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3D Bioprinting, A Powerful Tool for 3D Cells Assembling

1.1 What Is 3D Bioprinting?

3D printing, also known as additive manufacturing, is a layer-by-layer manufacturing approach, and it has been applied in many industrial applications and research fields. It could be thought of as an inverse process of potato cutting, assembling the chips or slices into integrity by certain rules. When 3D printing met biomedical engineering, 3D bioprinting was born. 3D bioprinting is an interdisciplinary science closely related to medicine biology, mechanical engineering, and material science. It can be divided into two concepts. Broadly speaking, 3D bioprinting refers to the use of 3D printing technology to achieve biomedical applications, such as the printing of medical aids, polymers, ceramics, or metal scaffolds [1–3]; in a narrow sense, this concept simply means 3D cells assembling through printing, therefore it can also be identified as cell printing or organ printing [4–6]. Here, this book is mainly focusing on the narrow viewpoint. A cartoon introduction of 3D bioprinting is illustrated in Figure 1.1.

In vitro bio-manufacturing of tissues/organs has always been a great dream pursued by mankind, driven by two needs: organ transplantation and accurate tissue models. First, there is a huge shortage of organs for transplantation. In 2016, there were 160 000 organ transplant recipients, but only 16 000 organ donors in the United States [8]. The complexity of human organs is not only reflected in the mechanism of organ growth that has not been revealed by biology, but also in the reproduction of fine structure manufacturing. The use of 3D bioprinting technology to solve the shortage of organ transplants is far too optimistic at the present stage. Second, traditional methods utilizing 2D cell culture were applied for drug screening and medical mechanism studies. However, microenvironment in vivo is far more complex than the 2D cell culture, and in some cases, 2D models may lead to opposite results. 3D bioprinting technology can realize spatiotemporal directional manipulation of various cells and has become the most ideal method to construct a 3D cell-laden structure in vitro.

In vitro models have undergone a meaningful revolution both in forms and functions: mini-tissue, organ-on-a-chip, and tissue/organ construct. Based on common bioprinting techniques, 3D mini-tissue in forms of spheres, fibers, or other geometric shapes could be fabricated [9, 10]. These models contribute to the simulation

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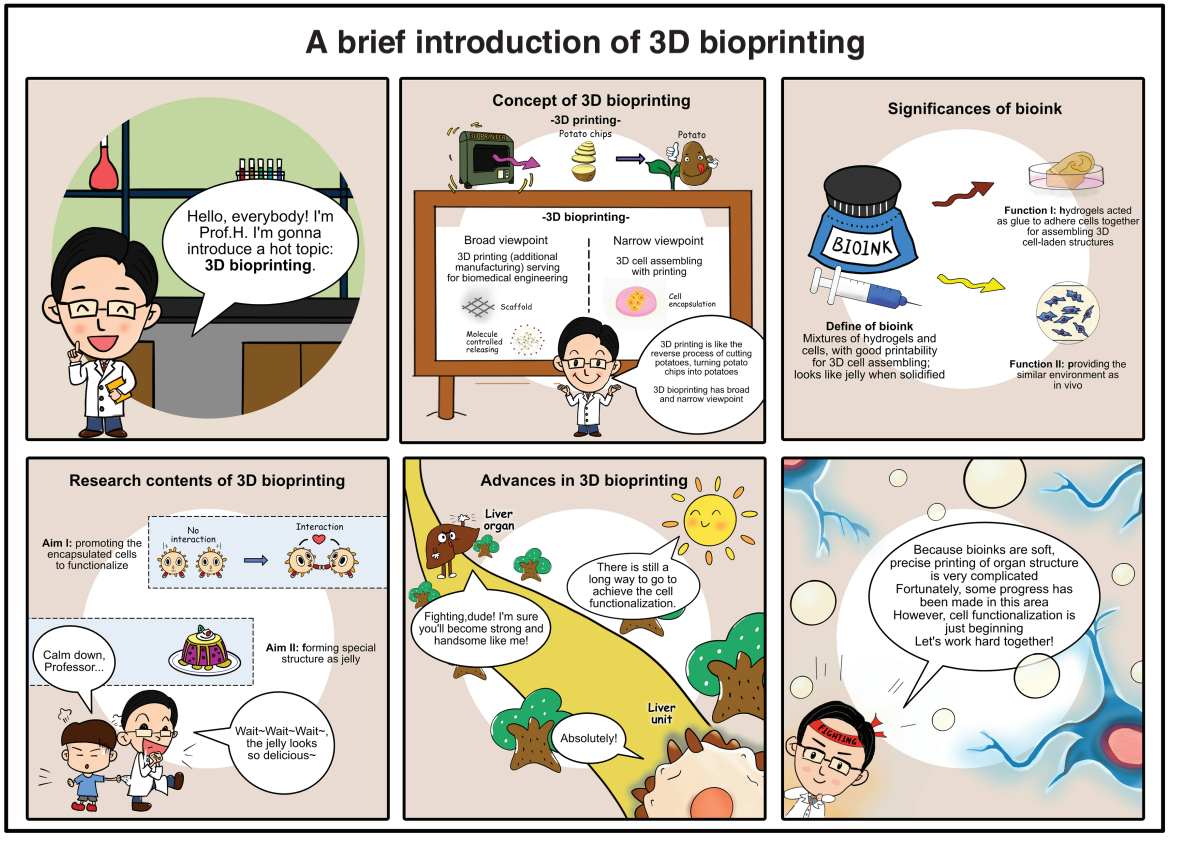

Figure 1.1 Introduction of 3D bioprinting. Source: He et al. [7]. Reproduced with permission of Springer Nature.

of functional units with simple composition and independent operation, which can be applied in high-throughput testing with a low dose. Besides, 3D bioprinting has been gradually involved in the setting up of organ-on-a-chip devices because of its excellent customizability and cell compatibility [11]. Modified microfluidic systems could be constructed with biomaterials through 3D bioprinting, on which specified cells are loaded and routine reactions were carried out. And the interactions and cross-talking between multiple organs can be well simulated by connecting different modules by means of microfluidic methods. Furthermore, 3D bioprinting has been further facilitated in the biofabrication of tissue/organ constructs with an inner channel network. A large number of 3D bioprinting strategies have been adopted in building 3D tissue/organ constructs with a vascular network, including coaxial bioprinting, projection-based bioprinting, as well as the integration of 3D bioprinting and sacrificial templates.

1.2 Evolution of 3D Bioprinting

As mentioned above, it is not practical to realize 3D bioprinting for full-function organ transplantation at present. However, it is an undeniable fact that bioprinting techniques have come a long way. Decades ago, pioneers such as Vladimir Mironov, Gabor Forgacs, and Thomas Boland saw the natural combination of technologies including cell patterning and others, such as commercial inkjet printing, to build living structures that might one day be used for human organ transplantation [6, 12, 13]. A timeline for the evolution of bioprinting technology up to state-of-the-art is illustrated in Table 1.1.

In 1984, Charles Hull invented stereolithography (SLA) for printing 3D objects from digital data, symbolizing the birth of 3D printing. Bioprinting was first demonstrated in 1988 while Klebe using a standard Hewlett–Packard (HP) inkjet printer to deposit cells by cytoscribing technology [14]. In 1996, Forgacs and coworkers drew a conclusion that apparent tissue surface tension was the macroscopic manifestation of molecular adhesion between cells and provided a quantitative measure for tissue cohesion [15]. In 1999, Odde and Renn first utilized laser-assisted bioprinting to deposit living cells for developing analogs with complex anatomy [16]. In 2001, direct printing of a scaffold in the shape of a bladder and seeding of human cells took place [17]. In 2002, the first extrusion-based bioprinting technology was reported by Landers et al., which was later commercialized as "3D-Bioplotter" [18]. Wilson and Boland developed the first inkjet bioprinter in 2003 by modifying an HP standard inkjet printer [19]. Their team implemented cell-loaded bioprinting with a commercial SLA printer a year after [20]. Also in 2004, 3D tissue with only cells (no scaffold) was developed. In 2006, electrohydrodynamic jetting was applied to deposit living cells [21]. Scaffold-free vascular tissue was engineered through bioprinting by Norotte et al. in 2009 [22]. In 2012, in situ bioprinting was attempted by Skardal et al. on mouse models [23]. The following years saw the introduction of many new bioprinting products, such as articular cartilage and artificial liver in 2012, tissue integration with the circulatory system in 2014, and so on [24, 25]. In 2015, coaxial

Table 1.1 Timeline for bioprinting evolution.

Year	Development
1984	Stereo lithography was invented, representing the birth of 3D printing
1988	Bioprinting was first demonstrated by 2D micro-positioning of cells
1996	Cells sticking together during embryonic development was observed
1999	First use of laser technology demonstrating 2D patterning of living cells
2001	3D printed synthetic scaffold for human ladder
2002	First extrusion-based bioprinter was achieved
2003	First inkjet bioprinter was developed
2004	3D tissue with only cells (no scaffold) was presented
2009	Scaffold-free vascular constructs were fabricated
2012	In situ bioprinting was realized on animals
2015	Tubular structure was printed by coaxial technology.
2016	Rapid continuous optical 3D printing based on projection (DLP) was applied
2016	Cartilage model was obtained by ITOP system
2019	Cardioid structure was first bioprinted
2019	Collagen human heart at various scales was built using FRESH technology

technology was adopted by Gao et al. for the fabrication of a tubular structure [4]. In 2016, Pyo et al. applied rapid continuous optical 3D printing based on digital light processing (DLP) [26]. In the same year, a cartilage model was manufactured by Anthony Atala's research group using an integrated tissue-organ printer (ITOP) [27]. In 2019, Noor et al. succeeded in manufacturing a perfusable scale-down heart [28], and a few months later, bioprinting of collagen human hearts at various scales based on the freeform reversible embedding of suspended hydrogels (FRESH) technology was achieved by Lee et al. [29].

1.3 Brief Classification of 3D Bioprinting

Based on different printing principles, cell-laden 3D bioprinting can be divided into three types: extrusion-based, droplet-based, and projection-based bioprinting. Extrusion-based bioprinting generates continuous fibers to set up the structures; droplet-based bioprinting produces droplets as the basic unit for biofabrication and projection-based bioprinting takes advantage of the properties of photosensitive materials by stacking 3D models layer-by-layer. Different approaches possess diverse characteristics aiming at various scenarios and have specific requirements for bioinks.

Extrusion-based bioprinting is the most widely used method, which is suitable for a wide range of biocompatible materials. According to different liquid dispensing

modes, pneumatic-driven, piston-driven, and screw-driven extrusion systems are applied to extrude cell-laden bioinks in the form of continuous filaments.

Droplet-based bioprinting which employs discrete droplets stacked into constructs can be roughly divided into inkjet bioprinting [30], electrohydrodynamic jetting (EHDJ) [31], and laser-assisted bioprinting (LAB) [32] based on different droplets forming principles. Thermal and piezoelectric-driven technologies are most commonly used in inkjet bioprinting. EHDJ uses a high voltage motivated electric field to pull droplets out of the nozzle orifice. Changes in voltage certainly affect the size of each droplet, where the higher voltage leads to smaller droplets [33, 34]. LAB is a non-contact, nozzle-free bioprinting strategy used precisely to deposit bioink droplets. LAB technique includes laser-guidance direct writing (LGDW) and laser-induced forward transfer (LIFT). LGDW employs a light trap to guild cells onto a substrate, while LIFT uses a focused pulsed laser to induce partial evaporation of bioink coating to propel the biomaterial toward the receiving layer.

Projection-based bioprinting solidifies light-sensitive biomaterials to form constructs under precisely controlled lighting with high printing precision and fast printing speed. The most common use of projection-based bioprinting is to print cell-free scaffolds, where cells would be seeded post-printing. Currently, however, cell-laden projection-based bioprinting has also been reported using DLP technology.

1.4 Evaluation of Bioinks

Generally speaking, 3D bioprinting has three steps: preparing bioinks, printing the soft live structures with multiple cells, and rebuilding the interaction among cells. And that is why developing appropriate bioinks has always been a significant part, as it affects every step that follows.

The performance of bioinks can be measured by three main factors: printability, biocompatibility, and mechanical property. Printability is to assess the formability of bioinks, where adjustable material viscosity, rapid transition from sol state to gel state, and a broad range of printing parameters are necessary. Biocompatibility is a measure of biomimicry that requires bioink and printed cells to be as similar as possible in the microenvironment in vivo. The mechanical property requires that the cured bioink be strong enough to hold subsequent culture and implantation. Perfusion and degradation might occur during bioprinted constructs culture in vitro, which requires considerable strength to support.

Therefore, the choice of bioink necessitates compromise among printability, biocompatibility, and mechanical property. Considering the requirements of the bioprinting process, cell growth and proliferation, and structural integrity, reasonable bioink design can be carried out according to the actual cell type and printing resolution requirements. But in fact, these three requirements of bioink are inherently contradictory in the mechanism. For example, the higher the viscosity of biological ink, the better the printability, and vice versa, the poorer the biocompatibility.

Hence, bioink selection to meet the specific needs of different applications is a key step in bioprinting.

An ideal bioink would certainly be close to the natural extracellular matrix (ECM), and it would need to be adapted to match different types of cells. Therefore, it could not be better to add specific substances in bioinks that cells possibly need during proliferation and functionalization. For example, when bioprinting chondrocytes, the addition of HA, a common component of cartilage, can significantly promote later culture and functionalization.

Typical bioinks applied in bioprinting may include hydrogels, decellularized matrix components, microcarriers, tissue spheroids and strands, cell pellet, and/or some advanced bioinks such as multi-material, interpenetrating network, nanocomposite, and supramolecular bioink, etc. [35, 36]. Among them, hydrogels are considered to be one of the most important biomaterials in bioinks, because of their outstanding capability of providing a viable microenvironment for cell adhesion, growth, and proliferation. Natural/synthetic hydrogels including alginate, fibrinogen, gelatin, collagen, silk fibroin, chitosan, agarose, pluronics, HA, GelMA, PEG, PEO, etc., have been found in countless applications in bioprinting. They are either ion-sensitive, photosensitive, thermosensitive, enzyme-sensitive, or pH-responsive, so they can be easily gelated to form constructs before, during, and/or after bioprinting [37].

1.5 Outlook and Discussion

3D bioprinting technologies still need further improvement. The complexity of tissues and organs has brought great difficulties to accurate bioprinting. One of the major disadvantages of current bioprinting technologies is the low accuracy of bioprinting compared to natural tissues/organs. Most tissues/organs are more delicate than current bioprinting devices. Another common drawback of bioprinting is the slow speed of bioprinting of complex scale-up structures, especially when it comes to multi-material alternate biofabrication.

Vascularization is the basis of bioprinted structures. Same as the challenge of tissue engineering and regenerative medicine, ensuring adequate vascularization in bio-manufactured structures is a key factor in 3D bioprinting. The effective construction of a multi-scale perfusion vascular network and the promotion of its vascularization by mechanical or chemical stimulation are the basis of the biological fabrication of scale-up constructs.

Functionalization is the primary goal for 3D bioprinting. Most of the current research is still focused on the manufacturing idea-oriented printing process and mechanism, while functionalization is the core factor leading 3D bioprinting from basic research to practical application. In order to be functional, bioink needs to have excellent biocompatibility and mechanical properties to meet the requirements of nutrient perfusion and implantation. In addition, the construction of microenvironments that mimic in vivo scenarios, including mechanical and

chemical stimuli such as perfusion culture and growth factors, is also critical for the functionalization of bioprinted structures.

Combined with the outlook of 3D bioprinting, there are several printing methods that are quite promising: DLP, coaxial bioprinting, and embedded bioprinting. Due to its intrinsic principle, DLP has a much higher printing resolution and speed than other bioprinting approaches. As a key application of 3D bioprinting technology, in vitro tissue models need to be standardized not only in sizes, but also in biological and mechanical properties, while DLP owns excellent uniformity and reproducibility compared to other methods. Additionally, coaxial bioprinting has become an increasingly popular extrusion-based bioprinting method since it was introduced into the field of tissue engineering in 2015 [4], especially in the area of blood vessel biofabrication/vascularization. The biggest advantage of coaxial bioprinting is its ability to construct hierarchical tubular structures with tunable biological/mechanical properties. It is well known that hydrogels with good biocompatibility tend to have insufficient mechanical strength. Coaxial bioprinting can partly solve the problem with its core-shell structure: core materials guarantee biocompatibility, while shell materials provide mechanical strength and vice versa. The use of sacrificial materials as the core material would also contribute to the convenient bioprinting of hollow tubular structures. Besides, embedded bioprinting allows anti-gravity writing of 3D freeform constructs within yield stress and gel-based supporting bath, which would be further removed post-printing to retrieve models with desired shapes or channels. Other than traditional bioprinting approaches, it can achieve the fabrication of discrete patterns, which are not mechanically supported [38–40].

In addition to the challenges including bioinks design, bioprinting techniques, vascularization, and functionalization, issues such as cell sources, bioreactor construction, and even ethical problems also require considerable attention. 3D bioprinted fully clinical translation could take a long time until bio-artificial tissues such as cartilage or skin, to be applied in transplantation. We all hope that 3D bioprinting can find its way from structural similarity into functional realization.

This book is organized into 14 chapters. This chapter "3D Bioprinting, A Powerful Tool for 3D Cells Assembling," covers the definition, evolution, and classification of 3D bioprinting. Chapter 2 "Representative 3D Bioprinting Approaches" and Chapter 3 "Bioink Design" demonstrates a variety of commonly used 3D bioprinting methods in detail, and introduces the principle of bioink design. In Chapter 4 "Coaxial 3D Bioprinting," Chapter 5 "Digital Light Projection-Based 3D Bioprinting," Chapter 6 "Direct Ink Writing for 3D Bioprinting Applications," and Chapter 7 "Liquid Support Bath-Assisted 3D Bioprinting," four types of promising 3D bioprinting technologies and their applications are highlighted respectively. Chapter 8 "Bioprinting Approaches of Hydrogel Microgel," and Chapter 9 "Biomedical Applications of Microgels" provides the manufacturing process and medical use of microgels. Chapter 10 "Microfiber-Based Organoids Bioprinting for in vitro Model" and Chapter 11 "Large Scale Tissues Bioprinting" are mainly concerned with biofabricated organoids and scale-up tissues. In Chapter 12 "3D Printing of Vascular Chips" and Chapter 13 "3D Printing of in vitro Models," vascular chips and

in vitro models by 3D printing approaches are well presented. Finally, Chapter 14 "Protocol of Typical 3D Bioprinting," comes up with an integrated blueprint for 3D bioprinting.

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