the Workplace and Risk Assessment of Carbon
		Nanomaterials 515
		Rui Chen and Chunying Chen
	16.1	Introduction 515
	16.1.1	Background 515
	16.1.2	Exposure Routes and Exposure Scenarios 515
	16.1.3	Exposure Metrics 516
	16.1.4	Occupation Exposure Limit for Carbon Nanomaterials 516

16.1.5	Strategy for Exposure Assessment of Carbon Nanomaterials	517
16.2	Potential Exposure in the Workplace 519	
16.2.1	Carbon Nanotubes 519	
16.2.2	Fullerenes, Metallofullerenes, and Graphenes 525	
16.3	Exposure Risk Assessment and Engineering Control 527	
16.3.1	Risk Assessment Strategy on Carbon Nanomaterials 527	
16.3.2	Inhalation Exposure Assessment Method 529	
16.3.3	Exposure Controls 530	
16.4	Summary and Outlook 531	
	Acknowledgments 531	
	References 531	

Index 535