INTEGRATED BIOMATERIALS IN TISSUE ENGINEERING

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Integrated Biomaterials in Tissue Engineering
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Preface

The last three decades have seen tremendous advances in the research and development of biomaterials suitable for engineering cells and tissues due to the advances in materials technology and cellular techniques. This book, *Integrated Biomaterials in Tissue Engineering*, attempts to convey the key aspects and recent development of biomaterials in the field of cell and tissue engineering. It consists of 11 chapters written by experts in biomaterials and tissue engineering fields around the world.

Chapter 1 deals with different protocols required for the fabrication of scaffolds for use in cell and tissue engineering. Chapter 2 discusses the recent developments and some of the key issues on using ceramic scaffolds for engineering cells and tissues. Chapter 3 focuses on the fabrication of porous scaffolds, particularly from ice particulate templates, suitable for tissue engineering applications. Chapter 4 describes the preparation and characterization of tissue engineering scaffolds by the emulsion freezing/freeze-drying technique. Chapter 5 deals with the interaction of electrospun nanofibers and stem cells and their effectiveness for use in tissue repair and regeneration. Chapter 6 focuses on the use of implant biomaterials and various aspects of how to improve the integration of the implants with host tissues in order to reduce implant failure. Chapter 7 provides an overview of fundamental developments in understanding human mesenchymal stem cell's differentiation and growth towards tissue repair and regeneration. Chapter 8 describes concepts and advances of endochondral bone tissue engineering in the context of biomaterials and stem cells, in particular endochondral ossification. Chapter 9 highlights the principle and technological advancement of craniofacial bone therapy. Chapter 10 reviews various aspects of small-diameter vascular graft regeneration with a special emphasis on tubular scaffolds and vascular cellular responses *in vitro* and *in vivo*. Chapter 11 discusses the role
and promise of vascular endothelial growth factor in soft and hard tissue engineering applications, particularly focusing on therapeutic angiogenesis.

All of these chapters make this book a self-contained source that updates the recent developments of biomaterials toward tissue engineering applications. The book is intended for a wide audience including students, researchers, professors, and industrial experts working in the fascinating field of biomaterials and tissue engineering.

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Protocols for Biomaterial Scaffold Fabrication

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Abstract
Scaffolds play a critical role in tissue engineering (TE), in particular scaffold-based TE, and they are designed to be biocompatible, with a suitable degree of porosity and surface chemistry to allow attachment, migration, proliferation, differentiation, and infiltration of the cells. Other important characteristics of scaffolds include having adequate mechanical properties, controlled biodegradability, and the ability to mimic in part the structure and biological function of the extracellular matrix. Keeping these key points in mind, this chapter focuses on the protocols for the preparation and characterization of conventional and novel scaffold biomaterials for the application of TE utilizing several synthetic and natural polymers.

Keywords: Scaffold, solvent casting, salt leaching, gas foaming, phase separation, electrospinning, self-assembly, rapid prototyping, membrane lamination, freeze drying

1.1 Introduction
Millions of people are suffering from tissue or organ failure and are waiting for some kind of tissue or organ transplantation.
Traditionally, tissue loss resulting from traumatic or nontraumatic destruction has been treated by methods such as autografting and allografting [1]. Although autogenic tissue transplantation is clinically considered as a gold standard, it has the limitation of donor site shortage. On the other hand, allogenic transplantations are more prone to immunogenicity as well as inducing other transmissible diseases. Because of these clinical limitations, the concept of tissue engineering was introduced nearly two decades ago [2], considerably saving numerous lives and improving the quality of life of patients. Tissue engineering involves the use of synthetic functional components (scaffolding material), culturing them with appropriate cells that are harvested from patient or donor, and then reimplanting the engineered constructs in the patient’s body where the tissue regeneration is required [3, 4]. There are four key factors to be considered for the success of any tissue development: (i) the cells that create tissue, (ii) the scaffold that gives structural support to cells, (iii) the bioactive signaling molecules that regulate the cellular processes, and (iv) cell-matrix (scaffold) interactions that direct the tissue development and remodeling. Therefore, to achieve the goal of generating functional tissues \textit{in vitro}, the specific cells, in particular anchorage dependent cells, should be combined with the right scaffolding material under appropriate conditions, meaning that the cells must be subjected to conditions highly mimicking the native microenvironments that lead to tissue formation (see Figure 1.1).

\textbf{Figure 1.1} Key factors constituting the concept of tissue engineering.
Significant attention has been paid to scaffolds for tissue engineering since they provide a biomimetic environment for cellular growth and tissue in-growth. Those scaffolds may be used in vitro or in vivo as supportive prosthetic materials and cell adhesive substratum to regenerate tissue. In addition to providing a physical support, scaffolds can be modified with bioactive molecules to have an active role in tissue regeneration. The interaction between the scaffolds and cells can be improved by functionalizing the surface of the scaffold to promote cell attachment [5]. Furthermore, signaling molecules such as growth factors can be incorporated in the scaffolds to enhance cell growth and morphogenesis, resulting in the regeneration of functionally organized tissues [6].

The critical structural and chemical requirements of scaffolding materials include: biocompatibility to the host tissue, having a three-dimensional architecture with a suitable degree of porosity and mechanical strength, possibility of surface modification with bioactive molecules, and controlled biodegradability. Such a scaffold allows maximal cellular attachment, growth, migration, differentiation and infiltration of cells, and facilitates proper transfer of nutrients and oxygen, while maintaining adequate mechanical properties. Safe implantation in the patient’s body without provoking immune response, and controlled biodegradability of those scaffolding materials are other important factors, which determine the successful integration of the tissue construct after implantation.

Scaffolds for tissue engineering have been developed through a variety of techniques, and have been fabricated from natural and synthetic materials. While scaffolds from naturally derived materials provide various biological functions, scaffolds from synthetic materials offer certain advantages due to their easy processability, controlled degradation, and susceptibility to modification [7].

This chapter focuses on providing an overview of synthetic and naturally derived scaffolding materials for tissue engineering, as well as various fabrication techniques including solvent casting, salt-leaching, gas foaming, phase separation, electrospinning, self assembly, rapid prototyping, membrane lamination, and freeze drying. The chapter concludes with future challenges and perspectives in the fabrication of novel scaffolds for successful engineering of tissue constructs.
1.2 Scaffolding Materials

Tissue engineering scaffolds have been fabricated using a variety of natural and synthetic materials, which include polymers, ceramics, and their composites. Owing to their high mechanical properties, ceramics and polymer-ceramic composites are mainly utilized to reconstruct hard tissues. Polymers on the other hand, are used for the reconstruction of soft tissues. Polymers provide unique functional properties and design flexibilities, which make them attractive candidates for fabricating tissue engineering scaffolds. Due to their wide range of application, in this section polymers applied for tissue engineering scaffolds are discussed in more details. Polymers used in scaffold engineering can be of natural and synthetic origins (Table 1.1). Novel hybrid polymeric scaffolds have also recently been developed by combining natural and synthetic polymers to mimic the extracellular matrix of a natural tissue.

1.2.1 Naturally Derived Materials

The most widely used natural polymers to fabricate tissue engineering scaffolds include fibrin, collagen, gelatin, chitosan, alginate, and hyaluronic acid [8-13]. Fibrin is a non-globular fibrous protein, and plays a critical role in blood clotting by polymerizing into a mesh over a wound site. It forms a tight complex with thrombin and has been used in mixtures with thrombin to produce an in situ forming gel [8]. Type I collagen, which is the most abundant collagen of the human body, is found in tendons, skin, artery walls, and fibrocartilage, and can be extracted from animal tissues. Collagen and gelatin, which is a denatured form of collagen, can form porous gel matrices, are also used to functionalize the surface of synthetic polymers to enable cellular attachment [9]. Chitosan is a cationic polysaccharide with hydrophilic properties, which is used as a scaffolding material to support cell adhesion and differentiation, and owing to its osteoconductive nature, it is particularly applied for bone tissue engineering [10, 11]. Alginate is an anionic polysaccharide, which is widely derived from cell walls of brown algae. In the presence of divalent cations such as Ca\(^{2+}\) it is capable of forming gels with a high swelling degree [12]. Hyaluronic acid is an anionic nonsulfated glycosaminoglycan, which is mainly found in connective, epithelial, and neural tissues. It forms crosslinkable hydrogels with various modifications and is highly cell repellent [13].
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<th>Material</th>
<th>Origin</th>
<th>Structure</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrin</td>
<td>Natural</td>
<td>Mesh of polymerized fibrillar proteins</td>
<td>• Cell adhesive&lt;br&gt;• osteoconductive&lt;br&gt;• Tunable mechanical properties</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>Natural</td>
<td>Elongated protein made of tough fibril bundles</td>
<td>• Non-immunogenic&lt;br&gt;• Cell adhesive&lt;br&gt;• Chemotactic&lt;br&gt;• Low mechanical properties</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Natural</td>
<td>Cationic polysaccharide made of D-glucosamine and N-acetyl-D-glucosamine</td>
<td>• Hemostatic&lt;br&gt;• Good osteoconductivity and mechanical properties when combined with hydroxyapatite</td>
</tr>
<tr>
<td>Alginate</td>
<td>Natural</td>
<td>Anionic polysaccharide made of blocks of β-D-mannuronate and α-L-guluronate</td>
<td>• Hydrophilic&lt;br&gt;• Low mechanical properties</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>Natural</td>
<td>Anionic polymer of non-sulfated glycosaminoglycans</td>
<td>• Minimal immunogenicity&lt;br&gt;• Cell repellant&lt;br&gt;• Low mechanical properties&lt;br&gt;• ECM mimicking viscoelasticity</td>
</tr>
</tbody>
</table>

(Continued)
Table 1.1 (cont.) The commonly used polymers for the fabrication tissue engineering scaffolds.

<table>
<thead>
<tr>
<th>Material</th>
<th>Origin</th>
<th>Structure</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (α-hydroxyesters)</td>
<td>Synthetic</td>
<td>Aliphatic polyester by polycondensation or ring-opening polymerization</td>
<td>• Biodegradable</td>
</tr>
<tr>
<td>(e.g. PLA, PGA)</td>
<td></td>
<td></td>
<td>• Tunable mechanical properties</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Degradation into acidic compounds</td>
</tr>
<tr>
<td>Poly (ε-caprolactone)</td>
<td>Synthetic</td>
<td>Aliphatic polyester prepared by ring opening polymerization of ε-caprolactone</td>
<td>• Biodegradable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Slow degrading</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Degradation products incorporated in the tricarboxylic acid cycle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• High mechanical properties</td>
</tr>
<tr>
<td>Poly (propylene fumarates)</td>
<td>Synthetic</td>
<td>Unsaturated polyester consisting of alternating propylene glycol and fumaric acids</td>
<td>• Degradable into fumaric acid and propylene glycol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Satisfactory biological results</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Tunabale mechanical properties</td>
</tr>
</tbody>
</table>