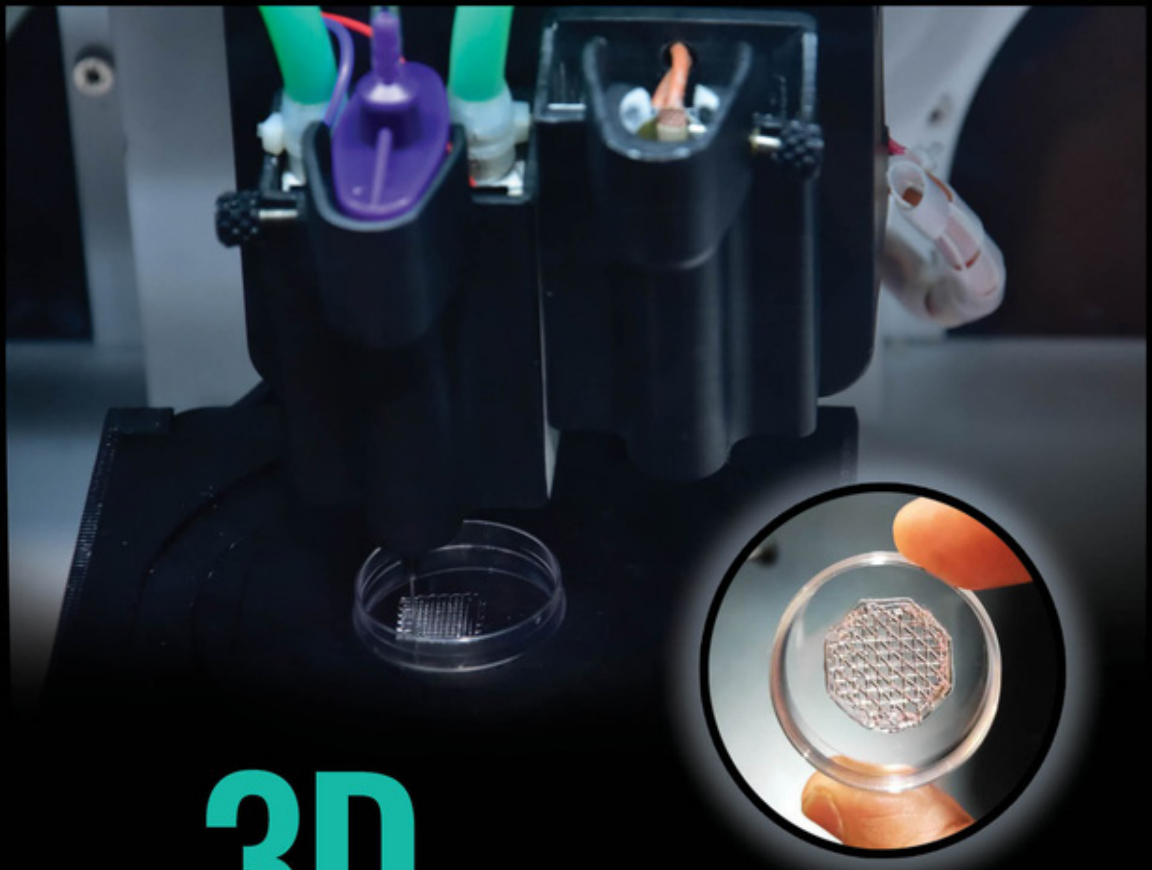

3D Bioprinting from Lab to Industry

Edited by Prosenjit Saha • Sabu Thomas
Jinku Kim • Manojit Ghosh

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Foreword

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Tissue damage due to disease or injury has been a major concern for the entire global population, and its remediation poses serious challenges to the healthcare providers, biomedical researchers, and professionals across the globe. The concern becomes manifold since some organs and tissues have very limited self-renewal or regenerative capacity. Thus for restoring such injured or degraded tissues, we need to regenerate functional living tissue or even the whole organ artificially. This has resulted in an extremely important and emerging field of tissue engineering and regenerative medicine, which offers the potential to provide solutions for bio-fabrication of functional tissues.

Bioprinting, the process of creating complex, living tissues using 3D printing technology has received paramount importance in recent times. The interdisciplinary technology, lying at the intersection of engineering and biology, is the backbone of regenerative medicine and tissue engineering and points to the future of modern medicine.

Using this technology, it is important to precisely position multiple cell types layer by layer using computer-aided additive manufacturing techniques. Bioprinting is essentially a computer-aided transfer process for assembling biologically relevant materials including biomolecules, cells, tissues, and biodegradable biomaterials, resulting in the formation of an engineered bio-functional construct. Indeed it is a revolutionary concept in the regeneration or repair of damaged tissues by automating the layer-by-layer hierarchical fabrication of cell-laden structures both in vitro and in vivo.

Some of the early models of 3D bioprinting technology used computer-controlled ink-jet printer or graphics plotter and were used for precisely positioning the cells on a 2D substrate. This work provided the fundamental platform for

3D bioprinting. From those early efforts in mid-1980s, the interdisciplinary endeavor in 3D bioprinting and regenerative medicine technology has progressed substantially over the past three and a half decades, making personalized medicine a reality.

Since the original native tissues of a patient have their own distinct complex architecture as well as 3D organization and distribution of cells and extracellular matrix, it remains a significant challenge to precisely understand the complexity of native tissues from a structural and functional perspective. It holds endless promise for revolutionizing healthcare and various industries. From regenerative medicine to pharmaceutical testing and beyond, the possibilities offered by bioprinting are vast and continually expanding. However, the growth in this field also holds the numbers of existing challenges that need to be addressed by the scientific community.

In this context, the book *3D Bioprinting from Lab to Industry*, edited by Prosenjit Saha, Sabu Thomas, Jinku Kim, and Manojit Ghosh, has opened up the exciting world of bioprinting and its modern industrial applications.

The book, written by some of the esteemed scientists in this field, will embark on a voyage through the intricate landscapes of bioprinting. The contributors of each chapter have shared their experiences to present the fundamental challenges along with the solutions that lie in each area. From the first chapter, where the authors explain the fundamental principles of 3D bioprinting, the readers will enjoy a journey through the diverse facets of bioprinting – controlling this translational technology to the design, fabrication, and applications of biomaterials and bioinks at industrial scale, the book covers all the important aspects of 3D bioprinting.

The broad overlap of additive manufacturing with tissue engineering has transcended the boundaries of conventional medicine, presenting enormous potential for a future where organs can be printed on demand at laboratory, customized to individual needs with precision. It holds endless promise for revolutionizing healthcare for various industries. From regenerative medicine to pharmaceutical testing and beyond, the possibilities offered by bioprinting are vast and continually expanding. However, the growth in this field also holds the numbers of existing challenges that need to be addressed by the scientific community.

Because of its affordability, commercial viability, and capability to fabricate complex and hollow constructs, the extrusion-based bioprinting, one of the most common techniques in bioprinting field, has been employed to print living cells, tissue constructs, organ modules, and even organ on-a-chip devices. In this book, the extrusion-based 3D bioprinting has been covered in adequate details for the readers. Bioinks are formulation of materials suitable for processing by an automated biofabrication technology. With the advancement of technology – especially the introduction of extrusion-based printing techniques that use a

small-diameter nozzle to deposit bioinks for the fabrication of complex constructs – individual cells and cell aggregates lack the ability to withstand the shear stress.

Starting with an introduction of the fundamental principles of bioink, the authors have presented here the translational technology for the design, fabrication, and applications of biomaterials and bioinks at industrial scale.

Furthermore, the book presents the industrial applications of bioprinting, showcasing how this technology can be used in personalized medicine, from planning to implementation, through case studies and laboratory-based research outcomes. The profound impact of AI and machine learning on the design of 3D bioprinting has also been included here. The processing parameters on the design of scaffolds, organoids, and cell/tissue models during printing process have also been discussed in detail by the authors.

With a lucid introduction to the realm of bio printing, followed by cellular requirements and preparation for bio printing, the authors have discussed various materials used for bioprinting. Bioprinting in regenerated organs, 4D bioprinted multiresponsive structure, the controlling factors in bioprinting, in situ bioprinting, machine learning, and in particular deep learning techniques in 3D bioprinting, nanomaterial and design of scaffolds, organoids, and cell/tissue models during printing process have been discussed in detail by the authors.

How to plan a bioprinting project and how it moves from laboratory to industry are two very important issues, which have been discussed here. The industrial applications of bioprinting, showcasing how this technology is utilized in personalized medicine from planning to implementation, have been discussed here through case studies and lab-based research outcomes. Finally, toxicity issues hold paramount importance in 3D bioprinting, and along with this comes the most pertinent ethical issues involved in this area. These have also been adequately discussed in this book.

For researchers, industry professionals, students, or anyone curious about the present and the future of 3D bioprinting and tissue engineering, this book will provide valuable insights into this rapidly evolving field. I hope that the book inspires and informs readers about the remarkable possibilities presented by 3D printing in shaping the future of modern medicine and healthcare industry.

Happy reading!

1

Introduction of 3D Printing and Different Bioprinting Methods

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1.1 Introduction of 3D Printing: Principles and Utility

3D printing (3DP), also known as additive manufacturing (AM), solid-freeform (SFF), and rapid prototyping (RP), is a fabrication technique using model data, where 3D structures are fabricated using controlled layer-by-layer deposition [1]. It was first described by Charles Hull in 1986, followed by production and commercialization by S. Scott Crump and his company Stratasys [2]. The basic subcategories of 3DP are stereolithography, fused deposition modeling, selective laser melting, electronic beam melting, and laminated object manufacturing [3]. 3DP involves scaffold construction by material addition, with high geometric precision reducing material waste. The primary procedure comprises data acquisition and synthesis of meshed 3D computer models in computer-aided design (CAD), followed by surface tessellation language (STL) file creation. This is followed by the slicing of mesh data into multiple 2D layer files and transferring them to a 3DP machine for fabrication [4]. Manufacture of complex designs, low cost, ease of access, and rapid and environment-friendly procedures are some of the advantages of 3DP in industrial, research, healthcare, and biomedical sectors.

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1.2 Ink Preparation and Printability

The choice of the base material as well as the recipe of its preparation to cater to the need for 3DP are of utmost importance. Bioink is the material used to produce either engineered or artificial living tissue using 3DP. It is the cell-trapping milieu composed of a multicomponent aqueous mixture that usually forms gels. This sol-gel transition of bioink is offered either by ionic bonds, covalent bonds, hydrogen bonds, or van der Waals interactions. Bioinks may be hydrogels, decellularized extracellular matrix, cell pellets, or tissue spheroids, of which hydrogels are the most common [5] due to their cell adhesion, growth, and proliferation capability since they absorb and retain large amounts of water. Ink for 3D bioprinting can be subdivided into two categories: cell-laden inks called bioink and cell-free inks called biomaterial ink. The bioinks usually consist of hydrogel precursors and are directly printed into Petri dishes filled with media and antibodies, whereas the biomaterial inks are usually utilized to print 3D scaffolds wherein the cells can be seeded on the scaffold under controlled conditions [6].

An ideal bioink should provide mechanical stability, stiffness, viscosity, surface tension, structural integrity, and biological ability – biocompatibility and biodegradability [7]. Many natural polymers like alginate, agarose, gelatin, chitosan, collagen, fibrin, and hyaluronic acid and synthetic polymers like polylactic acid (PLA), poly-D,L-Lactic acid (PDLLA), polylactic-co-glycolic acid (PLGA), polyvinyl alcohol (PVA), acrylonitrile butadiene styrene (ABS), polyethylene glycol (PEG), polyether ketone (PEEK), polycaprolactone (PCL), polybutylene terephthalate, and polyurethane (PU) [8] are used as bioinks for 3DP in the form of single or multicomponent.

Printability: The term "printability" is the ability of a bioink to form a 3D structure with accurate fidelity and integrity as per the design and the geometry. However, the terminology modulates itself according to the printing approach. For extrusion printing, the bioink must be able to form continuous filament; for the inkjet technique, it should form well-defined droplets while for laser printing, a prominent jet is required. The different printability indices [5] are the following:

1) *Extrudability*: The minimum extrusion pressure essential for printing at the desired flow rate.
2) *Strand printability*: Comparison of the diameter of printed strands with the CAD-generated parameters.
3) *Integrity factor*: Comparison of the thickness of printed scaffolds with designed geometries.
4) *Pore printability*: Comparison of the printed pores with designed internal geometry.
5) *Irregularity*: Comparison of outer geometry of scaffolds with designed parameters in *X*, *Y*, and *Z* directions.

Rheological properties and gelation kinetics determine the printability of a bioink, which is again dictated by the type of bioprinting. Low-viscosity bioinks are preferred in inkjet bioprinting; rapidly crosslinkable, shear-thinning bioinks are desirable for extrusion bioprinting and photo-crosslinkable bioinks are favorable for stereolithographic printing [9]. Rheological properties like viscosity, viscoelasticity, yield stress, shear thinning, elastic recovery, and viscoelastic shear moduli affect the printability of bioinks. The rheological properties of the crosslinked bioink must facilitate scaffold remodeling to mimic the ECM environment. This process provides physicochemical cues to the cells, promoting their spreading and proper distribution. For instance, substrates that mimic the mechanical properties and Young's modulus (~12 kPa) of native skeletal muscles offer better myogenic differentiation [10]. The key rheological parameters for a "good" bioink are described below:

Viscosity: Viscosity is the ratio of shear stress to shear rate and is governed by the molecular weight and concentration of the polymer. High-viscosity inks are preferable for high-fidelity printing but may limit cell growth within the substrate due to higher shear stress. This shear stress can be overcome by either using hydrogel inks having shear-thinning properties or using pre-gel solutions with lower viscosities. For e.g., alginate-based bioinks are directly extruded into calcium solution leading to ionic crosslinking. Due to higher surface tension, viscous bioinks prevent droplet formation without any merger of the columns with one another. Hence, crosslinking agents come into the picture with the caution of appropriate concentration so as to avoid phase separation and phase change [11]. Temperature-dependent hydrogen bonding or hydrophobic interactions may be exploited, as in case of gelatin, Pluronic, etc. Colloidal-like suspensions of densely packed microgels or jammed gels also prevent exposure of cells to high shear stress [12].

Viscoelasticity and yield stress: Viscoelasticity is the property of retaining elastic shape while allowing viscous flow. It is guided by three parameters – storage modulus (G′), viscous modulus (G″), and yield stress. Tan (δ), the ratio between G′ and G″, gives information about the rheological characteristics of the bioink. Yield stress is the stress limit beyond which deformation occurs. The parameters of G′ and yield stress are governed by the number of crosslinks within the bioink. These crosslinks offer resistance to shape change within the yield stress. Paradoxically, though yield stress of the bioink renders shape and stiffness to the substrate, it can also deter cell encapsulation and further growth. Hence, additives like carrageenan, gellan gum, and hyaluronan are added to the bioinks to improve yield stress [13, 14]. However, in stereolithography and light-assisted bioprinting, low-viscosity bioink is required for easy flow and for each layer to be crosslinked with each other.

Shear thinning: Shear thinning is the phenomenon where increase in shear rate results in the decrease in viscosity. Partially crosslinked hydrogels, colloidal suspensions, polymer melts, or polymer solutions above certain critical concentrations show shear-thinning properties with shape preservation. Shear thinning leads to decrease in viscosity in the extrusion phase, but rise in viscosity after extrusion results in shape preservation. For e.g., shape retention in printed calcium phosphate cement is due to high zero-shear viscosity [15]. PCL and PLA melts, used in polymer-based fused deposition modeling, possess intrinsic shear thinning properties due to shear-induced disentangling and alignment of long polymeric chains [16]. High resting viscoelasticity of pastes, solid suspensions, and colloidal dispersion bioinks arises due to the restoration of interaction between the suspended particles, which had been disrupted due to the shear-thinning process [17]. Hydrogels demonstrate non-Newtonian fluid behavior with shear-thinning features. So, the random polymer chains align themselves in one direction under shear force and become suitable for extrusion process.

Surface tension: Due to surface tension, there is an attraction between the liquid molecules, which ensures a contact angle between each printed strand. When the substrate has a higher surface energy than the surface tension of the bioink, the ink spreads; conversely, lower surface energy results in less spread [18]. For e.g., shape fidelity in printed constructs of ceramic slurries is reduced by both surface tension and gravity. It has been observed that a reduction in surface energy leads to droplet formation instead of a cuboidal structure [19].

Elastic recovery: This property explains how the bioink recovers its original solid-like property without any distortion after undergoing deformation or transition from liquid to solid state [20]. Due to this feature, multi-layered structures can be built up. Elastic recovery is the combination of both viscous flow and elastic recovery where the viscous modulus, G'', explains the fluid-like behavior of bioinks, and elastic modulus, G', defines the solid-like behavior of bioinks imparting elastic shape recovery. While the former allows mixing of cells and extrusion, the latter allows suspension of cells. Often, these moduli vary under different conditions of temperature, stress, and shear rate.

The recovery of solid-like behavior after extrusion through a needle must be fast to ensure good shape fidelity. The rheological evaluation of a bioink is done on the basis of the kinetics of yield stress and elastic recovery. The first step is to evaluate the effect of increasing shear stress and filament-forming capability, followed by the measurement of viscosity as a function of shear rate to evaluate the shear thinning property. Then, recovery tests are done to investigate whether the materials can restore their elastic properties on exposure to alternating low and high sheer stress [17].