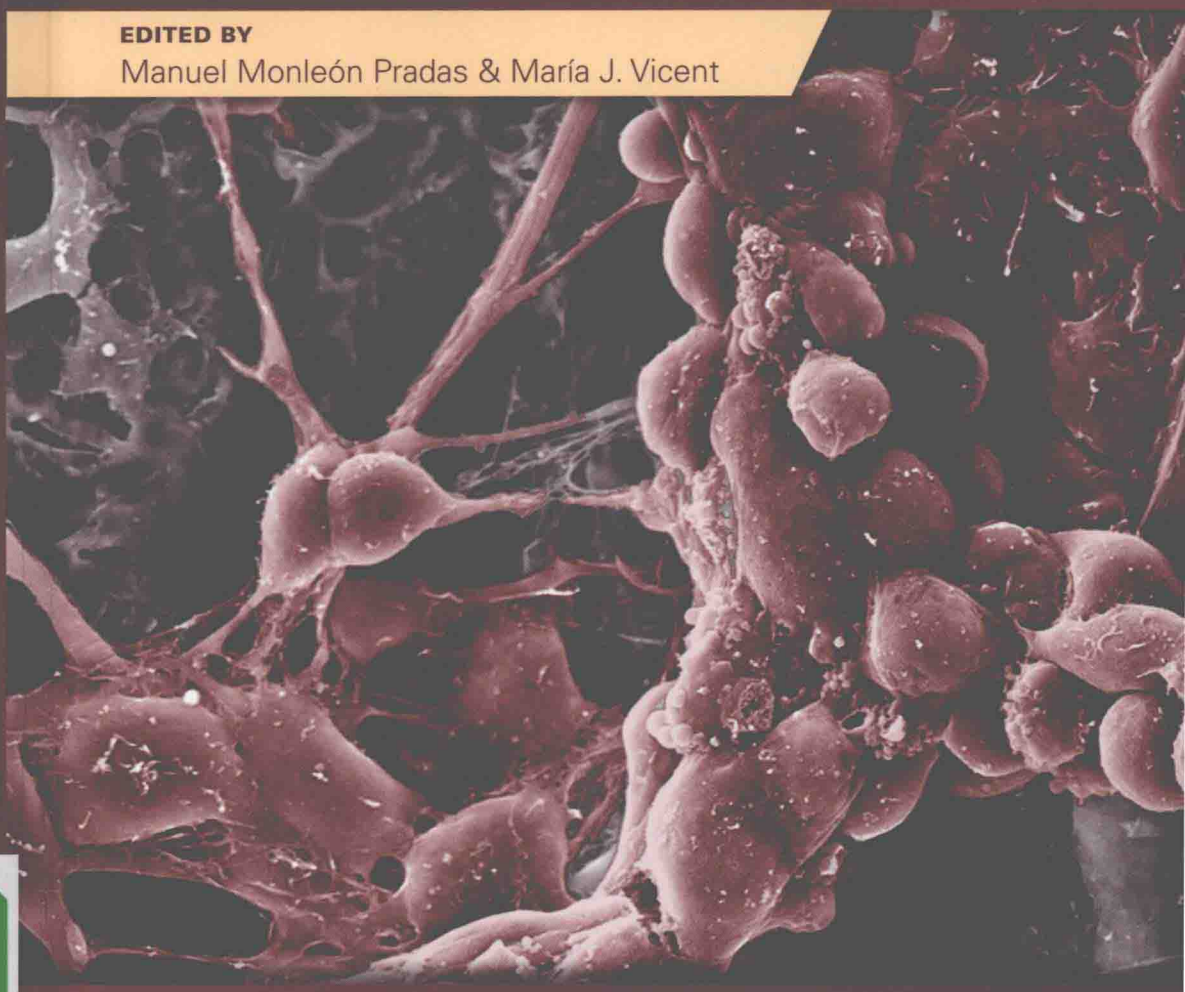
Polymers in Regenerative Medicine

Biomedical Applications from Nano- to Macro-Structures

EDITED BY
Manuel Monleón Pradas & María J. Vicent



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POLYMERS IN REGENERATIVE MEDICINE

PREFACE

Life expectancy has been continuously increasing and, consequently, human pathologies related to aging, such as musculoskeletal disorders, arthritis, nonhealing wounds, or neurodegenerative diseases, are becoming major health problems. Therefore, there is a need to identify novel strategies to improve the current therapeutic armory. This book presents a number of topics from polymer applications in the field of regenerative medicine, with a span from polymeric nanostructures to scaffolds. The full therapeutic potential of novel polymeric systems can only be developed through multidisciplinary collaborative research involving biologists, chemists, clinicians, and industries. This book tries to provide concepts and foundations to a general readership, as well as current applications and an overview of this exponentially growing field for experts.

Synthetic and natural polymers are compounds of great interest in many fields, especially in biomedical applications. In the past, they have been extensively used as excipients in traditional dosage forms, as materials for prostheses, valves, or contact lenses. More recently, their applications have been extended to sophisticated drug delivery systems and rationally designed scaffolds for cell therapy, so that interesting polymer structures for a variety of applications now cover the nanoscale in polymer therapeutics, the microscale in delivery systems, and the macroscale in hybrid cell-material constructs for tissue regeneration.

Polymeric materials are especially suited to interface with cells. Polymers are long-chain molecules that share basic features with biological macromolecules: both kinds of molecules deform with the inertial mechanism of conformational change and both are able to exhibit structure at a molecular level (the local sequence of different chemical monomers) and at a supramolecular and nano- to micrometer level (phase-separated domains, crystalline domains). More complex multimolecule

arrangements leading to the macroscopic network structure of the extracellular matrix (ECM) represent a third level of structure, with typical dimensions ranging from tens to hundreds of microns.

The contributions in the first part of the book, "Methods for synthetic extracellular matrices and scaffolds," comprise those topics that are more directly related to the tissue engineering and regenerative applications of polymer structures, where their micro- and macrostructures have more importance. Key questions permitting a rational design (Chapter 1) and selection of materials (Chapters 2 and 6) for scaffolds with adequate interactions with the biological interphase (Chapters 3–5) are addressed, as well as specific techniques and applications where scaffolds drive the therapeutic output, and organ replacement is discussed in Chapter 11. A closer look is then given in Part B, "Nanostructures for tissue engineering," to the effect of modifications at the nanoscale, a hot topic in the design of nanomedicines for tissue repair, a field of exponential growth. Here the selection of polymers as active components of nanostructures together with the understanding of the solution conformation of natural and synthetic materials (Chapter 8) with self-assembled properties at the nanoscale (Chapter 7) is of crucial importance to better design therapies in regenerative medicine. These materials should be able to efficiently deliver to the targeted site the bioactive agents of different nature including small drugs, peptides, proteins (Chapters 8 and 10), or even oligonucleotide sequences (Chapter 9).

Chapter 1 addresses the performance of polymers as materials for tissue engineering scaffolds. These synthetic tridimensional structures provide grafted cells with a *niche* and with adequate mechanical and chemical *stimuli* and thus can promote the process of tissue regeneration. Various mechanical, physicochemical, biological, and structural requirements posed on these structures are discussed, and how to match them through bulk and surface chemistry and by means of different porogenic techniques are elaborated. Questions arising from the interplay between composition, function, and structure are discussed, and the most important parameters for a physical and biological characterization of scaffold performance are presented. The possibilities afforded by polymerization chemistry and/or subsequent processing or treatment make polymers such unique materials for tissue engineering scaffolds.

Many polymers from natural sources have found application in tissue engineering and regenerative medicine. Chapter 2 presents a comprehensive overview of them, as well as examples of their application and clinical use. Their origin varies from marine crustacean and algae, as well as mammalian, plants, and microorganism-processed products. These polymers have good biodegradability, usually low-inflammatory response, and reduced cytotoxicity, which make them so interesting. The properties and main uses of naturally derived polyesters, polysaccharides (chitosan, agarose, alginates, starch, hyaluronate, and others), protein-based polymers (silk, collagen, fibrin, and others) are discussed, and emphasis is given to the responsive nature of these polymers and to their modification in order to obtain sensitive biomaterial systems for tissue engineering. Stimuli–responsive or "smart" polymeric systems are polymers that undergo strong physical or chemical property changes responding to small changes in environmental conditions of a physical (e.g., temperature, light, mechanical stress, or electric field) or chemical (e.g., pH or ionic strength) nature.

Various aspects of the interaction between polymer surfaces and cells are covered in Chapters 3 and 4. This is a central problem in the understanding of the regeneration process assisted by synthetic materials. The recognition of an alien surface by a cell is mediated through its membrane receptors that interact with the adsorbed protein layer on the surface. A thorough discussion of the processes of protein adsorption, cell adhesion, and matrix remodeling phenomena at the cell–material interface is presented in Chapter 3. Cell adhesion is the first step of the regeneration process and plays a fundamental role in subsequent cell differentiation, growth, viability, and phenotype expression. The nature of the adsorbed layer of proteins on a polymer surface dictates the initial cellular response and, eventually, the fate of a synthetic material when it is placed in a biological environment. The chapters review the role of surface chemistry and patterning on the phenomenon of fibrillogenesis of adsorbed ECM proteins such as laminin and fibronectin, and the different experimental techniques to follow protein adsorption. The fundamentals of cell adhesion on synthetic polymers are also presented in these chapters. The role of the different adhesion structures is examined, especially of focal adhesions, fibrillar adhesions, and focal complexes. These are multidomain molecules that can interact with several distinct partner molecules, and they are decisive for the proliferative or migratory response of cells and for the generation of the forces governing the mechanosensory processes in cells. The influence of mechanical, topographical, and chemical properties of the synthetic surface on focal adhesion kinase, a signaling protein contributing to integrin control of cell motility, survival, and proliferation, is specifically addressed in Chapter 4.

The processes of cell–material interaction *in vivo*, though, are much more complex than any of the experimental situations that can be reproduced *in vitro*. Many cell types coexist in any tissue, and the cross-talk processes between them through different kinds of signals are to a large extent unknown. An attempt to come closer to more realistic scenarios involves the use of bioreactors, where cells and materials can be combined with different signaling molecules under culture conditions that can be controlled in ways that try to resemble aspects of the natural cell microenvironment: nutrient flow, mechanical stresses, concentration gradients, different gas diffusion, etc., including coculture systems. This problem is addressed in Chapter 5, the last of the first, "macro" part of our book, with emphasis given to the dynamic character of the processes that lead to the consideration of the bioreactor, the cells, and the soluble and synthetic materials as a hybrid *system*.

The second part of the book, "Nanostructures for tissue engineering," includes contributions addressing topics where the molecular and nanoscale dimensions of the materials play a dominant role, as is the case of therapeutics. Bioactive nanostructures molecularly crafted to signal cells or carry therapeutic agents to specific cells have great potential to regenerate tissues and cure disease. The chemistry of such nanostructures should allow them to interact specifically with cell receptors or intracellular structures.

The first example of the importance of nanostructures shows the application of self-curing formulations for hard as well as soft tissue regeneration (Chapter 6), which react chemically in the human body and allow targeting and controlled release

of bioactive components. Self-curing systems based on macromolecular architectures can be applied locally and can act as antibacterial, antimicrobicide, or anti-inflammatory agents. Although very promising steps have been already achieved, to obtain real biomimetic systems that could be integrated in the natural ECM with adequate biofunctionality and biodegradability is still a challenge. The ECM is therefore a main target for consideration in future development of bioactive and biodegradable formulations that should be able to act as controlled reservoirs of bioactive agents, controlled release matrices and, in addition, as the adequate scaffolds for the development of natural regenerated tissues and organs. The application of physical interactions between macromolecular systems and the selective chemical reactions will be key factors for the evolution of these active materials at the nanoscale.

The organization of these nanostructures at larger length scales comparable to cells and large colonies of cells will also be critical to their function. Chapter 7 describes an extensive family of amphiphilic molecules that self-assemble into supramolecular nanofibers with capacity to display a large diversity of signals to cells. "Self-assembly" is the spontaneous arrangement of molecules into stable patterns by the driving force of noncovalent interactions such as hydrogen bonds, ionic bonds, electrostatic bonds, and van der Waals interactions. Regular alternating hydrophobic and hydrophilic residues in short peptide molecules create two distinct surfaces, one hydrophobic and the other hydrophilic, resulting in β-sheet structures in water. They are water soluble and form soft hydrogels when a change in ionic strength and/or the pH of the solution occurs due to salts or buffers. As a result, a network of interweaving nanofibers of around 10 nm diameter is formed, with many features in common with the ECM. Furthermore, the versatility of the modification of these materials permits their functionalization with signaling sequences to instruct cells in different ways. This chapter illustrates the use of these systems to regenerate axons in the central nervous system for spinal cord injuries, bone, and blood vessels in cardiovascular therapies. With the appropriate supramolecular design, these nanostructures could also be used in stem cell, cancer, and gene therapies.

Nanomedicine has been defined as "the use of nanosized tools for the diagnosis, prevention, and treatment of disease and to gain increased understanding of the complex underlying pathophysiology of disease. The ultimate goal is improved quality-of-life." Currently, about 40 nanoproducts for health care are in routine use. Among the nanotechnologies explained here, "polymer therapeutics" is blooming as the most successful first-generation nanomedicine (Chapter 8). Polymer conjugates differ from other nanopharmaceuticals that simply entrap, solubilize, or control drug release without resorting to chemical conjugation; it sums the advantage of small size, typically <25 nm, which enables better access to the biological targets that so many other nanocarriers cannot attain. Clinical proof-of-concept for polymer–protein conjugates is already a fact, but recent advances in polymer chemistry and the techniques available for physicochemical and biological characterization are enabling for the first time in-depth analysis of the critical polymer therapeutic characteristics governing their structure–activity relationships. Initial studies to date cover a broad spectrum of pathologies, trying to seek treatments for chronic and debilitating

diseases of increasing population of older age (i.e., diabetes, hypertension, infections, digestive track diseases, or rheumatoid arthritis). At present, Cimzia® (rheumatoid arthritis), Macugen® (age-related macular degeneration), and Krystexxa® (chronic gout) are in routine clinical use. Within this context, a very promising research approach looks at polymer therapeutics as a tool to promote tissue repair. Applications in wound healing, bone resorption, or ischemia/reperfusion injuries are described in Chapter 8. More ambitious targets, such as cardiac-tissue regeneration or neurodegenerative disorders, are the focus of ongoing research projects, and first studies already in process should come to fruition in the near future. Although still in its infancy, gene therapy can have a major role in this area. Indeed, stem cells can be genetically engineered by means of adequate nanovectors (viral or nonviral) to direct their *ex vivo* and *in vivo* behavior. Gene therapy as regenerative medicine is still facing considerable delivery challenges (e.g., safety and inefficiency); however, the latest advances in gene nanocarrier design have led to a few nanoconstructs with acceptable toxicities and efficacies and is described Chapter 9. This considerable progress has been fostered by new emerging materials designed in response to our deeper understanding of the biological barriers in gene delivery. Moreover, nanomaterials can now be used in combination with physical methods to increase further their efficacy *in vitro* and *in vivo*. With current techniques, and expecting further advances in the following years, successful application of synthetic gene nanocarriers to regenerative medicine seems to be both a desirable and a reasonable goal.

Organic polymeric materials can be combined with inorganic, giving rise to new "intelligent" hybrid materials possessing unique advantages. Chapter 10 discusses the use of mesoporous silica nanoparticles functionalized to become gated receptacles for controlled drug delivery. These mesoporous supports can be capped and synthesized as nanometric particles, resulting in suitable materials for the design of "nanodevices" for on-command delivery applications. The molecular "gates" are sensitive to a variety of stimuli, and the system is thus able to deliver active molecules or pharmaceuticals with high control.

As a concluding overview, Chapter 11 ponders the challenges and opportunities in the field. The chapter summarizes the requirements at the macro- and nanoscale for the polymers to be used in clinical applications and the technologies facilitating the process. Future advances in tissue engineering and regenerative medicine will depend on the development of "smart biomaterials" that actively participate in functional tissue regeneration. Engineering the mechanical, physical, and biological properties of these materials requires unique experimental, theoretical, and computational approaches necessarily based on a profound understanding of their structure at the nanoscale.

The volume of knowledge in these fields grows steadily, and proposals, alternatives, and solutions for many problems accumulate in the pages of journals. Although some tissue engineering approaches have already demonstrated practical application, clinical translation in regenerative medicine progresses only slowly, for various reasons. A much more dynamic translatory effort will surely result in real breakthroughs and, one must hope, actual advances in new health treatments.

The editors wish finally to express their deep gratitude to all authors and collaborators who have made this book possible.

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