THERAPEUTIC NANOMATERIALS
THERAPEUTIC NANOMATERIALS

Edited by

MUSTAFA O. GULER
Institute of Materials Science and Nanotechnology
National Nanotechnology Research Center (UNAM)
Bilkent University
Ankara, Turkey

AYSE B. TEKINAY
Institute of Materials Science and Nanotechnology
National Nanotechnology Research Center (UNAM)
Bilkent University
Ankara, Turkey

WILEY
CONTENTS

List of Contributors xi
Preface xiii

1 Nanomaterials for Medicine 1
   Mustafa O. Guler and Ayse B. Tekinay

1.1 Introduction, 1
1.2 Nanoscale Material Properties, 2
1.3 Nanomaterials for Understanding Disease Pathways, 2
1.4 Nanomaterials for Therapy, 3
1.5 Challenges and Future Prospects, 5

2 Nanosized Delivery Systems for Tissue Regeneration 7
   Goksu Cinar, Didem Mumcuoglu, Ayse B. Tekinay, and Mustafa O. Guler

2.1 Introduction, 7
2.2 Delivery of Protein Therapeutics with Nanocarriers for Tissue Regeneration, 10
   2.2.1 GFs and Cytokines, 10
2.3 Gene and siRNA Delivery with Nanocarriers for Tissue Regeneration, 13
   2.3.1 Gene Delivery, 13
   2.3.2 siRNA Delivery, 15
2.4 Systemic Targeting and Cellular Internalization Strategies for Tissue Regeneration, 15
   2.4.1 Targeted Delivery, 15
2.4.2 Cellular Internalization Strategies, 18

2.5 Future Perspectives, 20

References, 22

3 Nanomaterials for Neural Regeneration

Melike Sever, Busra Mammadov, Mevhibe Gecer, Mustafa O. Guler, and Ayse B. Tekinay

3.1 Introduction, 33
    3.1.1 Extracellular Matrix of Central Nervous System, 33
    3.1.2 ECM of Peripheral Nervous System, 37
    3.1.3 Urgent Need for Materials to Induce Regeneration in Nervous Tissue, 39

3.2 Nanomaterials for Neural Regeneration, 40
    3.2.1 Physical Functionalization of Nanomaterials to Induce Neural Differentiation, 40
    3.2.2 Effects of Mechanical Stiffness on Cellular Behavior, 40
    3.2.3 Effects of Dimensionality on Cellular Behavior, 42
    3.2.4 Effects of Substrate Topography on Cell Behavior, 43
    3.2.5 Effects of Electrical Conductivity on Cell Behavior, 44

3.3 Chemical and Biological Functionalization of Nanomaterials for Neural Differentiation, 45
    3.3.1 Effects of Biologically Active Molecules on Cell Behavior, 45
    3.3.2 Effects of Chemical Groups on Cellular Behavior, 46
    3.3.3 Effects of Biofunctionalization on Cellular Behavior Through ECM-Derived Short Peptides, 48

3.4 Conclusion, 50

References, 51

4 Therapeutic Nanomaterials for Cartilage Regeneration

Elif Arslan, Seher Ustun Yaylaci, Mustafa O. Guler, and Ayse B. Tekinay

4.1 Introduction, 59

4.2 Current Treatment Methods for Cartilage Injuries, 63

4.3 Tissue Engineering Efforts, 66
    4.3.1 Natural Polymers, 67
    4.3.2 Synthetic Polymers, 69
    4.3.3 Composite Materials, 70
    4.3.4 Physical Stimuli, 71

4.4 Clinical Therapeutics for Cartilage Regeneration, 72

4.5 Conclusions and Future Perspectives, 73

References, 78
5  Wound Healing Applications of Nanomaterials  
Berna Senturk, Gozde Uzunalli, Rashad Mammadov, Mustafa O. Guler, and Ayse B. Tekinay

5.1 Introduction, 87  
5.1.1 The Structure of Healthy Mammalian Skin, 88  
5.1.2 The Mechanisms of Wound Healing, 89  
5.1.3 Repair Process in Chronic Wounds, 94  

5.2 Applications of Nanomaterials for the Enhancement of Wound Healing Process, 95  
5.2.1 Artificial Skin, 96  
5.2.2 Natural Nanomaterials for Wound Healing, 97  
5.2.3 Synthetic Nanomaterials for Wound Healing, 100  
5.2.4 Wound Dressings Containing Growth Factors, 101  
5.2.5 Biomimetic Materials, 102  
5.2.6 Current Challenges in the Design of Nanomaterials for Chronic Wound Management, 103  

5.3 Peptide Nanofiber Gels for Wound Healing, 105  
5.3.1 Relevance of Nanofibrous Structure of Peptide Gels for Wound Healing, 106  
5.3.2 Engineered PA Nanofiber Gels for Wound Healing and Insights into Various Designs, 107  

References, 110

6 Nanomaterials for Bone Tissue Regeneration and Orthopedic Implants  
Gulcihan Gulseren, Melis Goktas, Hakan Ceylan, Ayse B. Tekinay, and Mustafa O. Guler

6.1 Introduction, 119  
6.2 Bone Matrix, 120  
6.2.1 Organic Matrix and Bioactivity, 120  
6.3 Inorganic Matrix, Mineralization, and Bone Organization, 122  
6.3.1 Mechanical Properties and Structural Hierarchy of Bone Tissue, 123  

6.4 Regulation of Bone Matrix in Adult Tissue, 125  
6.4.1 Angiogenic Factors in Bone Remodeling, 126  

6.5 Strategies for Bone Tissue Regeneration, 127  
6.5.1 Hard Grafts for Bone Regeneration, 127  

6.6 Soft Grafts for Bone Regeneration, 131  
6.6.1 Peptide-Based Bone Grafts, 132  
6.6.2 Polymer Nanocomposites as Bone Grafts, 134  

6.7 Future Perspectives, 138  
References, 138
7 Nanomaterials for the Repair and Regeneration of Dental Tissues

Gulistan Tansik, Alper Devrim Ozkan, Mustafa O. Guler, and Ayse B. Tekinay

7.1 Introduction, 153
7.2 Formation of Dental and Osseous Tissues, 155
7.3 Dental Implants, 156
   7.3.1 Metallic Implants, 158
   7.3.2 Ceramic Implants, 158
   7.3.3 Polymeric Implants, 159
7.4 Osseointegration of Dental Implants, 159
7.5 Uses of Nanotechnology in the Development of Dental Implants, 160
   7.5.1 Enhancement of the Osseointegration Process, 161
   7.5.2 Pulp and Dentin Tissue Regeneration, 162
   7.5.3 Whole Tooth Regeneration, 165
7.6 Conclusions and Future Perspectives, 166
References, 166

8 Nanomaterials as Tissue Adhesives

I. Ceren Yasa, Hakan Ceylan, Ayse B. Tekinay, and Mustafa O. Guler

8.1 Introduction, 173
8.2 Tissue Adhesives Based on Synthetic Polymers, 176
8.3 Naturally Derived Tissue Adhesives, 180
8.4 Bioinspired Strategies, 182
8.5 Nanoenabled Adhesives, 186
8.6 Conclusion and Future Prospects, 186
References, 189

9 Advances in Nanoparticle-Based Medical Diagnostic and Therapeutic Techniques

Melis Sardan, Alper Devrim Ozkan, Aygul Zengin, Ayse B. Tekinay, and Mustafa O. Guler

9.1 Introduction, 197
9.2 NPs used in MRI, 200
   9.2.1 $T_1$ CAs, 201
   9.2.2 $T_2$ CAs, 205
   9.2.3 Dual Modal Contrast Agents, 207
9.3 NPs used in Computed Tomography, 208
   9.3.1 Noble Metal-Based NPs, 209
   9.3.2 Heavy Metal-Based NPs, 211
9.4 NPs used in Optical and Fluorescence Imaging, 213
   9.4.1 Quantum Dots, 214
10 Biosensors for Early Disease Diagnosis

Ahmet E. Topal, Alper Devrim Ozkan, Aykutlu Dana, Ayse B. Tekinay, and Mustafa O. Guler

10.1 Introduction, 235
10.2 Biosensor Elements, 237
   10.2.1 Recognition Elements, 237
   10.2.2 Output Type and Detection Techniques, 239
   10.2.3 Optical Biosensors, 248
   10.2.4 Electrical and Electrochemical Biosensors, 250
   10.2.5 Mechanical Biosensors, 251
   10.2.6 Other Biosensor Types, 252
10.3 The Impact of Nanotechnology and Nanomaterials in Biosensor Design, 253
10.4 Early Diagnosis and Biosensor-Based Disease Detection, 255
10.5 Conclusion and Future Directions, 258
References, 259

11 Safety of Nanomaterials

Nuray Gunduz, Elif Arslan, Mustafa O. Guler, and Ayse B. Tekinay

11.1 Introduction, 271
11.2 Characterization, Design, and Synthesis of Nanomaterials, 272
   11.2.1 Chemical Identity and Physicochemical Properties, 272
   11.2.2 Biological Identity, 275
11.3 Interactions at the Cell–Material Interface, 277
   11.3.1 Intracellular Activity, 278
   11.3.2 Cellular Uptake Mechanisms, 283
11.4 Assays for Cell Viability/Proliferation, 283
   11.4.1 Assays for Oxidative Stress and Apoptosis Mechanisms, 284
   11.4.2 Evaluation of Uptake and Accumulation of ENMs, 284
   11.4.3 Genotoxicity Assays, 285
11.5 Animal Models and Long-Term Risk Assessment, 286
   11.5.1 The Blood–Brain Barrier, 286
11.6 Conclusions and Future Perspectives, 290
References, 291

Index
LIST OF CONTRIBUTORS

Elif Arslan
Hakan Ceylan
Goksu Cinar
Aykutlu Dana
Mevhibe Gecer
Melis Goktas
Mustafa O. Guler
Gülçihan Gulseren
Nuray Gunduz
Busra Mammadov
Rashad Mammadov
Didem Mumcuoglu

Alper Devrim Özkan
Melis Sardan
Berna Senturk
Melike Sever
Gülistan Tansık
Ayse B. Tekinay
Ahmet E. Topal
Gözde Uzunallı
I. Ceren Yasa
Seher Ustun Yaylacı
Aygul Zengin

Affiliation (all contributors): Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, Turkey
Interdisciplinary approaches through contributions from chemistry, biology, materials science, physics, engineering, and medicine offer a new generation of therapeutic methods, which can be used for the early diagnosis and treatment of many diseases and injuries that ail human population today. This book aims to provide a general perspective about nanomaterials and their use for therapeutic purposes for scientists, clinicians, patients, students, and novices in the field. It also provides detailed information on types of nanomaterials and their biomedical application areas for experts in nanosciences. Here we discussed how nanomaterials can be used for biomedical applications in addition to understanding side effects of these materials to humans and environment, and we also cautioned the lawmakers to make the necessary regulations.

This book discusses new materials for treatments of different types of tissues and organs. In addition to therapy, new methods for diagnosing diseases are briefly described. Main treatment methods were discussed under regeneration of tissues in situ. With the increase in aging population in the world, especially in developed countries, there is also an increased prevalence of degenerative disorders. Both degenerative disorders and accidental injuries can cause detrimental changes in various tissues, which result in not only deterioration of life quality of patients and caregivers but also a considerable amount of financial burden on the health systems of individual countries. Current treatment options for many of these injuries are insufficient. We believe that new generation of therapeutic materials
will be utilized extensively in the regenerative medicine field. Although there are many species that can perfectly regenerate the injuries in their tissues, *Homo sapiens* is not one of them. Therefore, when humans get injured, they need external help for repair of their injuries, with the fact in mind that they cannot fully regenerate. The deficiency of tissue repair mechanisms is more evident in some tissues like brain or cartilage, and the ability to repair is known to decrease with age.

**Mustafa O. Guler and Ayse B. Tekinay**
NANOMATERIALS FOR MEDICINE

Mustafa O. Guler and Ayse B. Tekinay

Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, Turkey

1.1 INTRODUCTION

Nanotechnology is an interdisciplinary research area that studies the characteristics of materials at nanometer scale and developing new materials with new functionalities. Advances in nanotechnology enable us to develop new molecules and materials with more controlled chemical, physical, and biological properties. The new techniques and materials produced by using nanotechnology provide a vast array of opportunities for diagnosis and therapy of many diseases that are still considered extremely challenging by medical professionals such as cancer, Alzheimer’s disease, Parkinson’s disease, diabetes, and aging-related disorders. In addition, nanotechnology provides us tools to study the in-depth mechanisms of the biological machinery enabling us to learn more about the pathophysiology of the diseases. These detailed analyses can be utilized to pinpoint the exact causes behind these diseases and correct the defects in the biological machinery. Since biological machinery works at nanoscale (e.g., the diameter of DNA is 2 nm; a typical ribosome’s diameter is 20–30 nm; individual collagen fibers of the extracellular matrix are ~1.5 nm in diameter and 300 nm in length), it can best be manipulated by using nanoscale materials with controlled functionalities.
Thus, nanomaterials with controlled physical, chemical, and biological characteristics can be used for the therapy of the specific causes of the diseases.

Overall, nanomaterials serve two important purposes for medical applications: They can be utilized to understand the pathophysiology of the diseases by enhancing detailed knowledge of biological machinery and increasing diagnosis efficiency, and they can provide us novel approaches to interrupt or correct the regular biological activity depending on the disease type and the treatment strategy.

1.2 NANOSCALE MATERIAL PROPERTIES

Nanoscale is generally considered as dimensions between 0.1 and 100 nm, and nanomaterials can display extraordinary characteristics compared to their micro- or macroscale counterparts. New synthesis techniques can control shape and function of materials at the nanometer level. There are several ways to develop new materials in nanometer scale. Mainly, top-down and bottom-up approaches are the two major techniques to produce nanomaterials. In the top-down techniques, bulk materials are tailored into specific shape and size with recent high-tech tools. For example, soft-lithography techniques can craft bulk surfaces into nanostructured textures to create a high surface area and molecular contact points with the biological materials. In bottom-up approaches, small molecular building blocks are used to form more complex and higher-scale nanometer-sized materials. Both techniques have advantages and disadvantages in terms of their fabrication method and product function. In theory, it is desired to utilize both techniques in conjunction so that we can eliminate the weaknesses of each technique. Depending on the application area, either one or both of these approaches can be used to develop materials that can be used in studying pathophysiology of diseases and their diagnosis and therapy. Especially, bioinspired and biomimetic strategies yield products that can replace or accommodate activities of the natural biomolecules. Nevertheless, for effective diagnosis and therapy of diseases, it is almost crucial to first understand the molecular reasons behind disease development.

1.3 NANOMATERIALS FOR UNDERSTANDING DISEASE PATHWAYS

Biological machinery is known for its perfect balance, and runs within a complex network, which enables it to tolerate irregularities up to a certain level. Diseases occur when these irregularities cannot be tolerated, and
several reasons might cause this, which are generally classified as hereditary or environmental reasons. In most cases, both of these components are the culprits behind medical problems, and it is always important to understand the changes in molecular level to decide the most appropriate treatment. For example, when an irregular activity of a protein, which can result in a disease, is detected, the necessary precautions can be taken or developed for the appropriate treatment. In some cases, protein production mechanism can be targeted to discontinue the disease-related activity. In other cases, the specific protein could be targeted and blocked; therefore the protein can be inactivated to stop the undesired activity. Since biological machinery works at the molecular level, these mechanisms can be best understood by using techniques that provide the highest sensitivity. Many of the current techniques that are used in biomedical research utilize microtechnology, which not only require higher amounts of biomolecules for analyses but also are only sensitive at microscale. On the other hand, techniques that utilize nanotechnology have recently been introduced in biomedical research and have revolutionized particular research areas. Developing DNA sequencing strategies for personalized medicine, biosensors with higher sensitivity that can be used for detection of low levels, or biomolecules and even nanoparticles that can be used for isolation for biomacromolecules such as DNA, RNA, or proteins are some of the examples of recent use of nanotechnology in understanding disease pathophysiology. On the other hand, there is an enormous amount of research in the recently published literature on developing better technologies for understanding biological events and pathways including nanomaterials for biocompatible labeling of biomolecules and cells for more efficient monitoring of activity, for tailoring nanomaterials for enhanced targeting ability (compared to regularly used antibodies), and for targeted blocking of biomolecular activity to understand their functionality in more detail. Application of these methods to biomedical research will yield in gaining more knowledge in the working mechanisms of biological machinery, and pathophysiology of diseases, and for enhancing diagnostic capabilities, all of which will in turn provide more opportunities for therapy.

1.4 NANOMATERIALS FOR THERAPY

To cure diseases with synthetic materials, the materials should be able to interact with specific biological actors in their natural environment. These biological actors can be cell surface receptors, which are mostly composed of proteins and carbohydrates; extracellular elements, such as growth
factors, cytokines, or structural components like collagens; or intracellular elements, such as DNA, ribosomes, RNA, enzymes, etc. The optimal venue of interaction with biomolecules would be similar to the way they interact with their natural binding partners, so that the balance of the biological machinery can be reinstated. Therefore, the materials to be used should carry physical properties to meet the requirements for appropriate interactions. In addition to these, the materials should be functionalized with bioactive molecules. The interaction between the bioactive domain of the material and the target protein determines the stability of the complex and determines the fate of the biological activity.

Nanomaterials are used for therapy of diseases through several ways such as targeted drug/gene delivery approaches and induction of regeneration of damaged tissues by using nanomaterials. For targeted drug delivery, nanomaterials can be used as targeting molecules, as carrier systems, or as the bioactive drug itself. Aptamers, for example, are one example of how tailored nanomaterials can be used for targeting purposes. On the other hand, most of the research on nanomaterials for drug delivery has focused on developing carrier systems such as liposomes, polymeric nanoparticles, or metal-based nanoparticles. Although small-molecule drugs are the most commonly used therapeutics used for drug delivery approaches, there have been serious advances in producing tailored nanomaterial drugs, mostly in the form of small peptides or their conjugates.

The nanomaterials can be also used in regenerative medicine applications. To regenerate the tissue defects caused by diseases, materials can form an artificial three-dimensional environment to fill the gap with the bioactive signals derived from the natural healing process. The soluble factors can diffuse inside this network, and the cells in the proximity can migrate to the defect side. If correct signals and the optimum environment are provided, the tissue defect can be healed and function of the tissue can be recovered. Many polymeric materials have previously been tailored to mimic the natural biomacromolecules both physically and chemically. These materials have also been further functionalized through addition of natural biological molecules such as growth factors. On the other hand, there is a growing area of nanomaterials that are synthesized by using natural biomaterials such as peptide nanofiber systems, which can be produced through bottom-up approaches. These nanomaterials can be specifically designed to mimic natural proteins and carbohydrates to distinctively interact with particular biomacromolecules so that they induce differentiation of stem cells into specific lineages and induce functional tissue regeneration.
1.5 CHALLENGES AND FUTURE PROSPECTS

Although there have been extensive advances in developing nanomaterials for biomedical purposes, only few of them have been translated into clinics. The major limitations behind this delay are about the biocompatibility and biodegradability of nanomaterials.

One of the desired properties of the nanomaterials in the biological environment is their physicochemical stability. When a nanomaterial is injected into the blood vessels, there are several biological macromolecules that can interact with it in the environment. The noncovalent interactions including hydrogen bonds, electrostatic interactions, and van der Waals forces cause the undesired interactions in the blood. These may cause problems in the blood flow, or simply the nanomaterials cannot travel in the blood vessels, and they fail to reach to the target. In some cases, the interaction of these random molecules in the blood changes the surface chemistry or bioactivity of the nanomaterials, and they may cause undesired side reactions.

Undesired accumulation of the nanomaterials in the body and side products produced by degradation of the nanomaterials is another drawback in the use of nanomaterials for therapeutic purposes since these may cause side effects. Major areas where nanomaterials are accumulated in the body are the liver, spleen, and kidneys, which might result in metabolic problems associated with these organs, which eventually can cause organ failure.

Beyond many advances in the field of molecular biology and medicine, most molecular interactions between biomacromolecules are unknown, and our knowledge pathophysiology of diseases and the mechanisms of tissue regeneration are limited. Thus, one of the major challenges in developing and using nanomaterials for therapeutic purposes lies in the lack information on appropriate target molecular mechanisms or pathways. With more advancement in understanding of these interactions and better control on production of nanomaterials, biocompatible and bioactive nanomaterials with tightly regulated characteristics can be developed to interact with biomolecules to correct and regulate the natural biological interactions to cure diseases in the future. Beyond diagnosis, these advances can also be used to design and fabricate nanomaterials that can deliver drugs or trigger natural key reactions for regeneration purposes.

It is important to stay up to date on how nanomaterials can be used for diagnostic and therapeutic purposes by presenting specific examples from the literature. The research on biomedical nanomaterials can be classified.
according to their medical applications. Since nanotechnology is a fairly new technology with many unknowns, several examples of nanomaterial–biological organism interactions in terms of nanotoxicology research were demonstrated in order to stress that although nanomaterials provide a vast array of opportunities for the diagnosis and treatment of diseases, the consequences of using these new types of materials should be carefully weighed prior to their use in medical practice.
2

NANOSIZED DELIVERY SYSTEMS FOR TISSUE REGENERATION

Goksu Cinar, Didem Mumcuoglu, Ayse B. Tekinay, and Mustafa O. Guler

Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, Turkey

2.1 INTRODUCTION

Repair and regeneration of damaged tissue is an important clinical need since millions of people all over the world are suffering from tissue and organ failure (Rice et al., 2013; Wei and Ma, 2008). Although tissue regeneration processes and components are quite different depending on tissue properties, the fundamentals of regeneration process involves cells, cellular microenvironment, and biological signals. Hence, the strategies in tissue regeneration focus on cell-based therapies, regenerative biomaterials as scaffolds, and delivery of biological signals to regenerating site. The integration of these strategies and the components with natural tissue healing stages is important to achieve successful therapies in clinical applications (Howard et al., 2008).

Controlled delivery of biologics such as growth factors (GFs), cytokines, nucleic acids, or siRNA is the focus of growing interest in tissue regeneration applications since these biomacromolecules serve highly specific and complex functions in cellular processes compared to small-molecule
synthetic drugs (McCall et al., 2011; Vermonden et al., 2012). Although therapeutic approaches in tissue regeneration focus on biologics, the clinical applications are highly limited due to the fragile nature and instabilities of the molecules. Delivery systems can increase therapeutic applicability of biologics sustaining a suitable environment for three-dimensional conformations and protection from enzymatic degradation during transportation to the regenerating site. In addition, these systems aim to mimic natural release mechanisms, conditions, and therapeutic dosage of biologics, which are controlled by distinct patterns and enzymatic reactions in cellular microenvironment (Tessmar and Göpferich, 2007). Stimuli–response of the nanosized carriers is also an important property to decrease side effects and effective dosage of the molecules necessary for the regeneration in tissue-specific conditions (Alvarez-Lorenzo and Concheiro, 2014).

Advances in material science and integration with nanotechnology provide us a new generation of biodegradable and biocompatible nanosized delivery systems with control over size, shape, and multifunctionality (Panyam and Labhasetwar, 2003; Zhang et al., 2013). Nanosized delivery systems can be designed as lipid-based, inorganic, polymeric, or multifunctional hybrid systems including liposomes, polymeric micelles, mesoporous silica nanoparticles, nanogels, or nanocomplexes with sizes varying between 1 and 200 nm (Khandare et al., 2012). Nanosized delivery systems provide tissue penetrating ability, reduced toxicity, and enhanced permeation and retention (EPR) in regenerating tissues (Gu et al., 2011). These systems can be injected to the bloodstream and extend the release of biologics protecting them from proteolytic cleavage and chemical degradation. EPR effect also provides passive targeting for the nanosized carriers increasing circulation time in the bloodstream. Improved solubility of hydrophobic compounds can be obtained via nanosized delivery systems (Mishra et al., 2010). In addition, nanosized delivery systems can be integrated into three-dimensional scaffolds sustaining controlled release and biocompatible microenvironment for regenerating cells (Wei and Ma, 2008).

Multifunctional nanosized delivery systems for biologics have been developed using both natural and synthetic polymers. Synthetic polymers enable sustained release of biologics over a period of days to several weeks based on different release mechanisms including concentration, degradation, affinity or stimuli controlled, and also combinations of them. FDA-approved synthetic polymers such as poly(lactide-co-glycolide) (PLGA) have been used for developing nanosized delivery systems due to their biocompatibility and tunable physical and chemical properties (Golub et al., 2010). PLGA nanoparticles can be hydrolyzed into biodegradable
metabolites: lactic and glycolic acid at acidic conditions (Kumari et al., 2010). In addition, there are examples of polymeric nanoparticles developed as biological delivery systems using poly(lactic acid) (PLA), poly(D,L-glycolide) (PLG), poly-ε-caprolactone (PCL), and poly(alkyl cyanoacrylates) for tissue regeneration (Kim et al., 2014). Although some of these synthetic nanosized polymeric systems reveal biodegradable properties, degradation products can be toxic compounds, and degradation process takes longer time periods. Moreover, formulation conditions and encapsulation steps may require organic solvents and high ionic strength, creating a harsh environment for biologics.

On the other hand, natural polymers can form biodegradable polymeric nanostructures for biomacromolecule delivery at mild conditions, and these nanostructures are functionalized with different delivery strategies for targeting and internalization. For these purposes, both protein-based natural polymers such as gelatin, collagen, albumin, and elastin and hydrophilic polysaccharides including alginate or chitosan have been used to obtain colloidal nanostructures. Compared to synthetic polymeric nanostructures, natural nanocarriers can be easily degraded by digestive enzymes and degradation products not harmful. However, the hydrophilic nature of these nanosized carriers leads to burst release of biologics when biologics are immobilized within the system via noncovalent interactions or short-term release profiles can be obtained due to easier biodegradability compared to synthetic polymeric nanosized delivery systems. In addition, contamination risk is higher since the sources of these compounds are natural organisms.

Molecular assemblies of both synthetic and natural compounds are intriguing nanosized delivery structures for biologics. Colloidal nanostructures such as liposomes, polymeric nanoparticles, block copolymer micelles, and dendrimers can be designed as stimuli responsive, and delivery mechanisms can be enhanced via internal and external factors including pH, temperature, or redox microenvironment (Fleige et al., 2012; Ganta et al., 2008). Intravenous administration of these colloidal nanosized systems also makes them suitable carriers for tissue regeneration applications (Alyautdin et al., 2014). In addition, the stimulated effects of gold nanoparticles between 20 and 50 nm size on osteogenic differentiation of stem cells and osteoblast-like cells have been showed in different studies (Heo et al., 2014; Ko et al., 2015).

In this chapter, we focus on advanced delivery of biologics including GFs, cytokines, genes, or siRNAs using a variety of nanosized systems for different regeneration applications focusing on bone, cartilage, nervous system, and muscle regeneration strategies. The limitations of biologics delivery and alternative strategies for overcoming recent problems are underlined presenting recent examples from the literature. In addition,
specific targeting and cellular internalization strategies of biologics delivery for tissue regeneration are discussed for providing future perspectives to the readers in this field.

2.2 DELIVERY OF PROTEIN THERAPEUTICS WITH NANOCARRIERS FOR TISSUE REGENERATION

2.2.1 GFs and Cytokines

As biological regulatory signals in variety of cellular responses, GFs are important components for controlling and directing tissue formation, maintenance, and regeneration. In addition, these biological signals direct crucial tissue regeneration processes such as angiogenesis and bone or granulation tissue formation (Eichmann and Simons, 2012; Laurencin et al., 2014; Ponte et al., 2007). Although the functions and importance of many GFs in tissue regeneration are well known, the clinical applications and therapeutic efficiency of these biologics are limited due to their short lifetime, production costs, and safety concerns for immunogenic responses (Martino et al., 2014; Rice et al., 2013). To increase their lifetime and facilitate controlled release of biologically active GFs over an extended time period for therapeutic applications, nanosized delivery strategies have been developed using different materials.

In living organisms, GFs are stabilized in extracellular matrix (ECM) via interactions of highly sulfated ECM components such as glycosaminoglycan (GAG) side chains. GFs can be immobilized on nanosized carriers by either noncovalent or covalent interactions (Chen et al., 2010). Polymeric nanoparticles with functional groups can be designed by sustaining these specific interactions for immobilization and affinity-controlled GF delivery (Wang and von Recum, 2011). Natural polyanionic polysaccharides such as heparin and chondroitin sulfate can form nanocomplexes with natural or synthetic polycationic polymers, and polyelectrolyte complex nanoparticles are used to deliver heparin-binding GFs including fibroblast growth factor (FGF) family and transforming growth factor-β (TGF-β) superfamily (Place et al., 2014). Natural polymers including proteins and polysaccharides can be modified with several functional groups and form colloidal nanostructures, which are also suitable candidates for delivering GFs via noncovalent interactions or covalent crosslinking. Gelatin is a well-known natural protein-based polymer with excellent biocompatibility and controllable biodegradability. It can be modified as either negatively or positively charged and enable polyion complexation with several GFs (Young et al., 2005).
Angiogenesis is a critical process in tissue restoration and constitutes of establishing a vascular system to supply required oxygen and nutrition to the regenerating site. Vascular endothelial growth factor (VEGF), which is a member of the cystine knot family, is an important biological factor for mediating angiogenesis in wound healing and myocardial ischemia treatments (Crafts et al., 2015). Different delivery strategies are developed for sustained release of bioactive VEGF to the regenerating site. In one study (Golub et al., 2010), VEGF-loaded PGLA nanoparticles enhanced blood vessel growth via sustained delivery for cardiovascular medicine applications. Polyelectrolyte nanosized complexes formed by coacervation of VEGF-bound dextran sulfate with different polycations such as chitosan, polyethylenimine, or poly-l-lysine were shown to have high encapsulation efficiency for the regeneration applications (Huang et al., 2007). In addition, it was shown that GAG-based polyelectrolyte nanocomplexes are quiet stable delivery systems at physiological conditions and show resistance to high ionic strength for in vivo applications (Novoa-Carballal et al., 2014). In another example, in vivo therapeutic revascularization was obtained via VEGF-loaded heparin-functionalized nanoparticle–fibrin complexes in a rabbit ischemic hind limb model (Chung et al., 2010).

Bone regeneration consists of cascades of complex biochemical processes that are coordinated via cells, ECM, and bioactive molecules such as osteogenic, angiogenic, inflammatory, and systemic GFs (Vo et al., 2012). The enhanced therapeutic effects of different GFs such as osteoinductive bone morphogenetic protein-2 (BMP-2) and mitogenic platelet-derived growth factor-BB (PDGF-BB) have been shown in clinical trials for bone defect treatments (Nevins et al., 2013; Shah et al., 2014). BMP-2 is an important member of TGF-β superfamily that includes major modulators of osteogenesis, which play important roles in the commitment and differentiation of osteoprogenitors. BMP-2 loaded 2-N,6-O-sulfated chitosan-based nanoparticles (S-NPs) were developed via complex coacervation of oppositely charged polyelectrolyte solutions as a delivery system for bone regeneration applications (Cao et al., 2014). In another study (Gan et al., 2015), pH-responsive chitosan-functionalized mesoporous silica nanoparticles were used for dual delivery of BMP-2 and dexamethasone (Dex) for osteoblast differentiation and bone regeneration at in vitro and in vivo conditions.

Neurotrophic factors (NF) including nerve growth factor (NGF), glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), or basic FGF-2 are regulatory biological signals for promoting the development, survival, and regeneration of neurons (Harvey et al., 2014; Ziv-Polat et al., 2014). The alterations in
cellular structure and metabolism of damaged neurons lead to impaired regeneration (Sivak et al., 2014). Biological factors can induce endogenous repair, enhance neural regeneration, and hinder inhibitor signals for the regeneration process (Donagheue et al., 2014). Delivery of these factors to the central nervous system (CNS) is a developing approach for treatments of traumatic brain, spinal cord, and peripheral nerve injuries and neural degenerative disorders (Mohtaram et al., 2013).

Different strategies have been developed for therapeutic delivery of biologics into the CNS such as liposomes, nanospheres, nanocapsules, dendrimers, and polymeric micelles (Orive et al., 2009). The design of nanosized delivery systems for the CNS focus on two important requirements: long circulation time of the nanocarrier systems consisting regenerating signals and ability to penetrate blood–brain barrier (BBB), which is formed by tight junctions preventing crossing of large neurotrophic protein molecules (Thorne and Frey II, 2001; Zhong and Bellamkonda, 2008). BDNF-bound magnetically guided nanoparticles overcame impermeability of BBB and decreased morphine-induced apoptosis in the CNS restoring the spine density and promoting regeneration of synaptic connections (Pilakka-Kanthikeel et al., 2013). In another study, bFGF-loaded PEG–PLGA nanoparticles were functionalized with Solanum tuberosum lectin (STL) for targeted delivery to brain tissue and enhanced spatial learning and memory of rats with Alzheimer’s disease (AD) promoting the survival and neurite growth of neurons (Zhang et al., 2014).

The delivery systems loaded with multiple GFs can mimic complex in vivo conditions releasing different therapeutic biologics for effective tissue regeneration (Chen et al., 2010). Time-dependent controlled releases of multiple GFs at different stages of tissue regeneration via nanosized delivery systems enhance the biochemical processes sequentially. In one study (Perez et al., 2013), bone regeneration was induced by initial release of the angiogenic factors like VEGF or bFGF and then supported via BMP-2 release as an osteogenic factor.

Platelet-rich plasma consist of biologically active proteins including several GFs, such as the isoforms of platelet-derived growth factor (PDGF), transforming growth factor (TGF), and FGF (Intini, 2009). The lysates of platelet-rich plasma are natural sources of multiple GFs that are important in bone regeneration (Visser et al., 2009). Hence, controlled delivery of plasma lysates via chitosan–chondroitin sulfate nanoparticles prepared by polyelectrolyte complexation was intriguing for bone tissue engineering applications promoting osteogenic differentiation via multiple factors (Santo et al., 2012).

Cytokines are small signaling molecules with sizes ranging between 8 and 40kDa and are responsible for regulating the immune response,
inflammation, neoangiogenic processes, and cellular differentiation (Ioannidou, 2006). Cytokines are also important biochemical factors in tissue regeneration controlling cellular activity (Gelain et al., 2010). However, short half-lives and serum-mediated degradation of cytokines prevent therapeutic applications and lead to developing different strategies for delivery of these small biologics. Cytokine delivery to injury site by controlled delivery systems can direct tissue regeneration in the absence of the transplanted cells (Roche et al., 2013).

Inflammation process is one of the critical steps of regeneration, and this complex step is controlled by secretion of GFs and pro- or anti-inflammatory cytokines. Cytokine-mediated signaling of inflammation involving activation and proliferation of satellite cells is an essential component of muscle repair, regeneration, and growth (Tidball, 2005). Delivery of cytokines such as tumor necrosis factor-α (TNF-α) (Chen et al., 2007), interferon-γ (Cheng et al., 2008), or interleukin-6 (IL-6) (Serrano et al., 2008) mediating inflammation in muscle regeneration processes can be an alternative approach for the treatments of acute or chronic muscle damages.

2.3 GENE AND siRNA DELIVERY WITH NANOCARRIERS FOR TISSUE REGENERATION

2.3.1 Gene Delivery

Gene therapy provides sustained expression of bioactive molecules including GFs and cytokines required for tissue regeneration (Bonadio et al., 1999) and can assist stem cells to differentiate into a variety of different lineages and cells types for regenerative medicine (Chen et al., 2011b). Low uptake across the cell membrane, limited stability of DNA molecules, and lack of nuclear targeting are the main difficulties in DNA delivery (Luo and Saltzman, 2000). Controlled delivery of DNA using nanosized carriers promotes gene delivery and extended transgene expression. In addition, targeted nanosized carriers for gene delivery can avoid side effects including immune response or distribution to the nontargeted tissue and cells (Pannier and Shea, 2004).

Delivery of DNA encoding for inductive biologics can spatially influence on cellular behavior and enhance the formation of complex architectures for tissue regeneration (De Laporte and Shea, 2007). Although viral and retroviral vectors have been showed to be highly efficient transfection agents for in vivo, nonviral systems provide lower immune response and controllable chemical and biological properties for gene delivery (Elangovan et al., 2014;
Leong et al., 1998). Nonviral delivery nanocarriers for DNA are developed using a variety of synthetic and natural polymers such as PLL, PLG, PVA, PEG, poly(ethyleneimine) (PEI), collagen, hyaluronic acid, gelatin, or chitosan (Pannier and Shea, 2004).

Cationic lipids consisting of a positively charged head group, a hydrophobic chain, and a linker that joins the polar and nonpolar regions can interact with negatively charged DNA molecules and form complexes called “lipoplexes” (Bhattacharya and Bajaj, 2009; Gao and Hui, 2001; Tros de Ilarduya et al., 2010). Similar to cationic lipids, cationic polymers can also form complexes with DNA and form “polyplexes,” which are capable of gene delivery into targeted cells (Zhang et al., 2004). PEI is a highly cationic polymer due to the presence of amino groups, and it can form nanoparticles by complexation with negatively charged plasmid DNA (pDNA) and prevents pDNA from lysosomal nuclease degradation (Pérez-Martínez et al., 2011). Chondrogenic differentiation of hMSCs was facilitated by the delivery of Sox9 gene, an important transcription factor in the process of chondrogenesis, complexed with PEI on PLGA nanoparticles (Jeon et al., 2012). In another study, pDNA encoding Runt-related transcription factor 2 (RUNX2) was delivered in liposomes, which were immobilized at the surface of polycaprolactone (PCL) nanofiber meshes sustaining physical support for hMSCs during osteogenic differentiation (Monteiro et al., 2014). Bone regeneration in rat cranial defects was also enhanced via localized delivery of PEI/pDNA nanocomplexes inducing expression of bone morphogenetic protein-4 (BMP-4) (Huang et al., 2005). On the other hand, cationic DNA/polymer nanocomplexes have some drawbacks including instabilities in physiological conditions and aggregations due to binding of serum proteins on the surfaces. Also cationic lipids and polymers (PEI) can be toxic. To eliminate these drawbacks, targeted delivery and internalization strategies have been developed for enhanced therapeutic activities.

Dendritic nanostructures are also promising gene delivery systems due to their highly branched structure, shape, and multivalency. These structures can be designed using biodegradable polymers providing non-toxicity and efficiency for gene delivery (Luo et al., 2014). Especially, polycationic dendrimers such as polyamidoamine (PAMAM) were shown to be particularly interesting in nucleic acid delivery providing high multivalent surface moiety, biocompatibility, and low cytotoxicity (Lee et al., 2014). Human bone morphogenetic protein-2 (hBMP-2) gene-containing PAMAM dendrimers were used as gene transfer carriers for inducing osteogenic differentiation of mesenchymal stem cells (MSCs) for bone regeneration applications (Santos et al., 2009).