

Edited by Viola Vogel

 WILEY-VCH

Nanotechnology

Volume 5: Nanomedicine



TB383
N18 6.18
V-5

G. Schmid, H. Krug, R. Waser, V. Vogel, H. Fuchs,
M. Grätzel, K. Kalyanasundaram, L. Chi (Eds.)

Nanotechnology

Volume 5: Nanomedicine

Edited by Viola Vogel



WILEY-VCH Verlag GmbH & Co. KGaA

The Editor

Prof. Viola Vogel

ETH Zürich

Laboratory for Biologically Oriented Materials

Department of Materials

HCI F443

Wolfgang-Pauli-Str. 10

8093 Zürich

Switzerland

Cover: Nanocar reproduced with kind permission
of Y. Shirai/Rice University

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 in the Internet at <http://dnb.d-nb.de>.

© 2009 WILEY-VCH Verlag GmbH & Co. KGaA,
Weinheim

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.

Typesetting Thomson Digital, Noida, India

Printing Strauss GmbH, Mörlenbach

Binding Litges & Dopf Buchbinderei GmbH,
Heppenheim

Printed in the Federal Republic of Germany

Printed on acid-free paper

ISBN: 978-3-527-31736-3

Nanotechnology

Volume 5: Nanomedicine

Edited by Viola Vogel

Related Titles

Nanotechnologies for the Life Sciences

Challa S. S. R. Kumar (ed.)

Volume 1: Biofunctionalization of Nanomaterials

2005

978-3-527-31381-5

Volume 2: Biological and Pharmaceutical Nanomaterials

2005

978-3-427-31382

Volume 3: Nanosystem Characterization Tools in the Life Sciences

2005

978-3-527-31383-9

Volume 4: Nanodevices for the Life Sciences

2006

978-3-527-31384-6

Volume 5: Nanomaterials - Toxicity, Health and Environmental Issues

2006

978-3-527-31385-3

Volume 6: Nanomaterials for Cancer Therapy

2006

978-3-527-31386-0

Volume 7: Nanomaterials for Cancer Diagnosis

2006

978-3-527-31387-7

Volume 8: Nanomaterials for Biosensors

2006

978-3-527-31388-4

Volume 9: Tissue, Cell and Organ Engineering

2006

978-3-527-31389-1

Volume 10: Nanomaterials for Medical Diagnosis and Therapy

2007

978-3-527-31390-7

Nanotechnology

Günter Schmid (ed.)

Volume 1: Principles and Fundamentals

2008

978-3-527-31732-5

Harald Krug (ed.)

Volume 2: Environmental Aspects

2008

978-3-527-31735-6

Rainer Waser (ed.)

Volume 3: Information Technology I

2008

978-3-527-31738-7

Rainer Waser (ed.)

Volume 3: Information Technology II

2008

978-3-527-31737-0

Viola Vogel (ed.)

Volume 5: Nanomedicine and Nanobiotechnology

2009

978-3-527-31736-3

Harald Fuchs (ed.)

Volume 6: Nanoprobes

2009

978-3-527-31733-2

Michael Grätzel, Kuppaswamy Kalyanasundaram (eds.)

Volume 7: Light and Energy

2009

978-3-527-31734-9

Lifeng Chi (ed.)

Volume 8: Nanostructured Surfaces

2009

978-3-527-31739-4

www.wiley.com/go/nanotechnology

List of Contributors

Kathryn T. Applegate

The Scripps Research Institute
Laboratory for Computational Cell
Biology
La Jolla, CA 92037
USA

Matthew L. Baker

National Center for Macromolecular
Imaging
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030
USA

Gang Bao

Georgia Institute of Technology and
Emory University
Department of Biomedical Engineering
Atlanta, GA 30332
USA

Ramille M. Capito

Northwestern University
Institute for BioNanotechnology
in Medicine
303 E. Superior St.
Chicago, IL 60611
USA

and

Northwestern University
Department of Materials Science and
Engineering
Evanston, IL 60208
USA

Shelton D. Caruthers

Washington University School
of Medicine
Consortium for Translational Research
in Advanced Imaging and
Nanotechnology
660 S. Euclid Avenue
CB 8215 Saint Louis, MO 63110
USA

Wah Chiu

National Center for Macromolecular
Imaging
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030
USA

Gaudenz Danuser

The Scripps Research Institute
Laboratory for Computational Cell
Biology
10550 N. Torrey Pines Road
La Jolla, CA 92037
USA

Paolo Decuzzi

University of Texas Health Science
Center at Houston
School of Health Information Sciences
Houston, TX 77030
USA

and

BioNEM—Center of Bio-Nanotechnology
and Engineering for Medicine
University of Magna Graecia
88100 Catanzaro
Italy

Dennis E. Discher

University of Pennsylvania
Biophysical Engineering Laboratory
Philadelphia, PA 19104
USA

Iain E. Dunlop

Max Planck Institute for Metals
Research
Department New Materials and
Biosystems
Heisenbergstr. 3
70569 Stuttgart
Germany

and

University of Heidelberg
Department of Biophysical Chemistry
Im Neuenheimer Feld 253
69120 Heidelberg
Germany

Michael L. Dustin

The Helen L. and Martin S. Kimmel
Center for Biology and Medicine at the
Skirball Institute for Biomolecular
Medicine
540 First Avenue
NYC 10016
USA

Adam J. Engler

University of Pennsylvania
Biophysical Engineering Laboratory
Philadelphia, PA 19140
USA

and

Present Address:
University of California San Diego
Department of Bioengineering
La Jolla, CA
USA

Mauro Ferrari

University of Texas Health Science
Center at Houston
Division of Nanomedicine
Department of Biomedical Engineering
1825 Pressler, Suite 537D
Houston, TX 77030
USA

and

The University of Texas MD Anderson
Cancer Center
Department of Experimental
Therapeutics
Houston, TX 77030
USA

and

Rice University
Department of Bioengineering
Houston, TX 77030
USA

Peter Fratzl

Max Planck Institute of Colloids and
Interfaces
Department of Biomaterials
Research Campus Golm
14424 Potsdam
Germany

Biana Godin

University of Texas Health Science
Center at Houston
Division of Nanomedicine
Department of Biomedical Engineering
Houston, TX 77030
USA

Anita Goel

Nanobiosym Labs
200 Boston Avenue, Suite 4700
Medford, MA 02155
USA

and

Harvard University
Department of Physics
Cambridge, MA 02138
USA

Himadri S. Gupta

Max Planck Institute of Colloids and
Interfaces
Department of Biomaterials
Research Campus Golm
14424 Potsdam
Germany

Michael S. Hughes

Washington University School of
Medicine
Consortium for Translational Research
in Advanced Imaging and
Nanotechnology
660 S. Euclid Avenue
CB 8215 Saint Louis
MO 63110
USA

Satoshi Jinno

Harvard-MIT Division of Health
Sciences and Technology,
Massachusetts Institute of Technology
Cambridge, MA 02139
USA

and

Center for Biomedical Engineering
Department of Medicine
Brigham and Women's Hospital
Harvard Medical School
Cambridge, MA 02139
USA

Ali Khademhosseini

Massachusetts Institute of Technology
Harvard-MIT Division of Health
Sciences and Technology
PRB 252
65 Landsdowne Street
Cambridge, MA 02139
USA

and

Center for Biomedical Engineering
Department of Medicine
Brigham and Women's Hospital
Harvard Medical School
Cambridge, MA 02139
USA

Klaus Klaushofer

Ludwig Boltzmann Institute of
Osteology at Hanusch Hospital of
WGKK and AUVA Trauma Centre
Meidling
4th Medical Department
Hanusch Hospital
Heinrich Collin Street 30
1140 Vienna
Austria

Philipp Kukura

ETH Zurich
Laboratory of Physical Chemistry
and Zurich Center for Imaging Science
and Technology (CIMST)
Nano-Optics Group
8093 Zürich
Switzerland

Robert Langer

Harvard-MIT Division of Health
Sciences and Technology
Massachusetts Institute of Technology
Cambridge, MA 02139
USA

and

Department of Chemical Engineering
and Department of Biological
Engineering
Massachusetts Institute of Technology
Cambridge, MA 02139
USA

Gregory M. Lanza

Washington University School of
Medicine
Consortium for Translational Research
in Advanced Imaging and
Nanotechnology
660 S. Euclid Avenue
CB 8215 Saint Louis
MO 63110
USA

Alvaro Mata

Northwestern University
Institute for BioNanotechnology in
Medicine
Chicago, IL 60611
USA

and

Nanotechnology Platform
Parc Científic de Barcelona
Baldri Reixac 10–12
08028 Barcelona
Spain

Michael P. Marsh

National Center for Macromolecular
Imaging
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030
USA

Jon N. Marsh

Washington University School of
Medicine
Consortium for Translational Research
in Advanced Imaging and
Nanotechnology
660 S. Euclid Avenue
CB 8215 Saint Louis
MO 63110
USA

Nitin Nitin

Georgia Institute of Technology and
Emory University
Department of Biomedical Engineering
Atlanta, GA 30332
USA

Bimal Rajalingam

Harvard-MIT Division of Health
Sciences and Technology
Massachusetts Institute of Technology
Cambridge, MA 02139
USA

and

Center for Biomedical Engineering
Department of Medicine
Brigham and Women's Hospital
Harvard Medical School
Cambridge, MA 02139
USA

Florian Rehfeldt

Georg-August Universität Göttingen
III. Physikalisches Institut
Friedrich-Hund-Platz 1
37077 Göttingen
Germany

Alois Renn

ETH Zurich
Laboratory of Physical Chemistry
and Zurich Center for Imaging Science
and Technology (CIMST)
Nano-Optics Group
8093 Zürich
Switzerland

Won Jong Rhee

Georgia Institute of Technology and
Emory University
Department of Biomedical Engineering
Atlanta, GA 30332
USA

Paul Roschger

Ludwig Boltzmann Institute of
Osteology at Hanusch Hospital of
WGKK and AUVA Trauma Centre
Meidling
4th Medical Department
Hanusch Hospital
Heinrich Collin Street 30
1140 Vienna
Austria

Jason Sakamoto

University of Texas Health Science
Center at Houston
Division of Nanomedicine
Department of Biomedical Engineering
Houston, TX 77030
USA

Vahid Sandoghdar

ETH Zurich
Laboratory of Physical Chemistry
and Zurich Center for Imaging Science
and Technology (CIMST)
Nano-Optics Group
8093 Zürich
Switzerland

Phillip Santangelo

Georgia Institute of Technology and
Emory University
Department of Biomedical Engineering
Atlanta, GA 30332
USA

Rita E. Serda

University of Texas Health Science
Center at Houston
Division of Nanomedicine
Department of Biomedical Engineering
Houston, TX 77030
USA

Michael P. Sheetz

Columbia University
Sherman Fairchild Center
Department of Biological Sciences
1212 Amsterdam Avenue, Room 713
New York 10027
USA

Joachim P. Spatz

Max Planck Institute for Metals
Research
Department New Materials and
Biosystems
Heisenbergstr. 3
70569 Stuttgart
Germany

and

University of Heidelberg
Department of Biophysical Chemistry
Im Neuenheimer Feld 253
69120 Heidelberg
Germany

Samuel I. Stupp

Northwestern University
Institute for BioNanotechnology in
Medicine
303 E. Superior
Chicago, IL 60611
USA

and

Northwestern University
Department of Materials Science and
Engineering
Evanston, IL 60208
USA

and

Northwestern University
Department of Chemistry
Evanston, IL 60208
USA

and

Northwestern University
Department of Medicine
303 E. Superior, Suite 11-129
Chicago, IL 60611
USA

Viola Vogel

ETH Zurich
Laboratory for Biologically Oriented
Materials
Department of Materials
8049 Zürich
Switzerland

Kirk D. Wallace

Washington University School of
Medicine
Consortium for Translational Research
in Advanced Imaging and
Nanotechnology
660 S. Euclid Avenue
CB 8215 Saint Louis, MO 63110
USA

Samuel A. Wickline

Washington University School of
Medicine
Consortium for Translational Research
in Advanced Imaging and
Nanotechnology
660 S. Euclid Avenue
CB 8215 Saint Louis, MO 63110
USA

Ge Yang

The Scripps Research Institute
Laboratory for Computational Cell
Biology
La Jolla, CA 92037
USA

Contents

List of Contributors XV

Part One Nanomedicine: The Next Waves of Medical Innovations 1

- 1 Introduction 3
Viola Vogel
- 1.1 Great Hopes and Expectations are Colliding with Wild Hype and Some Fantasies 3
- 1.2 The First Medical Applications are Coming to the Patients' Bedside 4
- 1.3 Major Advances in Medicine Have Always been Driven by New Technologies 5
- 1.4 Nanotechnologies Foster an Explosion of New Quantitative Information How Biological Nanosystems Work 6
- 1.5 Insights Gained from Quantifying how the Cellular Machinery Works will lead to Totally New Ways of Diagnosing and Treating Disease 7
- 1.6 Engineering Cell Functions with Nanoscale Precision 8
- 1.7 Advancing Regenerative Medicine Therapies 8
- 1.8 Many More Relevant Medical Fields Will be Innovated by Nanotechnologies 9
- References 10

Part Two Imaging, Diagnostics and Disease Treatment by Using Engineered Nanoparticles 17

- 2 From *In Vivo* Ultrasound and MRI Imaging to Therapy: Contrast Agents Based on Target-Specific Nanoparticles 19
Kirk D. Wallace, Michael S. Hughes, Jon N. Marsh, Shelton D. Caruthers, Gregory M. Lanza, and Samuel A. Wickline
- 2.1 Introduction 19
- 2.2 Active versus Passive Approaches to Contrast Agent Targeting 20
- 2.3 Principles of Magnetic Resonance Contrast Agents 21

2.3.1	Mathematics of Signal Contrast	22
2.3.2	Perfluorocarbon Nanoparticles for Enhancing Magnetic Resonance Contrast	23
2.3.3	Perfluorocarbon Nanoparticles for Fluorine (^{19}F) Imaging and Spectroscopy	24
2.3.4	Fibrin-Imaging for the Detection of Unstable Plaque and Thrombus	25
2.3.5	Detection of Angiogenesis and Vascular Injury	27
2.4	Perfluorocarbon Nanoparticles as an Ultrasound Contrast Agent	31
2.4.1	Entropy-Based Approach	33
2.4.2	The Density Function $w_f(y)$	33
2.4.3	Ultrasound in a Precancerous Animal Model	34
2.4.3.1	Image Analysis	36
2.4.4	Targeting of MDA-435 Tumors	38
2.4.5	<i>In Vivo</i> Tumor Imaging at Clinical Frequencies	42
2.5	Contact-Facilitated Drug Delivery and Radiation Forces	43
2.5.1	Primary and Secondary Radiation Forces	43
2.5.2	<i>In Vitro</i> Results	44
2.6	Conclusions	46
	References	47
3	Nanoparticles for Cancer Detection and Therapy	51
	<i>Biana Godin, Rita E. Serda, Jason Sakamoto, Paolo Decuzzi, and Mauro Ferrari</i>	
3.1	Introduction	51
3.1.1	Cancer Physiology and Associated Biological Barriers	51
3.1.2	Currently Used Anticancer Agents	53
3.1.2.1	Chemotherapy	54
3.1.2.2	Anti-Angiogenic Therapeutics	54
3.1.2.3	Immunotherapy	55
3.1.2.4	Issues and Challenges	56
3.2	Nanotechnology for Cancer Applications: Basic Definitions and Rationale for Use	57
3.3	First-Generation Nanovectors and their History of Clinical Use	59
3.4	Second-Generation Nanovectors: Achieving Multiple Functionality at the Single Particle Level	62
3.5	Third-Generation Nanoparticles: Achieving Collaborative Interactions Among Different Nanoparticle Families	65
3.6	Nanovector Mathematics and Engineering	69
3.7	The Biology, Chemistry and Physics of Nanovector Characterization	75
3.7.1	Physical Characterization	76
3.7.2	<i>In Vitro</i> Testing	76
3.7.2.1	<i>In Vitro</i> Toxicity Testing	79
3.7.3	<i>In Vivo</i> Animal Testing	79

3.8	A Compendium of Unresolved Issues	79
	References	82

Part Three Imaging and Probing the Inner World of Cells 89

4	Electron Cryomicroscopy of Molecular Nanomachines and Cells	91
	<i>Matthew L. Baker, Michael P. Marsh, and Wah Chiu</i>	
4.1	Introduction	91
4.2	Structure Determination of Nanomachines and Cells	92
4.2.1	Experimental Procedures in Cryo-EM and Cryo-ET	92
4.2.1.1	Specimen Preparation for Nanomachines and Cells	92
4.2.1.2	Cryo-Specimen Preservation	94
4.2.1.3	Low-Dose Imaging	95
4.2.1.4	Image Acquisition	95
4.2.2	Computational Procedures in Cryo-EM and Cryo-ET	95
4.2.2.1	Image Processing and Reconstruction	96
4.2.2.2	Structure Analysis and Data Mining	97
4.2.3	Data Archival	98
4.3	Biological Examples	98
4.3.1	Skeletal Muscle Calcium Release Channel	99
4.3.2	Bacteriophage Epsilon15	100
4.3.3	Bacterial Flagellum	101
4.3.4	Proteomic Atlas	102
4.4	Future Prospects	103
	References	104
5	Pushing Optical Microscopy to the Limit: From Single-Molecule Fluorescence Microscopy to Label-Free Detection and Tracking of Biological Nano-Objects	113
	<i>Philipp Kukura, Alois Renn, and Vahid Sandoghdar</i>	
5.1	Introduction	113
5.1.1	Linear Contrast Mechanisms	114
5.1.2	Nonlinear Contrast Mechanisms	117
5.2	Single-Molecule Fluorescence Detection: Techniques and Applications	117
5.2.1	Single Molecules: Light Sources with Ticks	118
5.2.2	The Signal-to-Noise Ratio Challenge	119
5.2.3	High-Precision Localization and Tracking of Single Emitters	120
5.2.4	Getting Around the Rayleigh Limit: Colocalization of Multiple Emitters	123
5.3	Detection of <i>Non</i> -Fluorescent Single Nano-Objects	127
5.3.1	The Difficulty of Detecting Small Particles Through Light Scattering	127
5.3.2	Interferometric Detection of Gold Nanoparticles	128

5.3.2.1	Is it Possible to Detect Molecule-Sized Labels?	131
5.3.2.2	The Needle in the Haystack: Finding and Identifying Gold	132
5.3.3	Combining Scattering and Fluorescence Detection: A Long-Range Nanoscopic Ruler	132
5.3.4	Label-Free Detection of Biological Nano-Objects	134
5.4	Summary and Outlook	137
	References	138

6 Nanostructured Probes for *In Vivo* Gene Detection 143

Gang Bao, Phillip Santangelo, Nitin Nitin, and Won Jong Rhee

6.1	Introduction	143
6.2	Fluorescent Probes for Live-Cell RNA Detection	145
6.2.1	Tagged Linear ODN Probes	145
6.2.2	ODN Hairpin Probes	146
6.2.3	Fluorescent Protein-Based Probes	150
6.3	Probe Design and Structure–Function Relationships	151
6.3.1	Target Specificity	151
6.3.2	Molecular Beacon Structure–Function Relationships	152
6.3.3	Target Accessibility	153
6.3.4	Fluorophores and Quenchers	154
6.4	Cellular Delivery of Nanoprobes	155
6.5	Living Cell RNA Detection Using Nanostructured Probes	158
6.5.1	Biological Significance	159
6.6	Engineering Challenges in New Probe Development	161
	References	163

7 High-Content Analysis of Cytoskeleton Functions by Fluorescent Speckle Microscopy 167

Kathryn T. Applegate, Ge Yang, and Gaudenz Danuser

7.1	Introduction	167
7.2	Cell Morphological Activities and Disease	168
7.2.1	Cell Migration	168
7.2.2	Cell Division	169
7.2.3	Response to Environmental Changes	170
7.2.4	Cell–Cell Communication	170
7.3	Principles of Fluorescent Speckle Microscopy (FSM)	171
7.4	Speckle Image Formation	172
7.4.1	Speckle Formation in Microtubules (MTs): Stochastic Clustering of Labeled Tubulin Dimers in the MT Lattice	172
7.4.2	Speckle Formation in Other Systems: The Platform Model	174
7.5	Interpretation of Speckle Appearance and Disappearance	175
7.5.1	Naïve Interpretation of Speckle Dynamics	175
7.5.2	Computational Models of Speckle Dynamics	175
7.5.3	Statistical Analysis of Speckle Dynamics	177

7.5.4	Single- and Multi-Fluorophore Speckles Reveal Different Aspects of the Architectural Dynamics of Cytoskeleton Structures	179
7.6	Imaging Requirements for FSM	180
7.7	Analysis of Speckle Motion	181
7.7.1	Tracking Speckle Flow: Early and Recent Developments	181
7.7.2	Tracking Single-Speckle Trajectories	183
7.7.3	Mapping Polymer Turnover Without Speckle Trajectories	185
7.8	Applications of FSM for Studying Protein Dynamics <i>In Vitro</i> and <i>In Vivo</i>	185
7.9	Results from Studying Cytoskeleton Dynamics	192
7.9.1	F-Actin in Cell Migration	192
7.9.1.1	F-Actin in Epithelial Cells is Organized Into Four Dynamically Distinct Regions	192
7.9.1.2	Actin Disassembly and Contraction are Coupled in the Convergence Zone	193
7.9.1.3	Two Distinct F-Actin Structures Overlap at the Leading Edge	193
7.9.2	Architecture of <i>Xenopus laevis</i> Egg Extract Meiotic Spindles	194
7.9.2.1	Individual MTs within the Same Bundle Move at Different Speeds	195
7.9.2.2	The Mean Length of Spindle MTs is 40% of the Total Spindle Length	195
7.9.3	Hierarchical Transmission of F-Actin Motion Through Focal Adhesions	199
7.10	Outlook: Speckle Fluctuation Analysis to Probe Material Properties	200
7.11	Conclusions	202
	References	203
8	Harnessing Biological Motors to Engineer Systems for Nanoscale Transport and Assembly	207
	<i>Anita Goel and Viola Vogel</i>	
8.1	Sequential Assembly and Polymerization	207
8.1.1	Engineering Principle No. 1: discrimination of similar building blocks	209
8.2	Cargo Transport	210
8.2.1	Engineering Principle No. 2: various track designs	212
8.3	Cargo Selection	215
8.3.1	Engineering Principle No. 3: barcoding	215
8.3.2	Engineering Principle No. 4: active transport of tailored drugs and gene carriers	218
8.4	Quality Control	218
8.4.1	Engineering Principle No. 5: error recognition and repair at the molecular level	219
8.4.2	Engineering Principle No. 6: error recognition and repair at the system level	220
8.5	External Control	220

8.5.1	Engineering Principle No. 7: performance regulation on demand	220
8.6	Concluding Remarks	223
	References	225

Part Four Innovative Disease Treatments and Regenerative Medicine 233

9 Mechanical Forces Matter in Health and Disease:

From Cancer to Tissue Engineering 235

Viola Vogel and Michael P. Sheetz

9.1	Introduction: Mechanical Forces and Medical Indications	235
9.2	Force-Bearing Protein Networks Hold the Tissue Together	237
9.2.1	Cell–Cell Junctions	237
9.2.2	Cell–Matrix Junctions	238
9.3	Nanotechnology has Opened a new Era in Protein Research	241
9.3.1	Mechanochemical Signal Conversion and Mechanotransduction	241
9.3.2	Mechanical Forces and Structure–Function Relationships	242
9.4	Making the Very First Contacts	244
9.4.1	Molecular Players of Cell–Extracellular Matrix Junctions	244
9.4.1.1	Fibronectin	245
9.4.1.2	Integrins	246
9.4.1.3	Talin	247
9.4.1.4	Other Scaffolding Proteins that Provide a Linkage Between Integrins and F-Actin	250
9.4.1.5	Cell Cytoskeleton	250
9.5	Force-Upregulated Maturation of Early Cell–Matrix Adhesions	250
9.5.1	Protein Stretching Plays a Central Role	250
9.5.1.1	Vinculin is Recruited to Stretched Talin in a Force-dependent Manner	251
9.6	Cell Signaling by Force-Upregulated Phosphorylation of Stretched Proteins	252
9.6.1	Phosphorylation is Central to Regulating Cell Phenotypes	252
9.6.1.1	Stretch-Dependent Binding of Some Cytoplasmic Proteins to Cytoskeletons	254
9.6.1.2	Tyrosine Phosphorylation as a General Mechanism of Force Sensing	255
9.7	Dynamic Interplay between the Assembly and Disassembly of Adhesion Sites	257
9.7.1	Molecular Players of the Adhesome	257
9.8	Forces that Cells Apply to Mature Cell–Matrix and Cell–Cell Junctions	261
9.8.1	Insights Obtained from Micro- and Nanofabricated Tools	261
9.9	Sensing Matrix Rigidity	263
9.9.1	Reciprocity of the Physical Aspects of the Extracellular Matrix and Intracellular Events	263