Biomimetic Design of 3D Printed Tissue-Engineered Bone Constructs

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Abstract: Surgery to repair damaged tissue, which is caused by disease or trauma, is being carried out all the time, and a desirable treatment is compelling need to regenerate damaged tissues to further improve the quality of human health. Therefore, more and more research focus on exploring the most suitable bionic design to enrich available treatment methods. 3D-printing, as an advanced material processing approach, holds the promising potential to create prototypes with complex constructs that could reproduce primitive tissues and organs as much as possible or provide appropriate cell-material interfaces. In a sense, 3D printing is a promising bridge between tissue engineering and bionic design, which can provide an unprecedented personalized recapitulation with biomimetic function under the precise control of the composition and spatial distribution of cells and biomaterials. This article describes recent progress in 3D bionic design and the potential application prospect of 3D printing regenerative medicine, including 3D printing biomimetic scaffolds and 3D cell printing in tissue engineering.

Keywords: Tissue engineering, biomimetic design, 3D bioprinting, biomaterials, human health, additive manufacturing.

1. INTRODUCTION

Bone diseases caused by physical and biological trauma have received considerable attention. Especially longitudinal segmental bone defects, generally resulting from oncologic surgery, high-energy trauma, inherent genetic disorders, and infection leading to diseased skeletal therapy an intractable clinical problem [1]. To address this dilemma associated with bone healing, there is an increasing interest in the research of bone grafts to restore damaged bone [2]. Traditionally, autogenous bone transplantation and allograft implantation are regarded as the “gold standard” treatment and the most effective approach for bone regeneration without any associated immune rejection. However, the application and availability of autograft are limited by donor supply and donor site complications [3, 4].

In the past decade, significant advances have been made in the development of valid bone substitute, which attempts to mimic the composition, structure and function of pre-injury bone tissue [5, 6]. There is no doubt that the exploration of biomimetic implants will expand the application of biomaterials, and biocompatible xenografts and synthetic bone substitutes have been developed as alternatives to autologous bone grafts to promote bone regeneration [1, 7, 8]. In the clinical setting, bone defect healing is a complex, dynamic and well-orchestrated physiological of bone tissue regeneration, which includes the proliferation, differentiation and extracellular matrix formation of osteoprogenitor cells [9, 10]. Therefore, it is difficult to precisely control the reconstruction process. In addition, reconstruction of bone tissue or organs is considered an unrepeatable procedure, which is tough to achieve in design and fabrication for conventional methods. Therefore, the research of tissue engineering and regenerative medicine aims at solving the limitations that traditional surgical methods are difficult to tackle. These strategies include providing regenerative stem cells, implanting cell scaffolds, and implanting bionic structures in tissue engineering [11].

3D-printing, a promising technology with the outstanding ability to fabricate intricately detailed three-dimensional structure through a layer-by-layer process, has attracted much attention in the biomedical engineering field [12, 13]. When making bone grafts, this technology can take outstanding advantage of its biomimetic design; this is the design of the implants on the macro, micro, and nano-level to facilitate nutrient transport and cell-matrix interactions [14]. As a state-of-the-art manufacturing method, 3D printing not only can create complexly biomimetic structures with high precision and accuracy, but also can be used in tissue engineering that encompasses damaged organs/tissues repair by bioprinting [13, 15]. It is noteworthy that all explorations seem to mimic the characteristics of natural bones, both in composition and structure, to improve the stability of implants and restore bone function. From the above, we can see that biomimetic design in this article means that human beings...
try their best to design substitutes for damaged tissues or organs to achieve the best therapeutic effect.

In this review, the current state of development and application of 3D Printing technologies in the biomedical engineering field have been reviewed, with emphasis on their unique ability of these manufacturing technologies in biomimetic design and tissue engineering. In addition, we will describe the potential application prospect of 3D biomimetic design in an effort to optimize tissue repair and restore organs function from biomimetic material, biomimetic structure and cell bio-printing.

2. 3D PRINTING BIONIC SCAFFOLD

Human tissue is a complex system, including the nervous system, metabolic system and blood circulation system. For example, natural bone, from a macroscopic point of view, consists of the outer compact bone and the inner spongy bone. Microscopically, it is a layered assembly structure, from collagen molecules to tropocollagen triple helix, then to collagen fibers and bone protein, assembled layer by layer. Collagen fibers are composed of nanocrystalline hydroxyapatite and collagen, as shown in Fig. (1) [16]. Due to this complexity of bone tissue, it poses a problem for the cure of orthopedic diseases, especially segmental bone defects, and we have to find a suitable bone substitute to fill the missing part of the bone.

3D printing, assisted with computer-aided design (CAD) technology or computed tomography (CT) scan, is based on the principle of material layer by layer overlapping manufacturing. In addition, as an efficient and flexible technology for the rapid fabrication of components with any complex shape without template, it can even create complex 3D structures on a micro-scale or even nano-scale [17, 18]. Numerous studies have shown that the interaction between cells and substances plays an important role in tissue engineering, which contributes to cell migration, proliferation and differentiation. Therefore, in addition to selecting ideal biomaterials, pore size, design and interconnection are also vital factors to be considered when making scaffolds [19]. Furthermore, in order to simulate natural tissues, it is necessary to find an appropriate balance between mechanical and biological properties, which are directly related to the structural characteristics, such as porosity, pore size and interconnection of their pores [20].

The type and structure of materials are the key factors to determine their functions. Especially for medical materials, exploring ideal biomaterials and suitable structures have been the focus of research [21]. Recent developments have been in the evolution of satisfied biomaterials or bionic design, which provide a bioactive microenvironment for cell reproduction and differentiation. At the same time, research on advanced biocompatible materials with fast crosslinking properties for bioprinting is an essential prerequisite for promoting the application of 3D printing in tissue engineering [22]. In general, according to the difference of printing target, biomaterial additive manufacturing technologies can be divided into two main categories: acellular and cellular

![Fig. (1). The chemical composition and multi-scale structure of natural bone [16]. (A higher resolution / colour version of this figure is available in the electronic copy of the article).](image-url)
technology. The acellular category involves printing biomaterials without any living cells. Cellular includes the printing of living cells and other biomaterials [23]. This section focuses on acellular 3D printing, including the selection and structural design of biological materials.

2.1. Biomat erial Selection

Biomaterials are natural or synthetic materials, and they are useful for repairing damaged parts of the body through interaction. These biomaterials are used to replace an integral part of the human body and to support physiological functions [24]. Therefore, biomaterials interact with human cells or tissues /organs, and shoulder the functions of original tissues. All kinds of materials, including metals, ceramics, polymers and composites, attract engineers and scientists in biomedical field. With the improvement of our understanding of the biological characteristics in biomedical application materials, researchers continue to pursue preferable material characteristics to treat various diseases in order to improve the quality of human life [25]. Generally, the manufacture of biomaterials has become an important issue to improve reliability and reduce the risk of infection or rejection. Continuously explore advanced manufacturing technologies and biomaterials to reduce costs, improve stability and maximize in vivo performance [26]. In addition to single component fabrication, advanced manufacturing technology is also developed to produce multi-component structures that are laborious by traditional routes. The gradient part of the structure or composition can be used as a multi-functional biomedical device to improve the performance in vivo [27].

Metals and ceramics with appropriate mechanical properties are currently used in high load applications, but their biological properties are limited. On the contrary, natural polymers are one of the most popular biomaterials because they are usually biocompatible, biodegradable and bioactive, but the mechanical properties of 3D printing polymer structure are undesired [28]. Therefore, the development of novel composite materials or hybrid materials and the popularization and application of materials are the main trend in the 3D printing biomaterial field.

2.1.1. Metallic Biomaterials

Metals and their alloys play a predominant status in all biomaterials worldwide, and these implants made of metal are commonly used for damaged joint replacement or filling in bone fracture sites to assist bone healing due to their excellent mechanical properties. Typically, these metal alloys, including cobalt-chromium, zirconium, titanium and stainless steel, are the main choices for implants [12, 29]. Until now, metal implants have been widely used in clinical medicine, such as ventricular assist device housings, dental implants, maxillofacial, craniomaxillofacial implants and spinal surgical screws [30]. It is worth mentioning that stress shielding may occur with respect to the superior elastic modulus of the metal implants. In this phenomenon, the bone tissue around the implant is unevenly stressed, which leads to increased bone dysplasia and fracture risk [31]. In spite of its inherent limitations, the development of advanced medical metals and alloys in cooperation with other materials has achieved success in manufacturing composite materials [32].

In the past few decades, titanium and its alloys have become a kind of popular biomaterials which not only has superior biocompatibility but also has excellent fatigue resistance and corrosion resistance [33]. In addition, the inherent low density of titanium-based materials is guaranteed to be lighter than the majority of metal materials, but has higher strength, which can be further enhanced by deformation [34]. Nevertheless, in practical applications, titanium-based materials still have some defects that need to be addressed, such as most of the titanium-based materials are applied to load-bearing implants due to inferior surface mechanical properties. Apart from this, the combination of human tissue and titanium-based implants is inadequacy, which can lead to infection and even implant dislocation [35, 36]. Additive manufacturing techniques can provide suitable solutions to these problems, whether surface modification of titanium-based materials improves surface topography and optimizes surface mechanical properties, or manufactures porous or multi-gradient structural parts to improve osseointegration [37, 38]. The second-generation β-titanium alloy has a higher modulus of elasticity than the application of more mature commercial pure titanium and Ti-6Al-4V, and the strain control and fatigue resistance of the β-titanium alloys are improved [39, 40].

To develop innovative metallic biomaterials with biodegradable/bioabsorbable nature has attracted numerous attention [41]. For permanent metallic materials, the lack of biodegradability makes them unsuitable for bone tissue engineering due to the desirable biomaterials to allow natural tissue to reabsorb and replace the implanted structure [42]. Generally, additional follow-up procedures are required to remove metal implants, especially when they are applied in pediatric patients who have not yet reached skeletal maturity [43]. Mg-based biomaterials have become the preferred biomaterial for degradable medical implant devices because of their low elastic modulus, low-density, biocompatibility and rapid corrosion characteristics [41]. However, achieving a perfect match between the corrosion rate and the effectiveness of the implant is the main concern. In order to solve this problem, the common methods add alloying elements and surface modification to control the corrosion rate [44]. For example, Aidin et al. successfully prepared MgO/ZnO composite coatings on Mg alloy matrix by micro-arc oxidation, and the corrosion resistance was enhanced. Furthermore, a betamethasone sodium phosphate is coated on magnesium microarc oxidation coatings as an anti-inflammatory drug carrier by dipcoating process. This composite coating provides continuous drug delivery and well corrosion resistance [45, 46].

2.1.2. Bioceramics

Bioceramics, including ceramic composites, amorphous glass and crystalline ceramics, have been shown to be highly mechanically active in bone tissue engineering with good bioactivity and biocompatibility [47]. Moreover, the degradation products of these ceramics can participate in human metabolism and produce an alkaline microenvironment for cells to enhance cell activity, and release bioactive ions can induce cell differentiation and accelerate bone regeneration [48-50]. The most representative crystalline bioactive ceramics in tissue engineering are calcium phosphates (CaPs),
mainly because its chemical composition is similar to that of the mineral content of natural bone tissue [51]. In addition, bioceramics also have a large degree of similarity in terms of bone structure, which allows ceramic implants to provide a biocompatible bioactive interface that promotes integration with host tissues without the formation of scar tissue biomimetics with high interconnectivity and high porosity [52].

Recently, the excellent biocompatibility, corrosion resistance and stiffness have made bioceramics widely used in clinical orthopedics. Patients benefit from ceramic components to replace damaged bone tissue, as well as bioceramics particles used to fill bone defects [53, 54]. However, in the context of bone tissue engineering, ceramic stents are prone to brittleness. In addition, like metal-based scaffolding, they also have a degradation rate that is difficult to control accurately. Therefore, researchers are paying more and more attention into the development of ceramic/polymer composite scaffolds with excellent mechanical properties [55]. On the other hand, ion release in ceramic materials, which inhibit the macrophage inflammatory responses, is believed to strongly influence cells bioactive and tissue regeneration. Cell adhesion, activity and proliferation on different components of ceramics are shown in Fig. (2).

2.1.3. Polymers

Due to the inherently advantageous properties of outstanding ductility, biocompatibility and biodegradability, the use of polymers has attracted widespread attention in the field of tissue engineering. Natural biopolymers are extracted directly from animal or plant tissues, mainly including collagen, alginate, chitosan, hyaluronic acid and cellulose [16]. It is worth mentioning that the surface of natural polymers usually contains biologically functional active molecules, which contribute to cell adhesion, proliferation and differentiation [56]. However, the application of natural polymers also encounters some challenges, including the presence of pathogenic impurities, sub-optimal mechanical properties, lack of degradation rates, and local cellular inhibition reactions that may be caused by degradation products [57].

Synthetic polymers commonly used in bone tissue engineering include poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly(caprolactone) (PCL) and poly (ethylene glycol) (PEG), while still a copolymer containing poly (lactic-co-glycolic acid) (PLGA). The biodegradable synthetic material can be employed as a supporting scaffold for tissues and organs, but the biodegradation of synthetic polymers currently takes a long time, which mismatches the tissue regeneration process [58]. Hydrogel-based bio-inks are the pre-
ferred material for bioprinting, providing a favorable environment for three-dimensional growth of cells while controlling the degradation rate to a certain threshold. The mechanical requirements of the skeletal supports are complex and must have the required mechanical capacity such as compression, tensile and fatigue properties. However, the use of synthetic polymers and natural polymers generally has relatively low load-bearing capacity and elasticity [59]. In addressing these limitations, a popular approach is to combine polymers with bioceramics to produce composite scaffolds. Typically, bioceramics or bioglass are added as a coating or filler to the polymer matrix to enhance biological activity and improve mechanical properties [60].

In general, the porometer can be incorporated into the structure prior to dissolution or prior to chemically triggering the release of gas to create pores. Micropores and macropores can be added by combining the use of a porometer with techniques such as 3D printing during the manufacturing process; alternatively, production of different porosity polymer ceramic gradient scaffolds with varying levels of precision by 3D printing technology, in which ceramics is used as reinforcements [61, 62]. However, they are hydrophobic that leads to a lack of cell adsorption capacity, and this limits their application in medical implant without secondary modification. Therefore, the polymer-based scaffold surface is also subjected to secondary chemical treatment to increase micro-porosity using an organic solvent. Generally, surface modification or supplementation with bioactive materials contributes to cell adhesion and proliferation. In addition, it is believed that the combination of 3D printing and electrocatalyst will be a powerful method for manufacturing devices for heterogeneous biological catalytic reactions [63, 64].

2.1.4. Trends in Biomaterials

There is no doubt that appropriate bone substitute material for biomaterials-based treatments is a pressing clinical need in orthopedics. Instead of blindly pursuing biological inert materials in the past, materials scientists today are more inclined to design biological active materials that are doped with some biologically active molecules or stem cells [5]. In the case of bone substitute, the ideal basic premise is that biomaterials should be osteoinductive, osteoconductive and osseointegration, while materials can be degraded and resorbed over time. Ongoing studies focus on the incorporation of antibacterial factors or osteoinductive molecules into scaffolds. In addition, cells are naturally sensitive to their surroundings microenvironment, such as a topographic reaction and scaffold structure, which affects cell adhesion and regulates the transcriptional activity and gene expression [65].

2.2. Biomimetic 3D-printed Scaffolds

3D-printed scaffold with multi-scale microstructures that resemble natural human bones could contribute to tissue regeneration. Therefore, the constructions of the biomimetic scaffold with heterogeneous multiple gradient structures have attracted extensive interests in the tissue engineering field [66, 67]. In clinical practice, computed tomography (CT) and magnetic resonance imaging (MRI) techniques are often used to pre-collect printed data; then the required structures are modeled and manufactured using computer-aided design and computer-aided manufacturing (CAD-CAM)) tools. In recent years, numerous studies have shown that diverse design of bio-scaffold characteristics, including porosity, interconnectivity and size of pores, causes different cellular responses, such as cells migrate, proliferate and differentiate [68]. However, certain scaffolds have unsatisfactory cell-material interaction. To deal with this problem, the prevalent strategy attempts to find appropriate biochemical or biophysical stimuli with bioactive material by surface modification [69]. Generally, the mechanical properties of polymers and ceramics scaffolds are too low to meet the mechanical requirements of the implant environment with severe load-bearing and inhomogeneous stress distribution. Synthetic polymer and bioceramics composite scaffolds with hybrid material seem to be an effective way to solve this problem [69, 70].

In addition to explore the suitable structure for achieving specific biofunctions, biological scaffolds should also match the properties of the native tissue to improve the stability and practicability of implants. For example, inspired by Elytrigia repens, Peng et al. [71], explore ultralight biomimetic hierarchical graphene scaffold with macroscopic hollow structures and cellular microstructure by ink-based 3D printing. As shown in Fig. (3), this nanomaterial significantly improves mechanical properties at a low density. 3D printing can fabricate versatile patient-specific structures with a high degree of freedom in structure design, which enables to construct complex multi-gradient scaffolds [72, 73]. Zhou et al. [74] successfully constructed a 3D biomimetic and biphasic scaffolds by 3D stereolithography printing technology, and confirmed that the cartilage bone scaffold can promote bone formation and cartilage regeneration. In particular, a typical schematic diagram of 3D printed biomimetic scaffold is shown in Fig. (4). Bio-inks of different compositions prepared in advance are used to print the upper and lower layers of the scaffold through the 3D printing model.

3. TISSUE BIOPRINTING

Tissue engineering is the outcome of significant advancements in interdisciplinary fields ranging from cell biology and medicine to biomaterials science and technology development. In a sense, tissue engineering has shown promising potential in building functional tissues/organs to alleviate the shortage of organ transplantation, and obtain a satisfactory application in pathogenesis research and treatment process simulation [75]. It has been demonstrated that the formation of neo-tissue can be affected by the control of tissue microenvironment, such as cell arrangement and extracellular matrix composition (ECM) [76]. In particular, ECM, as a unique composition and structure of each tissue, is a replicated structure composed of collagen, aminoglycan and growth factor, which can regulate cell migration, behavior and differentiation during tissue growth and development [77].

The convergence of 3D printing technology with cell biology has made a significant leap in tissue engineering. There is no doubt that cell printing is a promising avenue for artificial organs/tissue printing and regenerative medicine due to its flexibility, stability and reliability [78]. In general,
the printing matrix comprises a cell-loading material and cell growth and differentiation factor, and the matrix is mixed with the previously cultured cells to form a bio-ink, which is used to fabricate the required structure [79]. The introduction of living cells tremendously increases the complexity of bio-printing, such as biomaterial selection, cell printability, and technical challenges associated with living cell sensitivity. In order to address these complexities, it is necessary to optimize manufacturing conditions and the appropriate printing matrix should be controlled [80].

3.1. Tissue Bioprinting Strategies

Current mainstream cell bioprinting technology on tissue engineering can be classified into the following five methods: Micro-extrusion bioprinting, Inkjet bioprinting, Laser-assisted bioprinting, Stereolithography and Microvalve-based bioprinting [81]. In general, stem cells obtained from autologous tissues have the potential to differentiate into a variety of tissue cells with special functions, including pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), adipose-derived stem cell (ADSCs), amniotic fluid-derived stem cells (AFSCs) and muscle-derived stem cell (MDSCs), as shown in Fig. (5). Conceptually, it is feasible for cell printing to produce complex-shaped tissues for cell printing when appropriate cell printing techniques and stem cell types are selected [82]. In this section, we will discuss the current developments, applications, advantages and potential limitations of conventional
3D bioprinting methods and limitations of each bioprinting technology, respectively, and summarized in this section (Table 1).

### 3.1.1. Inkjet Bioprinting

Inkjet-based bioprinting, as a low cost and effective 3D printing technology with high printing speed, is widely employed in tissue engineering for medical applications [83, 84]. Generally, inkjet printing method, which deposits liquid biomaterials at pre-positioned locations by acoustic-, thermal- or electromagnetic-induced physical displacement, can be divided into three categories: electro-hydraulic jet biological printing, continuous inkjet biological printing and drop-on-demand inkjet bioprinting. The largest and most common way of printing is droplet piezoelectric inkjet bioprinting, which consists of thermal, piezoelectric and electrostatic inkjet bioprinting [85].

In inkjet bioprinting, the mechanical stress caused by the extrusion is quite unfavorable for this cell printing method, which causes the cell survival rate and cell density to be limited during printing. According to the report, only biological ink with viscosities lower than 10 MPa/s can be printed by inkjet printing due to the printing head that hardly provides continuous flow [86]. To some extent, low viscosity reduces shear stress and machine nozzle clogging and ensures great cell viability, but poses challenges for shape fidelity, especially affecting the tissue formation capability. In addition, it is necessary to ensure that gelation occurs after the material leaves the nozzle and coincides with the printing process because the nozzle is clogged when gelation has occurred inside the print head [87].

It is worth mentioning that the thermal inkjet system generates a pressure pulse by evaporating the bio-ink around the heating element to discharge liquid droplets from the printhead. As a result, the local high temperature reduces cell viability; sometimes, the heating element may reach above 200°C, even if the short exposure period (2 μs) during the printing process [88]. In order to have a better application of this technology in tissue engineering, exploring bioink with great thermosensitivity is a hot topic of current research [89]. For example, a thermoresponsive water-based biodegradable bioink was successfully synthesized with polyurethane, and this bioink may form a gel around 37°C without any crosslinker [90].

### 3.1.2. Micro-extrusion Bioprinting

Extrusion-based bioprinting as a rapid prototyping method is the most widely used in the field of bioprinting; currently, the main reason is that the printing precursor with cells is a liquid, which allows printing to provide continuous flow [91]. As a modification of inkjet printing, extrusion-based bioprinting has the advantages of ease of operation, the versatility of the available systems and ability to personalization. Compared to inkjet printing, extrusion-based bioprinting allows for the extrusion of viscous materials by increasing the extrusion capacity, thereby maintaining the high...
strength and ensuring better integrity of the 3D shape while containing high cell densities [92].

Extrusion-based bioprinting can accurately customize the shape of cell encapsulated hydrogels based on changing the physicochemical properties of biopolymers, thereby speeding up the gelling phenomenon, which consists of fluid dispensing system and automatic biological printer system for extrusion and biological printing [93]. According to the difference in the type of extrusion printing driving force, common extrusion-based bioprinting methods are categorized into piston-driven, pneumatic and screw-driven bioprinting [85]. Generally, pneumatic bioprinting supports a wider range of viscosities, but it is difficult to precisely control the quality of the deposition. Screw-driven bioprinting can be printed with mechanical driven and are much cheaper, but they encounter problems in high viscosity bioprinting [94].

As a versatile printing technique that develops cell-loaded biomaterials into specific shapes to produce living tissue or organ with a similar dimension and function of real body tissue/organ remains a promising approach. However, there are still facing numerous limitations and challenges, such as impediments to the manufacture of multifunctional solid organs, limited resolution and lack of a suitable printing solution transition the technology into practical applications [80, 95]. In addition, due to the increased squeezing force, the cell viability of micro-extrusion bioprinting is lower than inkjet-based bioprinting and is closely related to extrusion pressure and nozzle specifications. In fact, cell viability can be improved by optimizing bioprinting parameters such as temperature, extrusion pressure and deposition rate [81]. On the other hand, it is necessary to develop novel bio-inks that deliver cells quickly, safely and sustainably and provide a microenvironment with biocompatible and bioactive for cells.

3.1.3. Laser-assisted Bioprinting

Laser-assisted bioprinting, also known as laser-induced forward transfer (LIFT) and biological laser printing, is the process of implanting cells on a donor slide (containing an energy-absorbing layer) where cells can be safely propelled and encapsulated in droplets of biological material. When a focused laser pulse is employed to simulate a certain area of the absorbing layer, the cell-containing droplets will be deposited on the collector slide and subsequently crosslinked [96]. Laser-assisted printing can be divided into two types: laser-guided and laser-induced cell printing. The laser-guided cell printing method directly applies a laser beam to the bio-ink, and utilizes the refractive index difference between the cells and the medium to enable the laser beam to induce the cell-loaded bio-ink deposit on the receiving substrate [97].
As a nozzle-free bioprinting technique, LIFT is an effective process that offers a higher cell survival rate and allows high-resolution deposition of biomaterial. In general, the droplet resolution of laser-assisted bioprinting is affected by the rheological properties of the biomaterial, the donor-collector system and the laser characteristics [98]. Moreover, as a non-contact biological printing method between dispenser and bio-ink, laser-assisted cell printing hardly causes mechanical stress on cells, which leads to high cell viability, and easy to print various viscosity biomaterials with high strength to ensure the integrity of the printed structure [99]. Although cell transfer using laser-assisted bioprinting technology is successful, the 3D structure of complex cell alignment can be achieved with the aid of computer-aided design without negatively affecting cell viability or function [100]. However, relatively low cell concentrations are detrimental to cell differentiation, affecting the functionalization of printed tissues or organs. On the other hand, in order to avoid the adverse effects of the cross-linking process, lower printing velocity has to be selected. In this sense, it takes a lot of time to construct a clinically relevant 3D structure, which hinders a wide range of applications [101]. From a future perspective, the development of specific biomaterials and multi-nozzle hybrid bioprinting is a prospective approach to meeting these urgent demands [102].

### 3.1.4. Stereolithography

Stereolithography (SL) is a laser 3D printing system employed in bioprinting. A typical SL system utilizes a digital micromirror array to control the intensity of light in the printing area where light-sensitive polymer bioinks are polymerized layer by layer assembly [103]. SL bioprinting technology has a unique advantage to fabricate micro-macro-size 3D constructs compare to the other bioprinting technologies in straps or droplets. The total printing time is only related to the thickness of the component, which means that no matter how complex the single-layer structure is, the printing time for each layer is the same. Thus, stereolithography technology can not only be flexible in the design of 3D scaffold structures, but also can significantly reduce printing time [104]. In addition, SL as a nozzle-free bioprinting technology improves cell viability (> 90%) encapsulated in biomaterials, but results in resolution down to 100 μm [105]. Ultraviolet light is commonly used in the process of cross-linking curing, which has a significant impact on the viability of cells. Short-wavelength ultraviolet light has been reported to be harmful to the nucleus (DNA). Furthermore, increasing the intensity of light and the concentration of photo-initiator will also produce cytotoxic effects on living cells [106].

### 3.1.5. Microvalve-based Bioprinting

Microvalve-based, as one of drop-on-demand (DOD) printing approaches, is able to create spatially heterogeneous 3D bioengineered structure at pre-defined positions by accurately depositing a broader spectrum of biomaterials and cells types [107]. Generally, a standard microvalve-based bioprinting system consists of a computer-controlled triaxial
removable platform and a set of multi-electromechanical microvalve print heads connected with a gas regulator. The basic principle of microvalve assisted bioprinter is that the plunger is pulled up by a controllable magnetic field generated by applying a voltage pulse to the inductor coil, opening the nozzle orifice while adjusting the gas pressure regulator to generate the pneumatic pressure in order to overcome the viscosity and surface tension of the bio-ink at nozzle orifice [108]. The quality of printed bioengineering constructs is mainly determined by the viability and dispensation of cells. The touted advantages of microvalve based systems are that it can ensure uniform cell dispensation, which benefits from the automation of the printing process, and biomaterials and cells are ejected synchronously from different print heads. In addition, this approach can not only guarantee accurate cellular positioning and high throughput printing, but also improve the viability of cells (more than 86%) [108]. It is noteworthy that this technique can deposit a thin layer of material up to 2 microns thickness, which can be used to prepare artificial skin tissue. Although microvalve-based bioprinting is a promising cost-effective bioprinting technology, there are numerous challenges that bioprinting bioinks is limited to a range of viscosities (∼1 to 200 mPa s) and restricted cell concentration due to the small nozzle clogging issues [109].

3.2. Vascularization

Vascularization is crucial during tissue generation, and vascular networks not only provide indispensable nutrients for cells growth reproduction and differentiation, but also play an important role in regulating tissue metabolic balance [110]. The majority of implanted grafts failures are caused by inferior integration of neo-tissue and host tissue due to slow angiogenesis in a short time period. In general, when the thickness of the tissue structure exceeds 200 μm, vascularization is needed to transport oxygen and nutrients to cells and discharge waste from cells [111]. However, spontaneous blood vessel growth is slow and usually limited to a few tenths of one micrometer a day. Conventional research focuses on promoting vascularization by constructing osteoinductive scaffolds, combining vascularization-inducing factors and stem cell precursor differentiation or maturation [112, 113]. Nowadays, exploring advanced tissue scaffolds that combine both the structure of biomimetic features and the ability to regulate cell behavior remains a hot topic in this field. In the process of cell printing, the most important consideration is tissue vascularization, which affects long-range continuous mass transfer capability in complex three-dimensional tissues. Hence, an ideal vascular scaffold that integrates hierarchical networks with a functional transport
system should be designed to regulate cell metabolism and promote tissue generation [114]. Therefore, the researcher has been proposed to use the sacrificial template method to manufacture vascular biomimetic structures, as shown in Fig. (6a). This approach is intended to design a polymer scaffold with a well-organized hierarchical structure, as shown in Fig. (6b), resembling the native vascular hierarchical structure, including a 3D frame, a perfusion microchannel and a permeable porous wall, was established. This multi-level bionic structure that helps the artificial blood vessels exchange substances with the outside, and cell printing with this scaffold can significantly improve cell viability and survival rate [115].

Despite the considerable progress that has been made in bioprinting in recent years, the preparation of large-scale tissues is severely constrained by vascularization. The synergistic effect of the multi-gradient microchannel and functional cell printing is expected to promote the rapid vascularization of tissue [116]. In addition, the deficiencies of perfusion vascular structure and microporous permeation for artificial blood vessels is a challenge in bioprinting design. To further stimulate natural tissue, it seems an interesting way to print blood vessels and tissues directly in one step, that is, using two nozzles simultaneously; the printed blood vessels contain stromal cells, which can better regulate the intravascular and extracellular substances, as shown in Fig. (7).

### 3.3. Scaffold-free Tissue Engineering

As an emerging technology, cell printing aims to position a packaged biomaterial containing living cells (called bio-ink) layer by layer through an automated dispensing system. The major purpose of 3D bioprinting is to apply this cutting-edge tissue engineering technique to create an implantable tissue-related 3D structure that enhances tissue or organ reconstruction [118, 119]. The cells are cultured and packaged in a delivery medium or bio-ink with selected biological materials such as hydroxyapatite and hydrogel. Then, the cartridge containing the bio-ink is loaded into the 3D bioprinter to dispense bioink in a predetermined 3D geometry based on the CAD model [120]. 3D bioprinting offers a potential solution to help ease the burden of autologous transplantation. Different from traditional tissue engineering methods, which seeded cells onto prefabricated scaffolds, 3D bioprinting allows precise control of the location and content of cells, which can accelerate the growth of printed structures to functional tissues after bioprinting [121, 122]. The spatial control of structure and materials can be achieved if multiple print nozzles are printed simultaneously. The structure can be implanted directly into the patient or matured in vitro after bioprinting [123].

It is worth mentioning that cell density is critical to the function of the printed structure, and the shear stress, nozzle shape and printing holes during printing affect the cytotoxicity of printed structure and the building function [124]. As shown in Fig. (8) [93], in the case of low-density cells, the protective effect of encapsulating bio-ink is strong, and the proliferation rate after printing is increased, which leads to high cell survival. However, low cell density results in slower cell growth and differentiation. High cell density encapsulation will cause the 3D print chain to swell and become unstable. In addition, high cell concentration may result in significant cellular hypoxia and disruptive cell-to-cell interactions. In fact, the effect of different nozzle apertures on printing live cells is a significant discrepancy. Medium-sized nozzles ensure print fidelity and high cell viability during extrusion because the extruded cells are not affected by the specific force field created by the nozzle wall hydrogel flow. Large nozzle holes ensure high cell viability after printing, and reduce the shear force applied to the print unit, but at the expense of print resolution. In contrast, small-aperture nozzles can print high-precision structures but cause high stress on the printing unit during printing.

In 3D bioprinting, a combination of cells and biomaterials is commonly used as a printing precursor to create clinically relevant cell structures of size, shape and structural
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integrity [125]. Successful bioprinting relies in part on combining suitable bioprinting techniques with appropriate bio-inking. Therefore, the bio-printing community has paid considerable attention to the development of cell-friendly new bio-inks that can be 3D printed to create tissue structures [126]. Polymer hydrogels are often the main component of bio-inks because of their cell-friendly and degradable properties. At the same time, the hydrogel-based bio-ink pass contains growth cultures, cells, nutrients and growth factors to help cell proliferation and differentiation.

Cell printing with regard to the selection of suitable scaffolds and cell encapsulating biomaterial that involve many issues, such as cell-cell communication, cell-ECM/scaffold communication, toxicity, immunogenicity, inflammation, and mechanical incompatibility, all of which are directly determined by the characteristics of the scaffolds and biomaterials [32, 127]. Recently, the scaffold-free method has come to the fore. As a promising manufacturing technique, the living cell design 3D structure without any supporting structure can be fabricated in vitro using human cells [128]. Unlike the proliferation of cells in hydrogel scaffolds, we can obtain a considerable number of cells using cell printing, that is, close to natural tissue, and deposit cells in confined space to obtain ideal tissue as needed [82].

In order to explore synthetic mimics of cells as much as possible, Villar et al. [129] successfully printed the droplet network using micro 3D printing, which was used as a tissue engineering substrate, or as a living tissue simulant. As shown in Fig. (9), it is observed that there is an innovative scaffold-free spheroid-based bioprinting technology, which can achieve biomaterial-free, only cell bioprinting [130]. More specifically, the cells are cultured to form globular clusters, which are then picked up using a vacuum nozzle and assembled using a 3D bioprinter [131]. Furthermore, Arai et al. [132] utilized this novel scaffold-free cell printing technique to print aggregated spheroids onto the array of needles according to the desired 3D design. Direct cell printing in tissue engineering greatly facilitates human transplantation of human tissues and organs for medical applications [133].

CONCLUSION

This paper reviews the development of tissue engineering in 3D printing in recent years from the perspective of bionics, and discusses the application prospects of 3D printing in the medical field from the aspects of printing materials, bionic scaffolds design and bioprinting. In fact, 3D printing encounters numerous challenges in tissue engineering such as printing technology-related problems, undesirable vascularization, uncertain structure-function relationship, and impaired cell activity of 3D bioprinting. To deal with these challenges requires more research on the development of biomaterials, structure design and 3D bioprinting technology. In the coming days, biomaterials using in 3D printing is an important area that is expected to achieve multi-materials printing in one operation. Bionic scaffolds design is another area that plays an essential role in 3D printing using in medicine, and researchers tend to design high-precision low volume components with varying thickness. For bioprinting, almost all studies focused on single-cell bioprinting, but in order to further simulate complex tissue anatomy and physiology, multi-cell bioprinting should be explored to further enhance or activate some functions of tissues through cell interactions. Further expanding multifunctional biomaterials,
combined different printing technologies to develop more accurate bioprinting methods, seem to be an important area to promote the application of bioprinting in tissue engineering. All in all, 3D printing shows promising potential in the medical filed, and cell printing creates a beautiful blueprint for stem cells to be accurately deposited for tissue regeneration.

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CONFLICT OF INTEREST
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Fig. (9). Droplet networks printed in bulk aqueous solutions. (A) Schematic of printing in aqueous solution. Aqueous droplets are ejected into a drop of oil suspended in bulk aqueous solution. Excess oil can be removed after printing by suction through a printing nozzle. (B) Micrograph of a network printed in aqueous solution, viewed from above. A core of orange droplets is surrounded by a shell of blue droplets, which contain the fluorescent dye pyranine. Scale bar, 400 μm. (C) Horizontal sections of the network in (B) obtained by confocal microscopy, showing the fluorescent shell of droplets around the nonfluorescent core. The sections span approximately the bottom 150 μm of the network. Scale bar, 400 μm. (D) Micrographs of three other networks printed in bulk aqueous solution. Scale bars, 400 μm [129]. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
trices, and forces combine and control stem cells. 


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