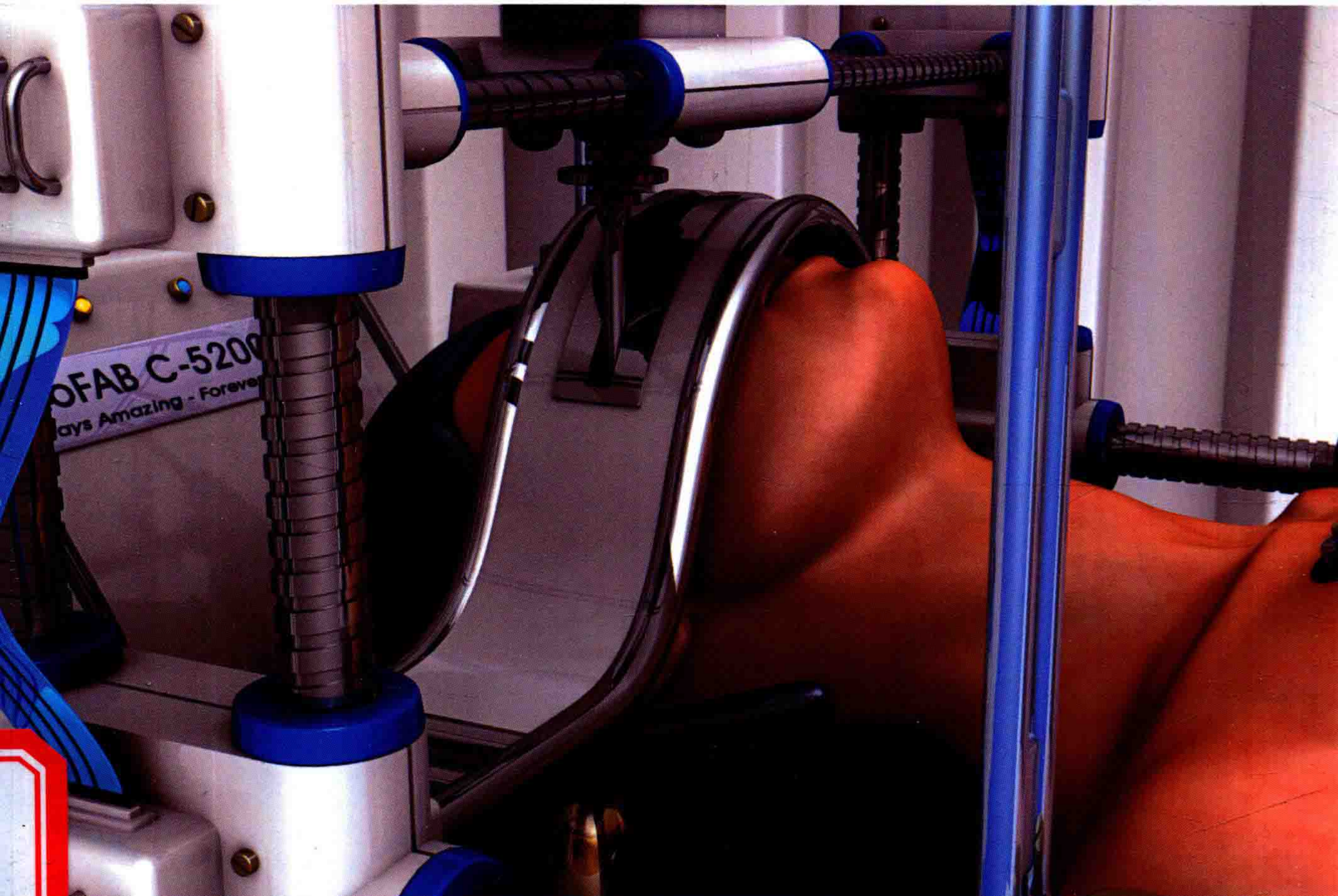
3D BIOPRINTING

Fundamentals, Principles and Applications



Ibrahim T. Ozbolat

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3D Bioprinting

Dedication

The author dedicates this book to his parents Guluzar and Suleyman

About the Author

Ibrahim Tarik Ozbolat is an associate professor of Engineering Science and Mechanics Department, Biomedical Engineering Department, the Huck Institutes of the Life Sciences, and the Materials Research Institute at The Pennsylvania State University, University Park, Pennsylvania, USA. Previously, he was a faculty member of The University of Iowa, Iowa City, Iowa, USA, and spearheaded Advanced Manufacturing Technology Group and the Biomanufacturing Laboratory. He received his PhD in tissue engineering from the Industrial and Systems Engineering Department at the University at Buffalo (SUNY) in Buffalo, New York, USA, and dual BS degrees in Mechanical Engineering and in Industrial Engineering from Middle East Technical University, Ankara, Turkey. Dr. Ozbolat is an internationally recognized expert in the area of 3D bioprinting. His research on bioprinting for tissue and organ fabrication has been published in several high quality of venues, received numerous national and international awards, and featured in national and international media, broadcast TVs, and press numerous times. He frequently gives invited talks at national and international forums, conferences, and seminars and organizes demonstrations and events to public and youth to encourage participation of future's engineers in medicine, engineering, and science.

Preface

Bioprinting is an emerging field that makes a revolutionary impact on medical sciences. It has attracted numerous researchers from various academic disciplines as well as the public. Bioprinting offers great precision on spatial placement of cells, proteins, DNA, drugs, and biologically active particles to better guide tissue generation and formation. This emerging technology appears to be more promising for advancing tissue engineering toward functional tissue and organ fabrication for transplantation, ultimately mitigating organ shortage and saving lives.

In this regard, development of a very comprehensive book covering all aspects of bioprinting is highly useful for the academic and educational circles, public, and the emerging bioprinting industry. The benefits of this book to the readership are in threefolds. First of all, the book is *a unique source* for academicians and researchers covering almost all aspects of bioprinting spanning raw materials, processes, machine technology, products, applications, limitations, and future perspectives. The growing research interest and the market in this field will make this book highly demandable. Secondly, it helps the bio-engineers, tissue and manufacturing engineers as well as medical doctors to understand features of each bioprinting processes, the bioink and bioprinter type, and select the appropriate process for a given application such as tissue engineering and regenerative medicine, transplantation, clinics, or pharmaceutics. Thirdly, it is a *great source* for upper undergraduate- and graduate-level courses in the area of bioprinting, biofabrication, and tissue engineering considering the great worldwide interest on new undergraduate and graduate programs in biofabrication.

The book is outlined in 10 chapters including Introduction, Design for Bioprinting, The Bioink, Extrusion-Based Bioprinting, Droplet-Based Bioprinting, Laser-Based Bioprinting, Bioprinter Technologies, Roadmap to Organ Printing, Applications of 3D Bioprinting, and Future Trends.

Chapter 1 introduces tissue engineering, 3D printing in tissue engineering, principles of bioprinting and its components, and historical evaluation and classification of bioprinting processes. Chapter 2 discusses design for bioprinting covering the steps taken from medical imaging to bioprinting. Chapter 3 covers the bioink materials; their physical, chemical, and biological properties; compatible bioprinting techniques; and the comparison of bioink types.

In Chapters 4–6, the author discusses three major groups of bioprinting modalities including extrusion-, droplet-, and laser-based bioprinting, respectively. In each modality, the process and its characteristics and main components are described, the underlying physics is presented in addition to suitable bioink and cell types printed, and major accomplishments achieved so far.

In Chapter 7, bioprinter technologies are highlighted covering the components of bioprinters as well as the commercially available bioprinters in the market along with a detailed discussion on the limitations of bioprinter technologies. Chapter 8 covers the organ printing topic, introduces the state-of-the-art followed by the roadmap for organ printing with a step-by-step description. Chapter 9 presents the application areas of bioprinting, including tissue engineering and regenerative medicine, clinics and transplantation, pharmaceutics, and cancer research. Finally, Chapter 10 discusses the future trends in bioprinting that will revolutionize the organ transplantation technology in the next couple of decades.

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Contents

1

Introduction

CHAPTER OUTLINE

All truths are easy to understand once they are discovered; the point is to discover them
Galileo Galilei

1.1 Tissue Engineering

Since the first successful kidney transplant in 1954 was performed between two identical twins (Merrill et al., 1956), organ transplantation has become a life-saving procedure for many disease conditions that hitherto were considered incurable. In the United States of America (USA), an average of 79 people receive transplants every day; however, the number of donors is much less than the number of patients waiting for a transplant (Ozbolat and Chen, 2013). Moreover, infections and rejection of the tissue by the host often make the transplantation process more challenging (Desmet et al., 2008). The solution to this problem, as with the solutions to other grand engineering challenges, requires long-term solutions by building or manufacturing healthy living organs from a person's own cells, which would relieve suffering and save lives.

Current medical procedures aim to restore tissue function to patients with diseased or damaged tissues through tissue transplantation and implants. Tissue engineering has grown as a multidisciplinary scientific field of biology, biomaterials, and engineering that has rapidly emerged and combines engineering principles with life sciences to replace

3D Bioprinting.

damaged tissues or restore malfunctioning organs by mimicking native tissue (Langer and Vacanti, 1993). One of the common strategies in tissue engineering is to develop engineered scaffolds that provide an optimum environment or housing for cell attachment and growth, tissue regeneration, fluid movement, and structural integrity. The main reason for the scaffolding approach is the need to maintain the shape and mechanical properties of the mimicked tissue engineered, to assist in cell attachment, and to provide a substrate for cell proliferation into three-dimensional (3D) functioning tissues (Hutmacher et al., 2004). Developed 3D porous engineered constructs enable cell attachment, proliferation, and regeneration. Upon implantation, scaffold material starts degrading, and cells grow and proliferate through pores. Eventually, degraded sites constitute new tissue and restore the functionality of the diseased or damaged tissue.

Several traditional fabrication techniques have been used for tissue scaffolds, including phase separation, membrane lamination, melt molding, fiber bonding, molding, gas foaming, solvent casting, freeze drying, and particulate leaching (Leong et al., 2003). Most of the abovementioned techniques are, however, limited in terms of manufacturing reproducibility and flexibility. Building patient-specific anatomically correct shapes with well-controlled internal geometry, including pore size and pore distribution, is highly challenging. In addition, they include manual interventions and inconsistent and inflexible processing procedures with the use of toxic solvents and porogens that limits the inclusion of cell and protein impregnation. 3D printing, also known as additive manufacturing, has been a game-changing technology in the rapid manufacturing of complex products and has been adopted in tissue engineering for biofabrication of 3D scaffolds (Hamid et al., 2011).

1.2 Three-Dimensional Printing in Tissue Engineering

3D printing opens a revolutionary era in medical product design and development and is widely used to fabricate 3D scaffolds by layer-by-layer deposition. In general, biomaterials are deposited through a dispensing unit to specific points on the space to create a scaffold with well-controlled geometry. Three-dimensional bioprinting has been extensively used for building tissue-engineered constructs due its repeatability and high accuracy in microscale fabrication resolution (Sachlos et al., 2003). 3D printing techniques such as fused deposition modeling, precision-extrusion deposition, selective laser sintering, three-dimensional printing, and stereolithography (SLA) have been used to fabricate biologically active tissue constructs by replacing the materials being processed with biocompatible materials such as synthetic and natural polymers, natural and inorganic ceramic materials, or recently developed biodegradable metals (Ozbolat and Hospodiuk, 2016). Adaptation of 3D printing into tissue engineering brings unique capabilities in rapid fabrication of tissue scaffolds with controlled porosity and internal architecture, tunable mechanical and structural properties, and the ability to load drug or protein molecules for enhanced cellular response and customized/multifunctional characteristics, which can guide the cellular environment for enhanced tissue regeneration. To achieve a truly interconnected internal architecture for cell growth and proliferation, an internal structure is formed by depositing cylindrical microfilaments parallel to each other in every layer using a certain lay-down pattern.

Despite its great benefits in the biofabrication of anatomically correct tissue scaffolds, 3D printing in tissue engineering faces several limitations in the generation of complex tissues and organs for transplantation or other uses. First of all, there is a lack of precision in cell placement due to manually driven seeding and placement of cells on the scaffold microarchitecture. It is highly challenging to manually seed, place, and pattern cells precisely in a scaffold construct; however, several cell type groups are organized and interact in very complex patterns in natural tissues and organs. In addition, seeding cells in high cell density is very limited because cells can only attach on the surface of the scaffold and cannot penetrate into the biomaterial in the scaffold. Scaffold biomaterials also occupy a significant volume of space in the scaffold, which do not let the cells grow into sufficient cell numbers. In addition, the need for a vascular network is essential to develop thick tissues and organs to facilitate an efficient exchange of media to keep the cells oxygenized, viable and functional, and it is very difficult to create such a network using 3D printing technologies alone. These difficulties have led many researchers toward the development of bioprinting technologies, where cells can be encapsulated in high cell density and printed and patterned into desired spaces to obtain anatomically correct tissue constructs with patterned cells interacting as in native tissue and organs.

1.3 Three-Dimensional Bioprinting

Three-dimensional bioprinting is an emerging field that makes a revolutionary impact on medical sciences. It has gained significant attention from numerous researchers from various academic disciplines as well as the general public. Bioprinting could be defined as a computer-aided transfer process for simultaneous writing of living cells and biomaterials with a prescribed layer-by-layer stacking organization to fabricate bioengineered constructs for tissue engineering, regenerative medicine, or other biological studies (Mironov et al., 2009; Ozbolat, 2015). The major difference between 3D printing for tissue engineering and 3D bioprinting is that bioprinting involves the printing of living cells and other biologics. It offers great precision on spatial placement of cells, proteins, DNA, drug particles, grow factors, and biologically active particles to better guide tissue generation and formation. This emerging technology appears to be more promising for advancing tissue engineering toward functional tissue and organ fabrication for transplantation, ultimately mitigating organ shortage and saving lives.

The 3D bioprinting process can be divided into three crucial technological steps: preprocessing, processing (actual printing), and postprocessing (Mironov et al., 2006). Preprocessing is a blueprint of tissue or organ design using imaging and computer-aided