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Current Progress in 3D Bioprinting of Tissue Analogs

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Abstract

Tissue engineering has progressed tremendously over recent decades through the generation of functional tissue analogs. Traditional approaches based on seeding cells into scaffold are limited in their capacity to produce tissues with precise biomimetic properties. Three-dimensional (3D) bioprinting is one kind of fabrication technology used to precisely dispense cell-laden biomaterials for the construction of functional tissues or organs. In recent years, much research progress has been made in 3D bioprinting technology and its application in generating tissue analogs, including skin, heart valves, blood vessels, bone, and cardiac tissue. However, it still faces many technical challenges. In this review, we introduce the current progress in 3D bioprinting technology and focus on biomaterials and their potential applications in regenerative medicine and drug discovery. Current challenges are also discussed.

Keywords

3D bioprinting, bioink, biomaterials, tissue engineering, tissue model

Introduction

Tissue engineering technology, by using scaffolds and seeding cells, can be used to repair and regenerate tissues and organs, and has been widely studied in cartilage, bone, skin, vascular tissue, nerve, heart and liver regeneration, and so forth.[1–9] Tissue engineering has achieved great success in the past few decades.[10] However, there are still limitations. For example, the precise distribution of various cells is hard to achieve. The biological function of reconstructed tissues and organs is limited. In a word, making complex and functional tissues or organs remains a huge challenge for tissue engineering.[11,12] In recent years, three-dimensional (3D) bioprinting has become more and more widely used in tissue engineering and regeneration.[13,14] As additive manufacturing, 3D bioprinting can print materials, cells, and growth factors by program controlling the structure of the scaffold, cell distribution, and biological signals, making it possible to generate multicellular tissues with normal structure and biological function.[15–17] In addition, 3D printing is contributing to the revolution of personalized and precision medicine, and has great development potential in the field of tissue engineering and regeneration.[18,19]

3D Printing Technology and 3D Bioprinting

3D printing is a rapid prototyping and additive manufacturing technique used to fabricate complex architecture with high precision through a layer-by-layer building process. This automated, additive process facilitates the manufacturing of 3D products with precisely controlled architecture, such as external shape, internal pore geometry, and interconnectivity, with high reproducibility and repeatability.[13,20] 3D printing includes many processes, such as light-mediated stereolithography (SLA), fused deposition modeling (FDM), selective laser sintering (SLS), inkjet printing, and extrusion printing.[21–23] 3D printing focuses on engineering technology, mainly for structural design, material selection, and engineering manufacturing. 3D bioprinting introduces concepts of developmental biology, tissue engineering, and regenerative medicine into 3D printing. 3D bioprinting enables precise control over multiple compositions, spatial distributions, and architectural accuracy and complexity, therefore achieving effective recapitulation of microstructure, architecture,

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mechanical properties, and biological functions of target tissues and organs.[24–27] 3D bioprinting offers precise spatiotemporal control on the placement of cells, proteins, DNA, drugs, growth factors, and other bioactive substances to better guide tissue formation for patient-specific therapy.[13]

Bioink

Print speed, print pressure, and moving distance directly affect the cell viability during 3D bioprinting. Researchers mixed the cells with bioink in order to maintain their viability.[28] The main role of bioink is to load cells and provide them with an external support environment similar to the extracellular matrix during printing.[29] It is important to choose bioink suitable for bioprinting, with good mechanical, biodegradable, and biocompatible properties. There are three parameters of bioink: viscosity, surface tension, and cross-linking properties. These parameters have a major impact on print accuracy and cell loading capacity. Take viscosity, for example; the polymer solution has a higher viscosity with poor fluidity, so that the shape structure can be maintained for a long time after printing, and the cell-binding property is excellent. However, high-viscosity solutions also require higher pressure but have a negative impact on cell activity.[30–32]

Materials that can be used as bioink include natural materials and synthetic materials. Natural materials include sodium alginate, silk fibroin, chitosan, and collagen.[33–36] The synthetic materials include polycaprolactone (PCL), polyethylene (poly(ethylene glycol) [PEG]), and hydroxyapatite (HA).[37–39] This section aims to highlight the achievement in 3D bioprinting by using different kinds of materials.

Collagen

Collagen is the most abundant and ubiquitous structural protein in the body, and it may be readily purified from both animal and human tissues. Collagen implants degrade through a sequential attack by lysosomal enzymes. Collagen contains cell adhesion domain sequences (e.g., RGD) that exhibit specific cellular interactions. Collagen is also used as a natural biomaterial in 3D bioprinting. However, collagen is prone to being temperature sensitive and degrades during most sterilization processes. It is better for collagen scaffolds to be cross-linked or combined with other materials.[40,41] Yeo et al. developed a new cell printing process supplemented with a core-sheath nozzle and an aerosol cross-linking method, to obtain a multilayered cell-laden mesh structure and a newly considered collagen-based cell-laden bioink. They used collagen bioink in the core region, and also used pure alginate in the sheath region to protect the cells in the collagen during the printing and cross-linking process. The 3D cell-laden mesh structure is supported by using a general alginate-based cell printing process, which showed significantly higher cell viability (92 $\pm$ 3%) compared with that (83 $\pm$ 4%) of the control. The results indicated that the selection of collagen bioink and the new printing strategy could lead to an efficient way to achieve 3D cell-laden mesh structures that mimic the anatomical architecture of a patient's defective region.[42] Akkineni et al. demonstrated that it is mechanically stable and robust for 3D scaffolds by combining high concentrated (16.7 wt%) alginate hydrogels as shell material with low concentrated, soft biopolymer hydrogels as the core, such as collagen, alginate, chitosan, gellan gum, and gelatin hydrogels.[43] Koch et al. investigated whether laser-assisted bioprinting (LaBP) was suitable for functional tissue substitutes in ex vivo engineering. They printed fibroblasts and keratinocytes embedded in collagen in 3D as an example for skin tissue.[44] Duarte Campos et al. showed that the conjugation of type I collagen to agarose with different ratios might be a suitable bioink for 3D-printed mesenchymal tissues.[45] Recently, Sorkio et al. produced 3D corneal tissue mimicking structures using laser-assisted 3D bioprinting and functional bioinks, which contained the recombinant human laminin and human-sourced collagen I.[46] Yang et al. used collagen type I or agarose mixed with sodium alginate to serve as 3D bioprinting bioinks and incorporated chondrocytes to construct in vitro 3D-printed cartilage tissue.[47] Although collagen has good biocompatibility, it is difficult for it to form a cell-loaded bioink with appropriate viscosity. Besides, collagen has low strength and is very sensitive to metalloproteinases. Further studies are needed for collagen-based composite bioink.

Gelatin

As a mixture of peptides and proteins produced by partial hydrolysis of collagen, gelatin has the characteristics of good biocompatibility, high water absorption, and low immunogenicity.[48] Yan et al. used a gelatin and chitosan composite system to mix and suspend liver cells, and then generated an active liver tissue constructed by 3D bioprinting and cross-linked by glutaraldehyde. They showed that the hydrogel composed of gelatin and chitosan has low mechanical strength and is easy to collapse. Although the shape of the hydrogel system is significantly improved after being cross-linked with glutaraldehyde, and the morphology and porosity of the scaffold remain intact, the use of glutaraldehyde reduces the biocompatibility of the system.[49] For this reason, pure gelatin is commonly used as a sacrificial material in 3D bioprinting. That is, gelatin gradually dissolves in the medium and forms a channel in the 3D scaffold during the culture to facilitate the transmission of oxygen and nutrients, thereby promoting cell survival, proliferation, and even differentiation.[50] In order to preserve the biocompatibility of gelatin and improve the mechanical strength, researchers have made several efforts to modify

and UV-cross-link gelatin.[51] Schuurman et al. found that gelatin-methacrylamide (gelMA) hydrogels were shown to support chondrocyte viability and differentiation and provided various mechanical properties depending on several cross-linking parameters. Polymer concentration, UV exposure time, and thermal gelation prior to UV exposure allow for control over hydrogel stiffness and swelling properties.[52] Skardal et al. synthesized the methacrylated ethanol amide derivative of gelatin (GE-MA), and the bioink made by partial photochemical co-cross-linking of GE-MA with methacrylated hyaluronic acid (HA-MA) was biocompatible, supporting cell attachment and proliferation of HepG2 C3A, Int-407, and NIH 3T3 cells in vitro.[53]

Alginate

Alginate, a polysaccharide isolated from seaweed, has also been commonly used in 3D bioprinting because of its gentle gelling properties in the presence of divalent ions such as calcium. Alginate is relatively biocompatible and is approved by the U.S. Food and Drug Administration (FDA) for human use as wound dressing materials.[54] Khalil and Sun generated tissue constructs by using endothelial cells and alginate via 3D bioprinting. Cell viability studies were conducted on the cell-encapsulated scaffolds for validating the bioprinting process and determining a cell viability of 83%.[55] Dolati et al. demonstrated a new practical technique for vasculature fabrication, where microvascular conduits were directly printed using a coaxial nozzle configuration. Vascular conduits based on alginate in this work were reinforced with carbon nanotubes (CNTs) to improve the mechanical properties.[56] In order to demonstrate the potential of laser printing as an effective bioprinting technique, Xiong et al. printed both straight and Y-shaped tubes using two different bioinks: 8% alginate solution and 2% alginate-based mouse fibroblast suspension. It has been demonstrated that the postprinting cell viabilities are above 60% immediately after printing as well as after 24 h of incubation for printed straight and Y-shaped fibroblast tubes.[57] Although calcium ions can rapidly cross-link alginic acid scaffolds, they are easily replaced with sodium ions in physiological environments, resulting in the degradation of scaffolds and subsequent deterioration of mechanical properties. The survival rate of the cells can be maintained only when the concentration of sodium alginate is low. At this time, the mechanical strength of the hydrogel is poor. In addition, cells are difficult to interact, proliferate, or even differentiate within the material because pure sodium alginate scaffolds lack good hydrophilic characteristics. Therefore, modification of sodium alginate, such as oxidation of sodium alginate or modification by RGD peptides and collagen, to improve cell adhesion and proliferation on sodium alginate scaffolds, has become the current trend of 3D bioprinting with sodium alginate.[58] Most recently, Ning et al. presented their study on bioprinting Schwann cell-encapsulated scaffolds using composite hydrogels of alginate, fibrin, hyaluronic acid, and/or RGD peptide for nerve tissue engineering applications. The data showed that the printed scaffolds can promote the alignment of Schwann cells inside scaffolds and thus provide haptotactic cues to direct the extension of dorsal root ganglion neurites along the printed strands, demonstrating their great potential for applications in nerve tissue engineering.[59]

Other Biomaterials

There have been many other natural as well as synthetic biomaterials applied in 3D bioprinting. Murphy et al. evaluated the characteristics of 12 hydrogels to determine their suitability for bioprinting applications. They found that many of the hydrogels screened may exhibit characteristics suitable for other applications. For instance, UV-cross-linked Extracel, a hyaluronic acid-based hydrogel, had many of the desired properties for bioprinting application.[60] Kesti et al. printed high-resolution scaffolds with good viability by blending the thermoresponsive polymer poly(*N*-isopropylacrylamide)-grafted hyaluronan (HA-pNIPAAM) with methacrylated hyaluronan (HAMA). They showed that HA-pNIPAAM can be used to support the extrusion of a range of biopolymers that undergo tandem gelation, thereby facilitating the printing of cell-laden and stratified cartilage constructs with zonally varying composition and stiffness.[61] Other biomaterials, such as silk fibroin, agarose, and gellan gum, are also used by researchers to prepare bioink.[62–64] Schacht et al. evaluated the potential of recombinant spider silk proteins as a new bioink system. The results demonstrated that cells were able to adhere and proliferate with good viability over at least 1 week in such spider silk scaffolds. The introduction of a cell-binding motif to the spider silk protein further enables fine-tuned control over cell–material interactions.[65]

It is often difficult for bioink composed of a single material to meet the requirements of tissue and organ 3D printing in terms of printability, cross-link ability, mechanical properties, and interaction with cells. Therefore, in order to meet more requirements, the construction of composite bioink is one of the important tasks of 3D bioprinting.

Application of 3D Bioprinting

3D bioprinting is an additive manufacturing technology that accurately distributes bioinks in living cells and generates complex artificial tissues and organs in computer-aided design.[66] So far, researchers have made much progress in 3D bioprinting of skin, heart valves, blood vessels, bone, cardiac tissue, and so forth. The advantage of this technology is that it can simultaneously print different types of cells in the corresponding spatial position and add suitable biomaterials

according to different tissue/organ characteristics to prepare artificial tissues/organs with similar strength, elasticity, and biological functions. 3D bioprinting has profound impacts on biomedical research and industry.[13] Potential future applications of 3D bioprinting include tissue/organ regeneration and in vitro experimental models.[67]

Skin

For printing the skin, the 3D bioprinting process must take into account the precise cell localization and the interactions between cell–cell and cell–matrix. Collagen type I, fibrin, and artificial acellular allogeneic dermis are commonly used as scaffolds in skin tissue engineering. Keratinocytes, fibroblasts, and stem cells are the main cell types for skin printing.[68] Lee et al. fabricated human skin by 3D bioprinting. Keratinocytes and fibroblasts were used as constituent cells to represent the epidermis and dermis, and collagen was used to represent the dermal matrix of the skin. The results showed that 3D-printed skin tissue was morphologically and biologically representative of in vivo human skin tissue. In comparison with traditional methods for skin engineering, 3D bioprinting offers several advantages in terms of shape and form retention, flexibility, reproducibility, and high culture throughput.[69] Recently, Min et al. reported a 3D bioprinting technique capable of producing a full-thickness skin model containing pigmentation. Multiple layers of fibroblast-containing collagen hydrogel precursor were printed and cross-linked through neutralization using sodium bicarbonate to constitute the dermal layer. Melanocytes and keratinocytes were sequentially printed on top of the dermal layer to induce skin pigmentation upon subsequent air–liquid interface culture.[70] Ng et al. also fabricated 3D pigmented human skin constructs using a 3D bioprinting approach. The 3D pigmented human skin constructs are obtained from three different types of skin cells (keratinocytes, melanocytes, and fibroblasts) derived from different skin donors, and they exhibit similar constitutive pigmentation (pale pigmentation) as the skin donors.[71] Although 3D bioprinting shows potential in engineering skin, it still remains to be explored. There are hurdles that need to be overcome, such as the resolution, vascularity, optimal cell and scaffold combinations, and cost of bioprinted skin. Small-scale 3D skin tissue models for the toxicity testing of cosmetics and drugs, as well as tumor modeling, are likely to be applied first, before this technology is used in reconstructive surgery.[72]

Heart Valve

Heart valve disease is an increasingly prevalent clinical condition. There is no clinically effective treatment. The only way to treat it is to replace the heart valve with a prosthetic one. However, these devices are unable to grow or respond biologically to their environments, thus leading to multiple resizing surgeries and lifelong coagulation treatment, especially in children.[73,74] 3D bioprinting is a better choice for heart valve disease because it provides a living valve conduit capable of growth and biological integration. Although the geometry of the heart valve is relatively special, one can be individually designed via 3D bioprinting technology by taking the complexity of the valve's microstructure into account to meet the biomechanical and hemodynamic requirements.[75] Hockaday et al. presented a novel simultaneous 3D printing/photo-cross-linking technique for engineering complex and heterogeneous aortic valve scaffolds. Native anatomic and axisymmetric aortic valve geometries were 3D printed with poly(ethylene glycol)-diacrylate (PEG-DA) hydrogels supplemented with alginate. Porcine aortic valve interstitial cell-seeded scaffolds maintained nearly 100% viability over 21 days, demonstrating that 3D hydrogel printing with controlled photo-cross-linking can rapidly fabricate anatomical heterogeneous valve conduits that support cell engraftment.[76] Duan et al. implemented 3D bioprinting to fabricate living alginate/gelatin hydrogel valve conduits with anatomical architecture and direct incorporation of dual cell types in a regionally constrained manner. Encapsulated aortic root sinus smooth muscle cells and aortic valve leaflet interstitial cells were viable within alginate/gelatin hydrogel discs over 7 days in culture. A cellular 3D-printed hydrogel exhibited a slightly reduced modulus, ultimate strength, and peak strain over the 7-day culture, while the tensile biomechanics of cell-laden hydrogels were maintained.[77]

Blood Vessels

The creation of vascularized tissue constructs has remained a principal challenge to date in the field of tissue engineering. However, given the myriad advantages over other biofabrication methods, it is expected that bioprinting can provide a viable solution for the vascularization problem and facilitate the clinical translation of tissue-engineered constructs.[78,79] Wu et al. showed that 3D biomimetic microvascular networks of nearly arbitrary design were patterned by omnidirectional printing of a fugitive organic ink into a photopolymerizable hydrogel matrix. Pluronic F127 was used as a representative sacrificial material for fabricating complex 3D constructs.[80] Miller et al. printed rigid 3D filament networks of carbohydrate glass and used them as a cytocompatible template in engineered tissues containing living cells to generate cylindrical networks that could be lined with endothelial cells and perfused with blood under high-pressure pulsatile flow. They also demonstrated that the perfused vascular channels sustained the metabolic function of primary rat hepatocytes in engineered tissue constructs, which otherwise exhibited suppressed function in their core.[81] Lee et al. developed a methodology using 3D bioprinting

technology to create a functional vascular channel in vitro with perfused open lumen using only cells and biological matrices. The fabricated vasculature has a tight and confluent endothelium lining, presenting barrier function for both plasma protein and high-molecular-weight dextran molecules.[82] Jia et al. reported the development of a versatile 3D bioprinting strategy that employed biomimetic biomaterials and an advanced extrusion system to deposit perfused vascular structures with highly ordered arrangements in a single-step process by using a specially designed cell-responsive bioink consisting of gelatin methacryloyl (GelMA), sodium alginate, and four-arm poly(ethylene glycol)-tetra-acrylate (PEGTA) in combination with a multilayered coaxial extrusion system to achieve direct 3D bioprinting.[83] Although much progress has recently been made in building perfusable tissues and branched vascular networks, generating perfusable hierarchical vascular networks is still a major challenge.[84,85] 3D bioprinting for vascularized tissue fabrication would require a high-throughput and high-resolution bioprinter capable of dispensing pro-vasculogenic bioinks to fabricate functional vasculature, ranging from capillaries to larger vessels within a tissue construct.[86]

Bone

In human tissues, bone tissue has the strongest mechanical properties. All biomaterials used for bone tissue engineering should be high-concentration and high-viscosity materials such as PCL, polylactic acid, polyglycolic acid, biphasic calcium phosphate (BCP), tricalcium phosphate, or combinations of these hydrogels. For the same reason, pressure-assisted printing technology is often chosen as the manufacturing method.[87,88] In addition, osteoblasts and mesenchymal stem cells (MSCs) could be embedded in the scaffold or seeded on the surface of the scaffold during printing to promote bone formation. Besides, growth factors such as bone morphogenetic protein (BMP) and vascular endothelial growth factor are often mixed with the scaffold material to enhance bone formation and angiogenesis.[89] Strobel et al. generated novel BCP matrices by 3D printing and characterized the porous BCP-scaffold properties and interactions of osteogenic cells and growth factors in vivo. Results showed that a combination of osteoblasts and BMP-2 synergistically enhanced bone formation in novel ceramic scaffolds.[90] Different cell types could be included in the printing process to improve the functionality of the bioprinted structures. Fedorovich et al. demonstrated the ability of the system to print intricate porous constructs containing two different cell types (endothelial progenitors and multipotent stromal cells) and showed that these grafts retained heterogeneous cell organization after subcutaneous implantation in immune-deficient mice. They found that cell differentiation leading to the expected tissue formation occurs at the site of the deposited progenitor cell type. While perfused blood vessels were formed in the endothelial progenitor cell-laden part of the constructs, bone formation occurred in the multipotent stromal cell-laden part of the printed grafts.[91]

Cardiac Tissue

Heart failure, especially myocardial infarction (MI), is one of the main causes of death in patients with heart disease.[92] Heart transplantation is a therapeutic option but limited by the lack of donor organs. Therefore, it is desirable to develop alternative strategies to repair MI to ameliorate the prognosis and life quality of patients. The emerging field of tissue engineering may offer promising alternatives. The ultimate goal in cardiac tissue engineering is to generate biocompatible and nonimmunogenic heart with morphological and functional properties of natural myocardium.[93] Recent advances in the 3D bioprinting strategy have shown its promise as a viable option for creating functional cardiac tissue constructs that are designed to regenerate or replace damaged tissues.[94] Gaetani et al. evaluated the therapeutic potential of a 3D-printed patch composed of human cardiac-derived progenitor cells (hCMPCs) in a hyaluronic acid/gelatin (HA/gel)-based matrix. They showed that the application of the patch led to a significant reduction in adverse remodeling and preservation of cardiac performance. Furthermore, the matrix supported the long-term survival and engraftment of hCMPCs in vivo, which exhibited a temporal increase in cardiac and vascular differentiation markers over the 4 weeks of follow-up.[95] To improve the contractility of generated cardiac tissue, Wang et al. designed a simple aligned cardiac structure composed of cardiomyocyte-laden hydrogel and a supporting PCL polymer frame that allowed bioprinted cardiac tissues to develop into dense and universally aligned cardiac muscle bundles with synchronous contraction.[96] Ideally, cardiac patches should be electrically conductive, mechanically robust and elastic, biologically active, and prevascularized. Most recently, Izadifar et al. fabricated a nano-reinforced hybrid cardiac patch laden with human coronary artery endothelial cells (HCAECs) with improved electrical, mechanical, and biological behavior. The study showed that the carboxyl functionalized CNTs provided a highly interconnected nanofibrous meshwork that significantly improved viscoelastic behavior and electrical conductivity of photo-cross-linked methacrylated collagen (MeCol). HCAECs presented significant cellular proliferation, migration, and differentiation (lumen-like formation) over 10 days of incubation in vitro.[97]

3D Bioprinting of Functional Tissue Models

3D bioprinting has enabled researchers to precisely position materials and cells to build functional tissue models for

drug screening and disease modeling in vitro, which hold great potential for the applications in medical research, drug discovery, toxicology, and other preclinical studies.[98] Massa et al. reported the development of a 3D vascularized liver tissue model to study drug toxicity through the incorporation of an engineered endothelial layer. Using a sacrificial bioprinting technique, a hollow microchannel was successfully fabricated in the 3D liver tissue constructs created with HepG2/C3A cells encapsulated in a GelMA hydrogel. After seeding human umbilical vein endothelial cells (HUVECs) into the microchannel, vascularized tissue constructs containing a uniformly coated HUVEC layer within the hollow microchannel were obtained.[99]

For many decades, cancer researchers have relied on studying the histopathology of tumors in the hope that it would provide clues to understanding the pathophysiology of cancer. Current preclinical research relies heavily on two-dimensional (2D) culture models. However, these models have had limited success in recreating the complex interactions between cancer cells and the environment in vivo. Thus, there is an increasing need to shift to 3D models, which more accurately reflect the physiological condition. With the more accurate in vitro tumor model, drug sensitivity could be tested to determine the best treatment option based on the tumor characteristics.[100] Recently, 3D bioprinting was used to create in vitro tumor models. Zhao et al. reported a method of 3D printing for Hela cells and gelatin/alginate/fibrinogen hydrogels to construct in vitro cervical tumor models. The results showed that Hela cells in 3D-printed models showed higher matrix metalloproteinase protein expression and higher chemoresistance than those in 2D culture.[101] Zhou et al. developed a biomimetic bone matrix using 3D bioprinting technology to investigate the interaction between breast cancer (BrCa) cells and bone stromal cells. When BrCa cells were introduced into the stromal cell-laden bioprinted matrices, the growth of BrCa cells was enhanced by the presence of osteoblasts or MSCs, whereas the proliferation of the osteoblasts or MSCs was inhibited by the BrCa cells. The results indicated that the 3D-bioprinted matrix with BrCa cells and bone stromal cells provided a suitable model with which to study the interactive effects of cells in the context of an artificial bone microenvironment, and thus may serve as a valuable tool for the investigation of postmetastatic breast cancer progression in bone.[102]

Conclusions

Compared with other in vitro construction techniques of tissue engineering, 3D bioprinting technology has great advantages with high precision and fast construction speed. Despite 3D bioprinting having developed rapidly in recent years, it still faces significant challenges, such as biomechanical control, selection of scaffold materials, assurance of aseptic environments, formation of printed constructs, blood supply, nutrient transport, and long-term survival of printed constructs. These challenges cause complications not for 3D bioprinting technology itself but for the materials.

Currently, only a few studies have involved the effects of bionic components and structures on cell behavior. In addition to carrying cells, bioinks have important implications for cell adhesion, migration, proliferation, and so on. Surface structures such as protrusions and grooves formed inside the bioink hydrogel could regulate cell behavior. In a word, the study needs to be improved for the understanding of the matrix environment in various tissues. In addition, one of the main issues of an in vitro culture system for 3D bioprinting is building a suitable tissue culture device to fully simulate the physiological state of cells in tissues and organs in vivo, so that the cells could redistribute on their own.

It is important to overcome these challenges and further our knowledge in every aspect of 3D bioprinting. Much more work needs to be done by multidisciplinary collaborations between biologists, bioengineers, and physicians in order to further apply 3D bioprinting in regenerative medicine and drug discovery.

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