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Advancing organoid development with 3D bioprinting

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Abstract

Organoids provide more realistic *in vitro* models that closely mimic their corresponding tissues or organs. Three-dimensional (3D) bioprinting allows for precise control over cell distribution, arrangement, and the regulation of cell behavior and interactions, offering flexibility and repeatability. By combining 3D bioprinting with organoid culture, optimal extracellular matrix conditions can be created, better replicating the complex interactions between cells and their environment. The integration of 3D bioprinting amplifies the scalability of organoids, facilitating the development of large-scale organ models that significantly enhance research capabilities. This review explores the crucial role of 3D bioprinting in organoid development, emphasizing its contributions to improving organoid structure and functionality. We discuss how this innovative approach supports the creation of well-defined physiological microenvironments, aids in the development of functional organoids, and enables high-throughput cultivation. Furthermore, we highlight the advantages of optimizing bioprinting strategies and bioinks to advance organoid applications. In addition, we examine the importance of refining 3D bioprinting devices to enhance organoid fabrication and application. Finally, we outline strategies for leveraging 3D bioprinting to further advance organoid research and its implications for regenerative medicine and disease modeling.

Keywords: 3D bioprinting; Organoid; Bioinks; Stem cells

1. Introduction

Over the past decade, organoids have gained significant attention in stem cell research and biomedical sciences. Organoids are three-dimensional (3D) multicellular aggregates derived from stem cells or organ-specific progenitor cells, which can differentiate and self-organize to replicate specific structures and functions of corresponding organs.[1,2] In 2009, Hans Clevers reported the first small intestinal organoid derived from leucine-rich repeat-containing G-protein coupled receptor 5-positive intestinal stem cells. This work revealed cell composition and structural characteristics similar to those of the natural small intestine.[3] Since then, various organoids, including liver, stomach, kidney, brain, and heart organoids, have been developed.[4-9] Due to their similarity to real tissues and the aggregation and interaction of multiple organ-specific cells, organoids possess broad biomedical application prospects and have been studied for drug screening, disease modeling, tissue development research, and tissue regeneration.[10-12]

Given their importance as biomedical research tools, promoting the development and application of organoids is essential. 3D bioprinting is a promising fabrication technology that has received increasing attention in

biomedical research and applications.[13-15] 3D bioprinting involves printing bioink containing living cells to form a 3D construct according to a preset structure.[16] The key elements of 3D bioprinting include the printing equipment, printing models, and bioink.[17] Printing devices are primarily divided into extrusion 3D printing, droplet 3D printing, and stereolithography.[18] Extrusion 3D bioprinting is the process of continuously micro-extruding or microfluidic extrusion of bioink, followed by layer-by-layer printing to form constructs.[19] This technology is easy to operate and has a wide range of applications, but its resolution is relatively low. Droplet 3D printing involves generating droplets of bioink for printing, offering fast printing speed and high resolution, but it requires high precision in the characteristics of the bioink.[20] Stereolithography utilizes photosensitive materials for light curing and layer-by-layer stacking, offering high printing accuracy and structural diversity.[21] Different printing devices are selected based on the biomaterials and structures to be printed. Meanwhile, bioink primarily consists of biomaterials, active factors, and live cells, and its composition design is crucial for the formation and development of 3D constructs. Given the variety of available printing conditions, 3D bioprinting offers flexibility and stability.

Given its ability to form multicellular 3D constructs and the flexibility and designability of printed structures, 3D bioprinting offers significant potential for organoid research.[22,23] First, 3D bioprinting serves as an effective method for the formation and high-throughput cultivation of organoids, while also enabling the development of 3D growth platforms for organoids.[24] Notably, by adjusting the printing parameters, it is possible to regulate the migration, differentiation, function, and other behaviors of cells, which is important for organoid development.[25] In addition, 3D bioprinting allows the distribution of stem cells forming organoids according to a predetermined structure, thereby facilitating the formation of specific structures within the organoids.[26] Moreover, 3D bioprinting holds the potential to manufacture large tissue structures by printing organoids, further expanding their applications.[19] In turn, the cultivation platform created by 3D bioprinting enhances the versatility of organoid applications, including drug screening, disease research, and multiorgan interaction studies.[27-29]

In this review, we explore recent advancements in the development and application of organoids combined with 3D bioprinting (Figure 1). We summarize the role of 3D bioprinting in developing and culturing various organoids, particularly those of the internal tissues/organs, musculoskeletal system, and tumors. Furthermore, we highlight the advantages of 3D bioprinting in enhancing organoid development and application, underscoring the complementary relationship between organoids and 3D bioprinting. We also discuss how 3D bioprinting can be applied to promote the development of miniature organoids into larger tissue structures. Finally, we summarize and envision future research strategies that combine 3D bioprinting for organoid development, considering the future prospects for this innovative approach.

2. Data retrieval strategy

The relevant data for this review were obtained by searching PubMed and X-MOL. The main search keywords included "organoids," "3D bioprinting," "organoid structures," "organoid development," "large-scale organ models," "high-throughput culture," "organoid vascularisation," "organoid applications," "organoid monitoring," "tissue regeneration," and "disease modeling." Only papers published in English between 2009 and 2024 were selected.

3. Merging organoids with three-dimensional bioprinting

Three-dimensional bioprinting is an additive manufacturing process that enables the formation of 3D structures by printing living cells and other bioactive materials based on designed models.[18] The addition of biological factors and the regulation of printing parameters can optimize these functions. In recent years, 3D bioprinting has evolved into an effective technology for

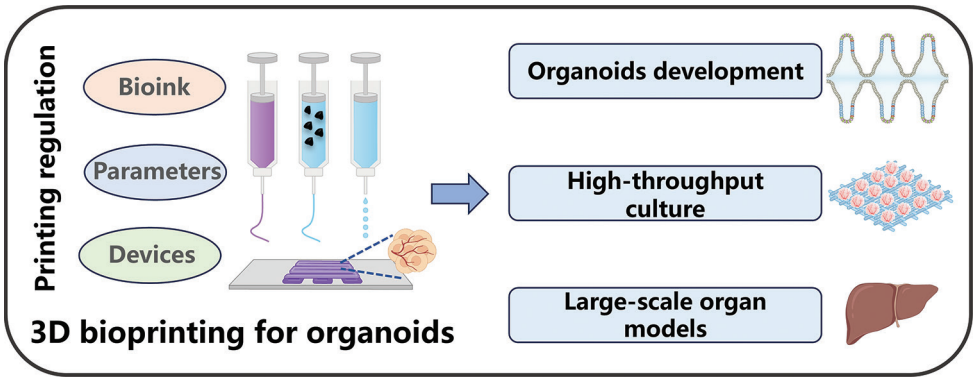


Figure 1. Overview of the development and application of organoids in combination with three-dimensional (3D) bioprinting.

3D cell culture. It allows for precise control over cells in specific microenvironments and holds promise for creating ideal organoid development models and large tissue structures.[30] In organoid development, 3D printing serves as a powerful tool, providing an optimal microenvironment for organoid production and facilitating high-throughput cultivation. Furthermore, adjusting parameters such as bioink composition and printing structure can further promote the development of organoids. In this section, we summarize the close relationship between 3D bioprinting and organoids, focusing on the generation of various organoid types and emphasizing the role of 3D bioprinting in organoid development.

3.1. Organoids derived from internal tissues/organs

3.1.1. Intestinal organoids

The intestine is a structurally complex tissue in the human body, responsible for functions such as digestion, absorption, and excretion.[31,32] The development of intestinal organoids provides a reliable platform for understanding intestinal development and disease mechanisms. Intestinal tissue has a typical crypt-villus structure. Intestinal stem cells reside in crypts and support the self-renewal of the intestinal epithelium. This crypt-villus structure not only protects the intestinal epithelium but also serves as the primary site for nutrient absorption. By regulating the structural parameters and cell density in 3D bioprinting, a large intestine with structural characteristics can be developed. For example, Brassard *et al.*[33] used organoid stem cells as a component of bioink. These cells function as building blocks, aggregating and arranging in space to form specific cellular structures (Figure 2). The generated tissue exhibited centimetre-scale dimensions and featured a lumen, epithelial cells, and crypt-villus structure, resembling that of the intestine. This study combined 3D biomanufacturing with organoids, enabling the development of organoids in various dimensions and providing strategies for developing engineered organoids.

3.1.2. Liver organoids

Recently, an increasing number of studies have utilized liver organoids to explore liver diseases and address liver regeneration challenges. The further development of liver organoids has capitalized on this trend.[34-36] Notably, the integration of 3D bioprinting has further optimized the development of liver organoids. Yang *et al.*[37] developed a 3D bioprinted liver organoid using HepaRG cells and bioink, following a predetermined printing program. After a period of *in vitro* differentiation, the 3D bioprinted liver organoid exhibited certain liver functions, including albumin secretion, drug metabolism, and glycogen storage. After *in vivo* transplantation, the 3D bioprinted liver organoid demonstrated ideal liver function, potentially providing a suitable donor for liver transplantation. Furthermore, one study developed optimized bioprinting materials and incorporated primary stem cells to create artificial livers.[38] After *in vitro* cultivation, the artificial liver exhibited specific liver functions. Notable, when transplanted into mice with 90% liver resection, the artificial liver successfully connected with the host, aiding liver function recovery and reducing liver damage. In addition, 3D bioprinting technology has the potential to overcome the limitations of conventional organoid culture methods. One study utilized microarray 3D bioprinting to generate a large number of small liver organoids on column plates.[39] The organoids produced by this method not only exhibited superior liver function but also demonstrated significant advantages for large-scale production.

3.1.3. Kidney organoids

The combination of 3D bioprinting has a positive impact on the development of kidney organoids. 3D bioprinting allows for the rapid and high-throughput production of kidney organoids with normal cell viability.[40] These organoids are expected to serve as a model for drug testing. This study demonstrates that 3D bioprinting can control the quality of organoids, including their size and cell count, thus providing a strategy for manufacturing kidney tissue slices with functional proximal tubular segments. In addition, Lin *et al.*[41] designed a 3D proximal renal tubular model, which exhibits albumin and glucose reabsorption through tubular-vascular exchange. Furthermore, this model can be used to investigate the crosstalk between renal tubular epithelium and vascular endothelium under high blood glucose conditions. This study utilized 3D bioprinting to develop a vascularized renal tubular model, providing a platform for studying kidney function and pathology.

3.1.4. Cardiac organoids

A major challenge in the large-scale development of cardiac organoids is promoting the transport of internal nutrients and oxygen to maintain cell vitality.[42] A more effective strategy is to develop an internal vascular network. Fang *et al.*[43] encapsulated human induced pluripotent stem cells in biphasic bioink and printed them to form a cardiac organoid. In this process, sacrificial ink was printed into the uncrosslinked structure to generate a natural tubular vascular network, which was later removed after *in situ* crosslinking. Through 3D bioprinting, perfusable vascular structures can be formed in cardiac organoids, promoting their development. Moreover, 3D bioprinting provides an excellent technology for the development of engineered heart tissue from cardiac organoids. A study designed an endothelialized microfiber scaffold using 3D bioprinting, where the structure and size of the microfibers were precisely controlled to guide the formation of myocardial

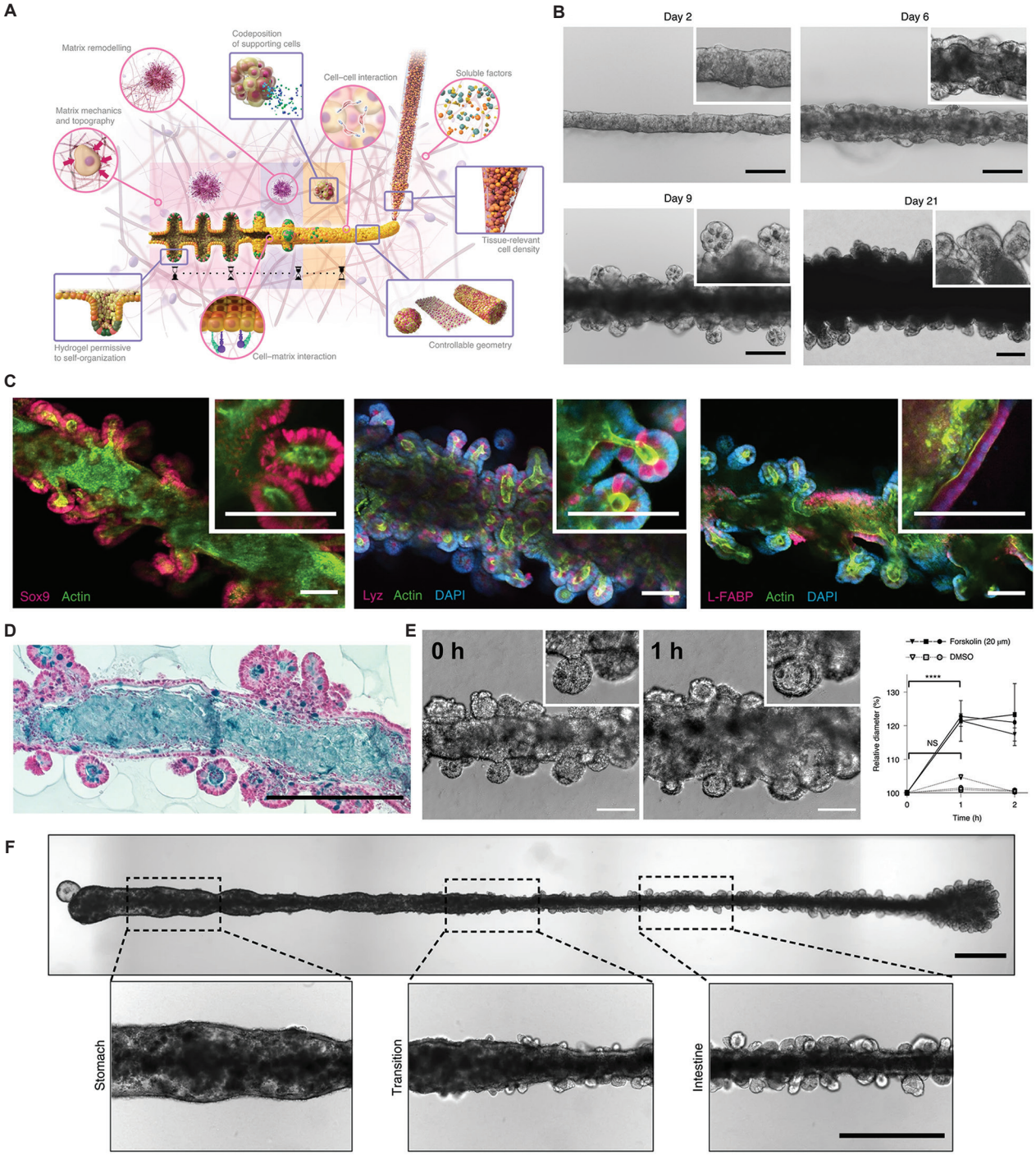


Figure 2. Intestinal tube bioprinting. (A) Schematic diagram of intestinal tube formation. (B) Bright-field image of intestinal development. Scale bar, 200 μm. (C) Stained images of stem cells and progenitor cells (labeled with Sox9), Paneth cells (labeled with Lyz), and intestinal epithelial cells (labeled with L-FABP). Scale bar, 100 μm. (D) Alcian blue and nuclear solid red staining of intestinal slices show epithelial cells and goblet cells. Scale bar. (E) Changes in intestinal tube diameter over time after treatment with Forskolin. Scale bar, 100 μm. (F) Images of the stomach, transition zone, and intestinal region after co-printing with intestinal stem cells and mouse intestinal mesenchymal cells. Scale bar, 500 μm; magnification. Reprinted from Brassard *et al.*[33] Copyright 2020, with permission from Springer Nature.
Abbreviations: DAPI: 4',6-diamidino-2-phenylindole; Lyz: Lysozyme; L-FABP: Liver-type fatty acid binding protein; Sox9: SRY-box transcription factor 9.

organoids similar to natural myocardium through inoculated myocardial cells.[44] These studies demonstrate the functionality of 3D bioprinting in guiding the generation of vascularized cardiac organoids.

3.2. Organoids from the musculoskeletal system

Bone tissue is a crucial organ in the human body, responsible for various important functions.[45] Due to its inherent structural complexity and diverse cellular composition, developing bone organoids is extremely challenging.[46] However, 3D printing technology can reconstruct these complex structures.[47,48] For example, Zhang *et al.*[49] developed a Haversian-simulated bone structure scaffold, which includes cortical and cancellous bone structures, as well as Haversian and Volkmann tubes. 3D bioprinting integrates cells into specific bone tissue structures, providing a clear direction for the development of bone organoids. A team led by Prof Jiacan Su at Shanghai University mixed inorganic hydroxyapatite with organic polymers to create a bioink, which was printed using digital light processing (DLP)-based 3D bioprinting to create a bone organoid scaffold.[50] This study showed that the resulting construct provides functional mechanical support, supports long-term cell culture, and exhibits self-mineralization ability both *in vitro* and *in vivo*. After *in vivo* cultivation, the organoids further developed and displayed a trabecular bone structure (Figure 3). This study offers a clear strategy for developing bone organoids using 3D bioprinting, influencing the development of bone tissue-related biomedical research. In another study, the hydrogel microspheres encapsulating bone marrow mesenchymal stem cells were prepared in batches through the DLP-based bioprinting technology. These microspheres were cultured in the cartilage-forming medium for 3 weeks, leading to the development of osteo-callus organoids.[51] The *in vitro* development pattern of these osteo-callus organoids resembles natural endochondral ossification. When used to repair large bone defects *in vivo*, the osteo-callus organoids accelerate *in situ* bone regeneration. 3D bioprinting provides a desirable strategy for the preparation of bone-related organoids, which not only simulate the structure and developmental patterns of bone tissue but also facilitate the large-scale production of bone-related organoids.

3.3. Tumor organoids

Tumors have long been a major medical challenge, seriously threatening human life and health. Recent research has shown that tumor organoids derived from patients retain a high fidelity to the original tumors, providing an excellent model for personalized treatment and the study of tumor pathology.[52] However, limitations in tumor organoid development persist, including the lack of important extracellular matrix components, the challenge of simulating unique physiological microenvironments, and the need for high-throughput culture systems. The application of 3D bioprinting aims to address these issues. For example, one study developed a vascularized organoid model composed of patient-derived gastric cancer organoids, endothelial networks, and decellularised gastric extracellular matrix, all constructed via bioprinting.[53] The highly vascularised approach achieved through 3D bioprinting provides a superior simulation of the tumor microenvironment. In addition, the decellularised gastric extracellular matrix provides a suitable microenvironment for gastric cancer organoids, facilitating the study of tumor-extracellular matrix interactions. Similarly, using 3D bioprinting, patient-derived glioblastoma cells, vascular endothelial cells, and brain tissue decellularized extracellular matrix were printed to form a tumor-matrix concentric ring structure.[54] These tumor organoids maintain a radial oxygen gradient, reflecting the structural properties of natural tumors (Figure 4). Importantly, the development of these specific malignant tumor organoids offers great potential for patient treatment. High-throughput culture of tumor organoids is essential for evaluating and developing anti-tumor drugs. At present, Matrigel is the primary matrix material used for tumor organoid culture; however, its limited mechanical properties and variability in tumor models present challenges. 3D bioprinting offers a more suitable microenvironment, enabling repeatable and high-throughput cultivation of tumor organoids. Research has shown that colorectal cancer organoids can grow successfully in composite bioinks, allowing for the generation of large numbers of organoid microspheres for anti-tumor drug screening.[55]

In this section, we have discussed the development of various organoids by 3D bioprinting. The role of 3D bioprinting technology in organoid development is also highlighted. The accuracy and flexibility of 3D bioprinting are crucial in enhancing organoid structures, creating ideal microenvironments for organoid growth, high-throughput cultivation, and enabling the scaling-up of organoids.

4. Harnessing three-dimensional bioprinting for organoid development

Various organoids have been integrated with 3D bioprinting to develop different tissues and organs. The precision and flexibility of 3D bioprinting play an important role in improving organoid structures, creating optimal microenvironments for organoid growth, facilitating high-throughput cultivation, and enabling organoid enlargement. In this section, we review the advantages of 3D bioprinting in organoid development, including the optimization of organoid structure and function, the vascularization of organoids, and strategies for enhancing organoid development and application through 3D bioprinting.

4.1. Optimization of organoid structure and function via three-dimensional bioprinting

One of the key features of organoids is their ability to simulate the structures and functions of real tissues.[56] These

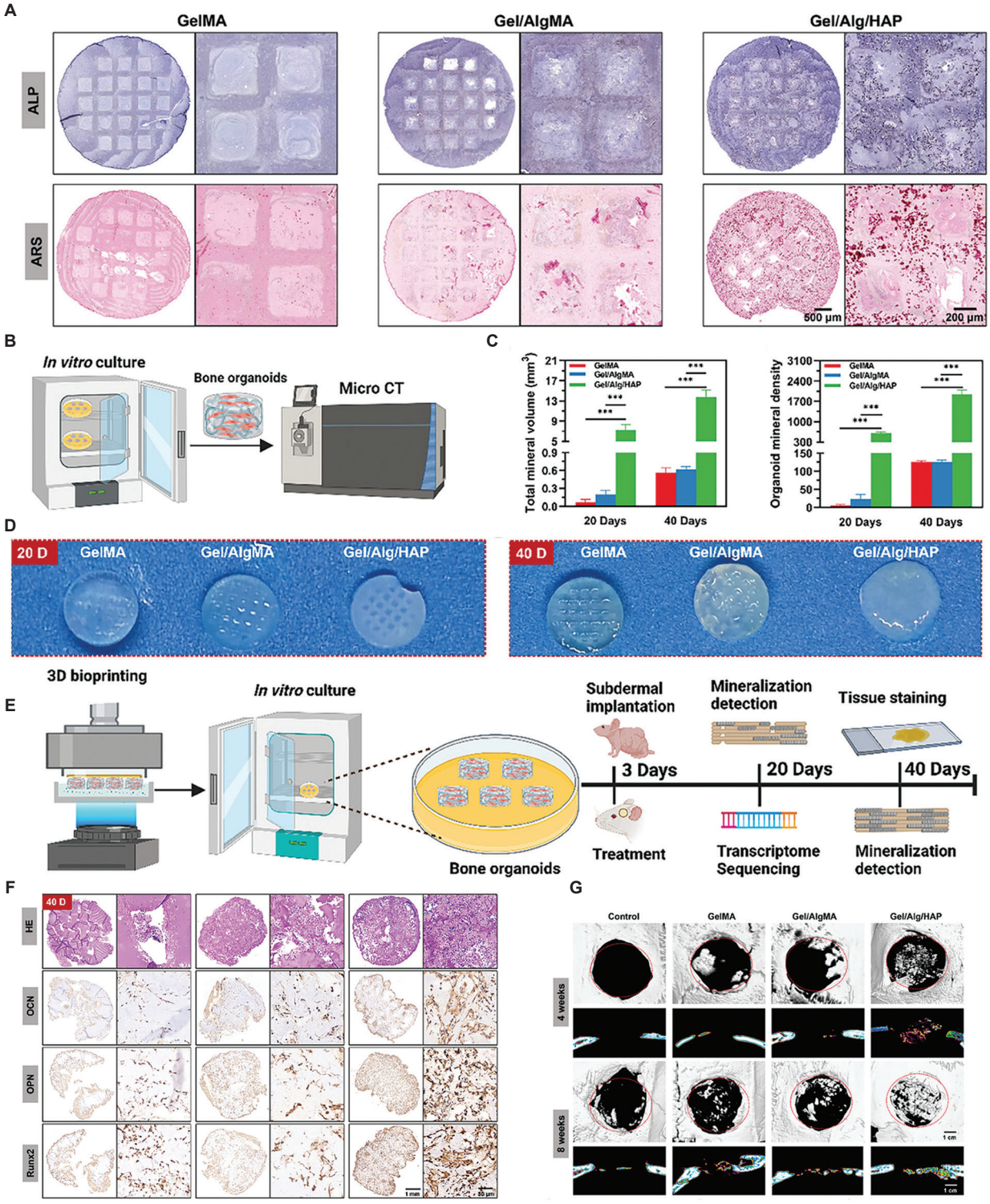


Figure 3. Development of bone organoids by three-dimensional bioprinting. (A) *In vitro* osteogenic ability of optimized bioink. (B) *In vitro* self-mineralization of bioprinted bone organoids. (C) Total mineral volume and mineral density of organoids. (D) Macroscopic images of bone organoids cultured for 20 days and 40 days *in vitro*. (E) Enhanced osteogenic ability of bioprinted bone organoids *in vivo*. (F) Immunohistochemical staining after 40 days of *in vivo* culture. Scale bar, 1mm and 50 μm, respectively. (G) CT reconstruction of repaired rat skull defects after bone organoid implantation. Reprinted from Wang *et al.*[50] Copyright 2024, with permission from Wiley-VCH GmbH.

Abbreviations: Alg: Alginate; AlgMA: Alginate methacryloyl; CT: Computed tomography; GelMA: Photocrosslinked gelatin methacryloyl; HAP: Hydroxyapatite; HE: Hematoxylin and eosin; OCN: Osteocalcin; OPN: Osteopontin; Runx2: Runt-related transcription factor 2.

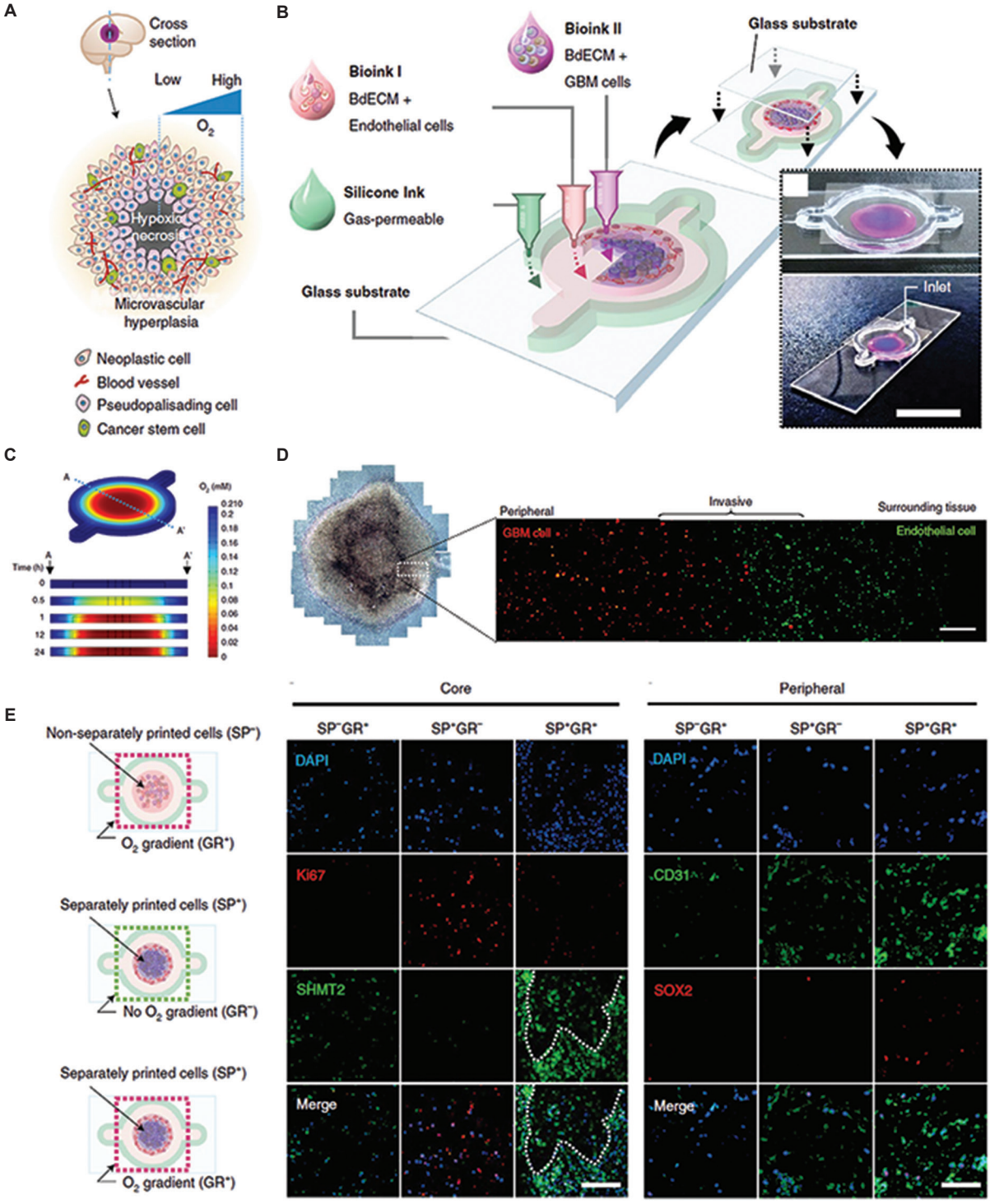


Figure 4. Development of tumor organoids by three-dimensional bioprinting. (A) Schematic diagram of the microenvironment and structure of natural glioblastoma. (B) Schematic diagram of the bioprinting process to form glioblastoma (GBM) and construct partition structures. Scale bar, 2 cm. (C) Color map image of oxygen concentration along cross-section A-A', simulated by a computer, shows the delayed injection. (D) Representative images of GBM-on-a-chip observed through phase contrast optical microscopy (left) and fluorescence microscopy (right). Scale bars, 500 μm. (E) Cell staining images of the core and peripheral regions under different printing conditions show the development of GBM. Scale bars, 200 μm. Reprinted from Yi *et al.*[54] Copyright 2019, with permission from Springer Nature.

Abbreviations: BdECM: Biomimetic decellularized extracellular matrix; DAPI: 4',6-diamidino-2-phenylindole; SHMT2: Human serine hydroxymethyltransferase 2.

structures and functions are typically complex and diverse. 3D bioprinting has been widely studied for creating 3D scaffolds with intricate structures and specific functions, making it a powerful tool for optimizing organoid structure and function. To mimic the complex cellular composition, multiscale spatial structure, and extracellular matrix characteristics of liver tissue, Jian *et al.*[57] optimized the bioink formulation to maintain structural integrity and manufactured liver organoids with biomimetic lobular structures using 3D droplet bioprinting. After seven days of cultivation, the lobular-like structure of the liver organoids remained intact, displaying characteristics of multicellular distribution.

The multicellular printing capability of 3D bioprinting, coupled with the flexibility of printing programs, greatly benefits the development of hierarchical tissue organoids. For instance, a study developed a skin organoid containing human keratinocytes, fibroblasts, and endothelial cells through extrusion 3D bioprinting combined with stepwise photocrosslinking under the influence of a temperature field.[58] This improvement in the preparation process preserved the structural characteristics and mechanical properties of 3D bioprinted skin organoids, which could be customized for wound healing applications.

In addition to optimizing structure, 3D bioprinting has shown significant potential for improving the function of organoids. In a study, a 3D scaffold with an elastic modulus matching that of natural heart tissue was designed to form a cardiac organoid model by printing a mixture of cardiomyocytes, fibroblasts, and microvascular endothelial cells.[59] *In vitro* experiments revealed that this cardiac organoid model exhibited cardiac-specific cellular functions, with myocardial cells displaying contractile activity during cultivation.

4.2. Vascularization of organoids via three-dimensional bioprinting

Vascularization is crucial for the development and application of organoids.[60] Fortunately, 3D bioprinting provides a method for creating organoids with perfusable vascular functions. Skylar-Scott *et al.*[61] assembled stem cell-derived organoids into high-density living matrices and then introduced perfusable vascular channels into them through embedded 3D printing. The resulting cardiac organoids fused and began beating within 7 days (Figure 5). This manufacturing method not only promotes organoid growth but also incorporates vascular channels, which are essential for the functional development of the organoids. Furthermore, a heart organoid model was developed by self-assembling and fusing 3D bioprinted spheres into high-density microstructures.[62] The self-healing properties of hydrogel microspheres allowed them to fuse under controlled conditions to form a stable structure. The developed cardiac organoid, with a controlled ratio of myocardial cells and fibroblasts, was able to simulate the structural and functional characteristics of cardiac tissue after myocardial infarction, including decreased contractility and irregular electrical activity.

The dense microvascular network is also an important feature of the intestine. Li *et al.*[63] printed bioink containing endothelial cells and composite hydrogel precursors to form a 3D scaffold with a macro tubular structure and internal circular folding. They then seeded Caco-2 cells onto the internal structure of the scaffold, and, under the influence of the structure and the maturation of the internal endothelial cells, they developed an intestinal construct with dense microvasculature and an intestinal epithelioid structure. Overall, 3D bioprinting offers an effective approach for the large-scale development of intestinal organoids and is valuable for developing organoids with a more comprehensive intestinal tissue structure.

5. Improving three-dimensional bioprinting technology for organoid development

Three-dimensional bioprinting has demonstrated considerable advantages in organoid development, inspiring researchers to explore better methods for leveraging this technology to advance organoid applications. Consequently, enhancing 3D bioprinting techniques for organoid development has become a key focus of research. This section highlights how advancements in 3D bioprinting strategies can foster the development and application of organoids.

5.1. Developing advanced bioinks

The physical and chemical properties of bioink directly influence the behavior of printed cells or organoids.[64-66] Therefore, an optimized composition of bioinks is essential for advancing organoid development. Wang *et al.*[67] designed a specific bioink composed of pancreatic extracellular matrix and hyaluronic acid methacrylate to create 3D bioprinted islet organoids. This bioink enhances the functional characteristics of pancreatic islets *in vitro*. *In vivo* experiments also confirmed that the hydrogel formed by this bioink regulates insulin levels and promotes angiogenesis, offering a promising therapeutic approach for islet transplantation.

For tumor organoids, bioinks must not only meet the printing performance requirements but also simulate the cell-extracellular matrix interactions to accurately replicate the tumor microenvironment.[68] Consequently, researchers have developed a low-concentration collagen I-based bioink that is compatible with breast tumor cells and cancer-related fibroblasts.[69] This bioink regulates the phenotype and carcinogenic behavior of the cells, and

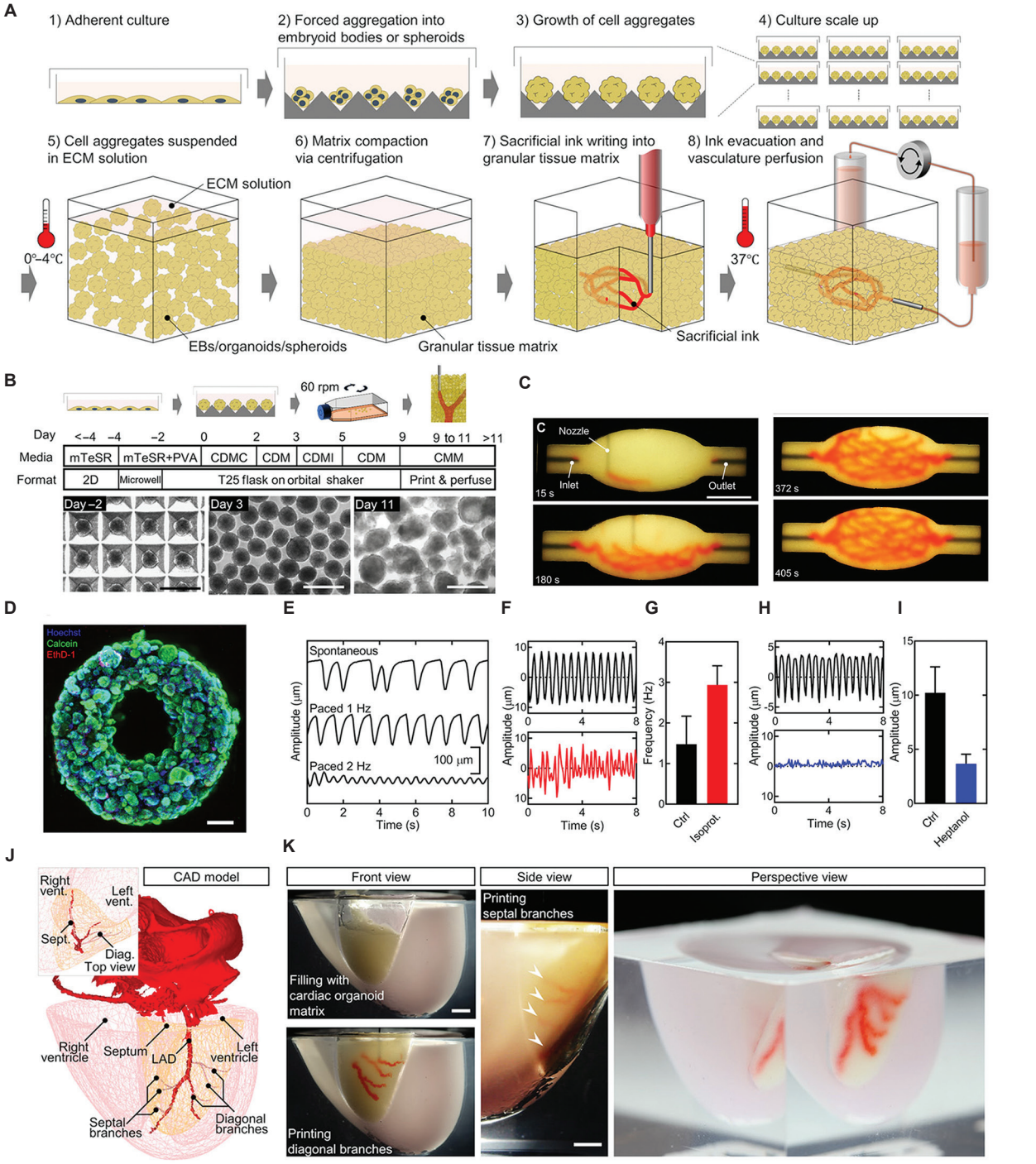


Figure 5. Vascularization of organoids by three-dimensional (3D) bioprinting. (A) Schematic diagram of the "sacrificial writing into functional tissue" process. (B) Design plan for cardiac organoids. Scale bar, 50 μm. (C) Branching vascular networks by 3D printing. (D) Cross-sectional cell viability of cardiac tissue after 24 hours of perfusion. Scale bar, 500 μm. (E) Spontaneous contraction and electrical pacing (1 and 2 Hz) of printed cardiac tissue. (F) Spontaneous contractions before and after administration of 10 μM isoproterenol. (G) Average systolic frequency under isoproterenol treatment. (H) Spontaneous contraction patterns before and after application of heptanol. (I) Maximum peak-to-peak shrinkage amplitude under heptanol treatment. (J) Schematic diagram of the structure of an artificial heart model. (K) Images of printed heart tissue. Scale bar, 5 mm. Reprinted from Skylar-Scott *et al.*[61] Copyright 2019, with permission from the American Association for the Advancement of Science.

Abbreviations: CAD: Coronary artery disease; CDM: Cardiomyocyte differentiation medium; CDMC: CDM and 5 μM CHIR99021; CDMI: CDM and 2 μM iWR1; CMM: Cardiomyocyte maturation medium; Ctrl: Control; Diag: Diagonal; EBs: Embryoid bodies; ECM: Extracellular matrix; EthD-1: Ethidium homodimer-1; Isoprot: isoproterenol; LAD: Left anterior descending artery; mTeSR: Refers to the name of a culture medium produced by STEMCELL Technologies; PVA: Polyvinyl Alcohol; Sept: Septum; vent: Ventricle; 2D: Two-dimensional.

tumor organoids printed with it retain features similar to primary breast tumors, reconstructing key aspects of the tumor microenvironment. Overall, optimizing bioinks for 3D bioprinting significantly aids the construction and development of organoids.

Moreover, to better regulate cell behavior and promote the development of multicellular 3D bioprinted scaffolds, bioinks containing functional materials have proven to be a viable strategy. For example, research led by Prof Chunying Chen at the National Center for Nanoscience and Technology of China demonstrated that carbon nanotubes could enhance the mechanical properties of the extracellular matrix, activate the mechanical sensing signaling pathway of cells, and regulate cellular metabolism, including promoting mitochondrial respiration and nutrient absorption, thereby facilitating the development of intestinal organoids (Figure 6).[70] Another bioink, incorporating iron oxide nanoparticles, enables the manipulation of organoid deposition and movement using a magnetized 3D printing device.[71] This platform allows for the controlled and precise arrangement and combination of neural organoids derived from human induced pluripotent stem cells and glioma organoids derived from patients (Figure 7). In addition, the inclusion of engineered extracellular vesicles, known for their therapeutic efficacy in treating diseases, can also enhance the development of functionalized bioinks.[72]

Inorganic bioactive materials possess excellent biological functions, not only guiding the behavior of stem cells but also regulating the interactions between cells, thereby positively affecting the development of stem cell-derived organoids.[73,74] For example, Ma *et al.*[75] developed a composite hydrogel composed primarily of calcium silicate and methacrylate gelatin to support the development of organoids. Research has shown that bioactive ions released from calcium silicate promote the key signaling pathways for organoid development, highlighting the potential application of inorganic biomaterials in organoid research.

Zhang *et al.*[76] showed that MXene promotes the maturation of cochlear organoids, with regenerated hair cells exhibiting electrophysiological characteristics similar to those of natural hair cells. They also demonstrated that MXene activates the mammalian target of rapamycin signaling to promote hair cell differentiation. Furthermore, in the co-culture system of cochlear organoids and the modiolus, neural innervation was established between regenerated hair cells and spiral ganglion neurons, promoting synapse formation. These findings suggest that inorganic biomaterials, through their influence on ion release, regulation of matrix mechanical properties, and promotion of organoid-cell functional co-culture systems, are becoming increasingly integral to organoid research.

Therefore, designing bioinks that incorporate inorganic biomaterials to generate functional 3D bioprinted organoids is highly valuable. For example, Zhang *et al.* designed a bioink containing calcium silicate to print a multicellular scaffold that mimics the distribution of bones and nerves.[77] Calcium silicate significantly promotes osteogenic differentiation of stem cells and the neural differentiation of Schwann cells. Inorganic biomaterials, such as calcium silicate-based bioinks, present an effective strategy for designing complex tissue constructs, such as bone organoids, through 3D bioprinting.

5.2. Optimizing bioprinting parameters

In the process of using 3D bioprinting to develop organoids, the design of bioinks is not the only crucial factor; the setting of the printing device also plays a significant role. The adjustment of printing parameters is directly linked to the structural characteristics of 3D bioprinting constructs. In addition, optimizing printing device settings enhances the production and application of organoids.

To maintain an optimal 3D culture environment for liver cells derived from human induced pluripotent stem cells, DLP-based 3D bioprinting has been used to develop microscale hexagonal constructs.[78] This cultivation model significantly promotes the phenotype and function of liver cells, mimics the structure of liver modules, and provides strategies for designing biomimetic 3D bioprinted livers. The key to this strategy is the use of hexagonal building blocks, which correspond to physiological scales similar to lobular units. High-resolution 3D bioprinting allows the production of finer structural features, including micropore size and curvature. Research has demonstrated that the micro-porous culture platform, designed by high-resolution 3D bioprinting, successfully generates mature human brain organoids.[79] These organoids displayed key structural features of the brain. In addition, by adjusting curvature and resolution, the development of organoids can be further optimized, highlighting the benefits of modifying the 3D printing structure for developing improved organoid culture platforms. Moreover, regulating printing pressure and needle settings during 3D bioprinting aids in the standardized production of organoids. A study has shown that after incubation at 7°C for 30 min, printing paste can produce tumor organoids < smaller than 200 μm on a standard glass substrate under a pressure of 7 – 15 kPa.[80] Furthermore, a microchannel scaffold with a top channel and four interconnected side channels has been designed by adjusting printing parameters. This scaffold facilitates the loading and embedding of organoids. Importantly, the different channels provide conditions for inoculating various cell types, which is beneficial for studying the interactions between cells and organoids.[81]

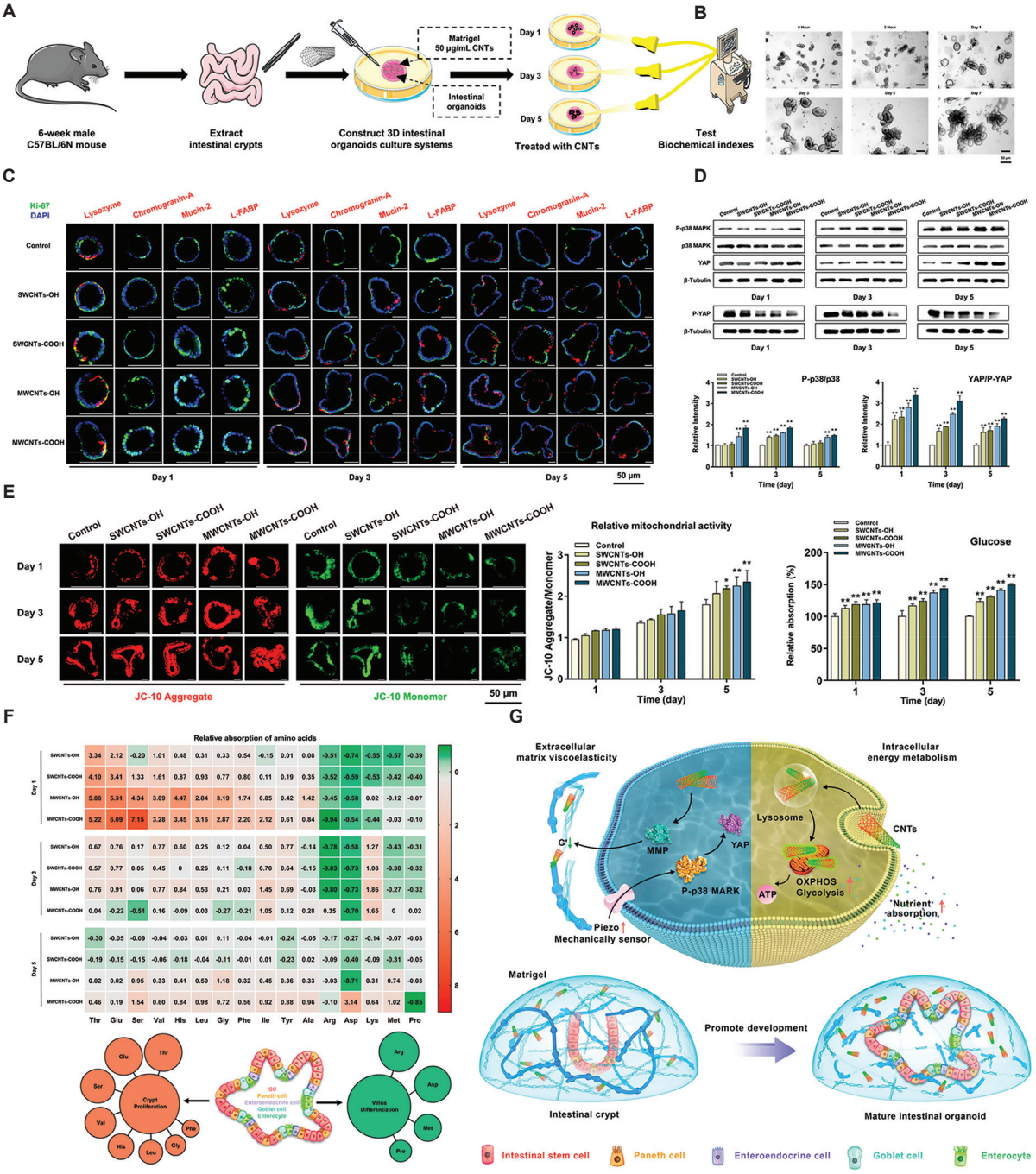


Figure 6. Effects of nanocarbon tubes on the proliferation and differentiation of intestinal organoids. (A) The experimental process of using carbon nanotubes to promote the development of intestinal organoids. (B) Optical images of intestinal organoids develop over time. Scale bar, 50 μm. (C) Representative stained images of different epithelial cells in intestinal organoids treated with carbon nanotubes. Scale bar, 50 μm. (D) Western blot analysis of Piezo2 and Piezo1 expression in intestinal organoids treated with carbon nanotubes. (E) Confocal images of mitochondrial membrane potential and relative mitochondrial activity statistics of intestinal organoids treated with single-walled carbon nanotubes and multi-walled carbon nanotubes over time. Scale bar, 50 μm. (F) Heatmap of the relative absorption of amino acids by intestinal organoids treated with carbon nanotubes over time. (G) Schematic diagram of the role of carbon nanotubes in promoting intestinal organoid development. Reprinted from Bao *et al.*[70] Copyright 2021, with permission from the American Chemical Society.

Abbreviations: Arg: Arginine; Asp: Aspartic acid; ATP: Adenosine triphosphate; CNTs: Carbon nanotubes; DAPI: 4',6-diamidino-2-phenylindole; Glu: Glutamine; Gly: Glycine; His: Histadine; ISC: Intestinal stem cells; Leu: Leucine; L-FABP: Liver-type fatty acid binding protein; Met: Methionine; MMP: Matrix metalloproteinases; MWCNTs: Multi-walled carbon nanotubes; Phe: Phenylalanine; Pro: Proline; Ser: Serine; SWCNTs: Single-walled carbon nanotube; Thr: Threonine; Val: Valine; YAP: Yes-associated protein; 3D: Three-dimensional.

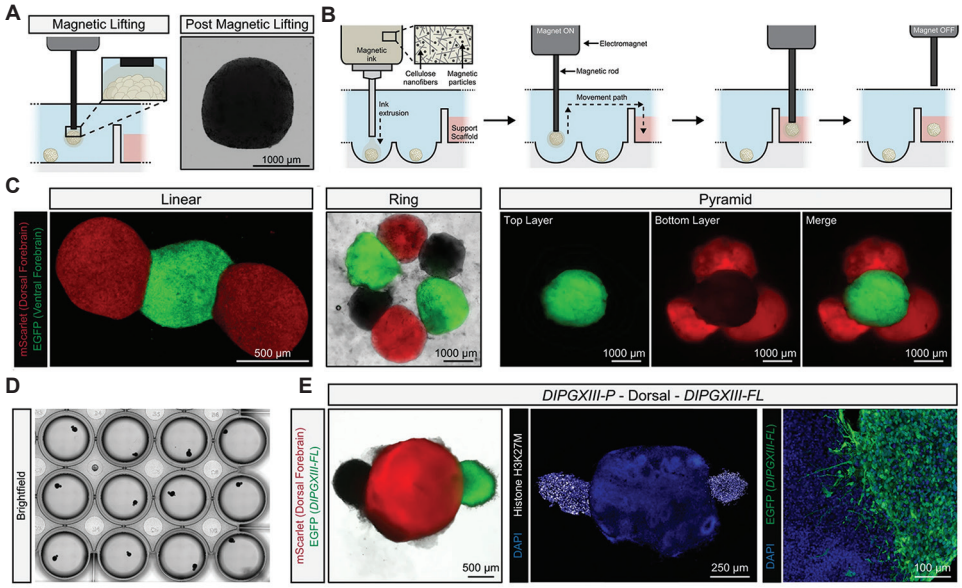


Figure 7. Development of neural organoids by three-dimensional bioprinting. (A) Schematic diagram of magnetic enhancement of neural organoids and bright-field image of neural organoids after magnetic enhancement. Scale bar, 1,000 μm. (B) Schematic diagram of the spatially patterned organ transfer platform. (C) Fusion and free assembly of organoids. (D) Fusion of neuromorphic organoid and diffuse pontine glioma organoid. (E) Bright-field images and immunofluorescence staining of organoid complexes. Reprinted from Roth J. G. *et al.*[71] Copyright 2021, with permission from Springer Nature. Abbreviation: EGFP: Enhanced green fluorescent protein.

5.3. Enhancing three-dimensional bioprinting devices and methods

The optimization of 3D bioprinting equipment is also important for the development and application of organoids. Microfluidic chips designed using 3D printing can explore interactions between organoids and endothelial cells forming a vascular network, which is essential for developing functional organoids. Salmon *et al.*[82] utilized this platform to promote interactions between brain organoids and vascular cells, leading to the formation of neurovascular organoids. The 3D bioprinting platform can be adapted for various organoid types, offering strategies for vascularized organoid development and enabling the exploration of organoid-cell interactions. Specifically, adjusting printing equipment enhances the potential applications of organoids.

Several improved 3D bioprinting systems have shown promising results in microsphere preparation, demonstrating significant potential in the development of organoids. Xie *et al.*[83] developed an electro-assisted bioprinting method to prepare hydrogel microspheres. Under the influence of electrostatic attraction, uniform-sized photo-crosslinked gelatin methacryloyl microspheres can be rapidly printed. In addition, the separation method of microspheres is relatively gentle on cells, minimizing damage and reducing printing costs while improving printing efficiency, providing valuable strategies for organoid development. Furthermore, hybrid bioprinting, which combines suction power to apply liquid absorption and control 3D printed constructs, has been developed.[84] This printing method allows for the precise control of structure formation, as well as the accurate positioning of printed spheres, which facilitates the controlled assembly of organoids into large-scale organ models.

Acoustic bioprinting can manipulate the generation and arrangement of individual organoids.[85] Chen *et al.*[86] employed acoustic bioprinting equipment to construct 3D structures with precisely arranged tumors and healthy organoids to simulate the colon of patients. This model was used to analyze tumor spread and invasion, as well as to conduct drug screening, thereby assisting healthcare providers in making treatment decisions.

5.4. Incorporating monitoring measures

Although the application of organoids in the biomedical field is becoming increasingly common, there remains a lack of monitoring methods for certain physiological characteristics of their development. One study designed a high-resolution 3D printing combined with liquid metal microelectrodes to monitor the development of ganglion cells in early to mid-stage retinal organoids.[87] This device can accurately locate the inner layer of the retina within the organoid and record signals within the retina, thus avoiding the risk of damaging the organoid (Figure 8). In addition, inkjet 3D printing has been used to prepare microelectrode arrays capable of recording electrophysiological signals from cortical organoids, again avoiding damage to the organoids.[88] Moreover, 3D-printed electrochemical porous plates have been applied to monitor

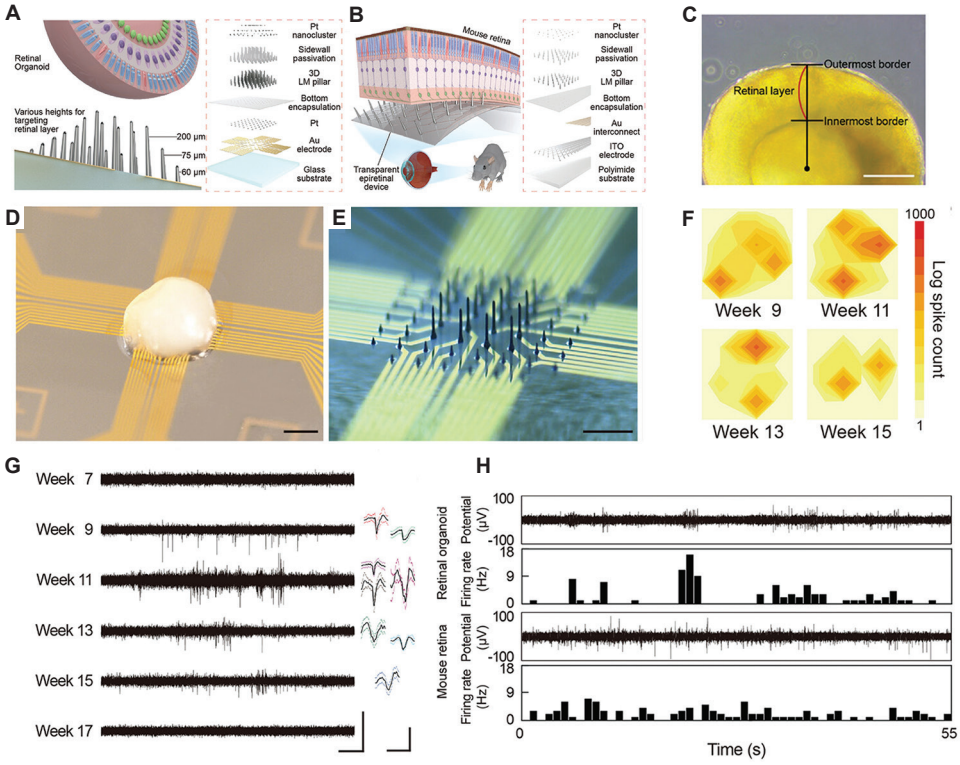


Figure 8. Development analysis of retinal organoids by three-dimensional (3D) bioprinting. (A) Schematic diagram of a microelectrode array with 3D liquid metal electrodes. (B) Schematic diagram of a 3D anterior retinal membrane device with 3D liquid metal electrodes. (C) Image of the retinal layer in a human retinal organoid. Scale bar, 200 μm. (D) Retinal organoids placed on a 3D liquid metal microelectrode array. Scale bar, 500 μm. (E) A 3D liquid metal microelectrode array with different heights targeting various retinal cell layers. Scale bar, 300 μm. (F) Contour map shows changes in discharge rate of retinal organoids during 16 culture cycles targeting RGCs. (G) Changes in single-unit activity of RGCs in retinal organoids during the cultivation period. (H) Single-unit activity and discharge rate comparison between retinal organoids at week 11 and the retina of wild mice. Reprinted from Lee *et al.*[87] Copyright 2024, with permission from Wiley-VCH GmbH.
Abbreviations: Au: Gold; ITO: Indium tin oxide; LM: Liquid metal; Pt: Platinum.

Table 1. The role and advantages of three-dimensional (3D) bioprinting in organoid development and application

Organoid model	Role of 3D bioprinting	Regulation of printing parameters	Application prospects	References
Intestinal organoids	Syringe-based extrusion bioprinting combined with microscopy; optimizing the structure and function	Structural parameters and cell density	Drug discovery, diagnostics, and regenerative medicine	33
Liver organoids	3D extrusion bioprinting; constructing large-scale organ models	Regulation of bioink and printed cells	Artificial organ transplantation	37,38
	Droplet-based printing technology; high-throughput cultivation of organoids	Microarray 3D bioprinting	Drug discovery	39
	Droplet-based printing technology; rapid, digital 3D bioprinting; optimizing the structure and function of organoids	Bioink regulation; high-resolution 3D bioprinting	Disease modeling and tissue regeneration	57,78
Kidney organoids	3D extrusion bioprinting; high-throughput cultivation of organoids; control of scale and structure	Organoid size, cell number, and conformation	Drug discovery	40
Cardiac organoids	Expanding embedded 3D bioprinting; 3D extrusion bioprinting; optimizing structure and function of organoids, creating suitable physiological microenvironments	Bioink regulation and design of the printing structure	Regenerative medicine and drug screening	43,44,59,61

(Cont'd...)

Table 1. *(Continued)*

Organoid model	Role of 3D bioprinting	Regulation of printing parameters	Application prospects	References
	Expanding embedded 3D bioprinting; high-resolution 3D printing; optimizing the structure and function of organoids	Self-assembly and manipulation of organoids	Disease research and tissue regeneration	61,62
Bone-related organoids	Digital light processing bioprinting; optimizing structure and function of organoids and constructing large-scale organ models	Bioink regulation	Tissue regeneration	50
	Digital light processing bioprinting; optimizing the structure and function of organoids; achieving high-throughput cultivation of organoids	Regulation of bioink and printed cells	Tissue regeneration	51
Tumor organoids	Temperature-controlled extrusion 3D bioprinting; optimizing the structure and function of organoids; creating suitable physiological microenvironments	Regulation of bioink and printed cells	Tumor treatment and studying tumor pathology	53
	Embedded 3D bioprinting; 3D extrusion bioprinting; optimizing structure and function of organoids; creating suitable physiological microenvironments; achieving high-throughput cultivation	Regulation of bioinks, printed cells, and structure	Development of anti-tumor drugs	54,68,69
	Magnetized 3D printing; 3D extrusion bioprinting; achieving high-throughput cultivation of organoids	Regulation of bioink; organoid manipulation	Anti-tumor drug screening	55,71
	Acoustic bioprinting; creating suitable physiological microenvironments for organoids	Improvement of printing device	Analyzing the spread and invasion of tumors; drug screening	86

serotonin levels in intestinal organoids exposed to different food components, providing a high-throughput platform for rapid screening of food intolerance.[89]

6. Conclusion and prospectives

The development of organoids has received significant research attention due to their authenticity and complexity in simulating tissue structure and function, as well as their ability to display cell-cell interactions and cell-matrix interactions.[90-92] However, several challenges remain in the development of organoids. The structure and function of real tissues or organs are complex and closely intertwined. As such, the structure and function of organoids must be further optimized to better match corresponding tissues or organs. In addition, current organoid research lacks adequate interaction with other cells, and the development of organoids is not yet regulated by local microenvironments – such as vascular networks, neural structures, and immune cells – which significantly differ from real tissue development. Finally, considerable variability between batches of organoids limits their broader application.

Three-dimensional bioprinting technology can precisely place cells within a specific microenvironment and create constructs of appropriate structures and sizes based on actual needs.[93,94] Therefore, 3D bioprinting can be used to prepare impeccable models and address the limitations faced by organoid development. In this review, we have summarized the role and advantages of 3D bioprinting in organoid development and application (Table 1). Overall, 3D bioprinting aids in optimizing the structure and function of organoids, creating suitable physiological microenvironments, enabling high-throughput cultivation, and advancing large-scale organ models. Particularly, when combined with new 3D bioprinting approaches, the development and application of organoids can be further enhanced. For example, hybrid 3D bioprinting, which combines suction and magnetic-assisted bioprinting, can accurately manipulate the position and behavior of organoids, offering strategies for precise localization of organoids within manufactured structures. In addition, incorporating microelectronic devices in 3D bioprinting allows for monitoring the dynamic changes and secretion of organoids. Furthermore, research has shown that 3D bioprinting based on suspension baths can effectively create engineered vascular structures, providing an effective strategy for further functionalization of organoids.[95]

In addition, optimizing the parameters or equipment of 3D bioprinting also contributes to organoid development.[96-98]

First, the meticulous design of 3D bioprinting bioinks has significant regulatory effects on stem cell behaviors and intercellular interactions. Specifically, the incorporation of bioactive components, such as inorganic bioactive materials, in bioinks holds promise for enhancing organoid development. Moreover, various printing parameters, including needle selection and printing pressure, are crucial for developing organoids with ideal structure and function. Furthermore, optimized 3D printing devices are not only useful for organoid development but also for assembling and applying organoids by manipulating their behaviors.

The development and application of organoids, combined with 3D bioprinting, have seen significant advancements, demonstrating immense potential for various applications. The following strategies are proposed for the future development of organoid 3D bioprinting: (i) In the development of stem cell-derived organoids, the adjustability of 3D bioprinting can be used to regulate stem cell behaviors and create ideal physiological microenvironments for cells, including structural and mechanical cues; (ii) To enhance the application of 3D bioprinted organoids as *in vitro* models, the 3D bioprinting process must be further standardized and systematized, ensuring not only the accurate simulation of organoid structures during production but also the maintenance of inter-batch stability of organoids; (iii) The design of bioinks can be further optimized by incorporating key factors that promote organoid development. For example, bioactive ions released by silicate biomaterials are expected to serve as effective additives, promoting the printing of organoids and the production of large organ models; (iv) The integration of 3D bioprinting with other technologies should be the primary strategy to promote the application of organoids. For instance, organoid development that requires flow shear forces can benefit from the combination of 3D bioprinting and perfusable devices, which effectively maintain the structure and function of organoids.[99] Moreover, the addition of perfusable systems is essential for drug screening in organoids; (v) Organs in the human body do not function independently, and there is interaction between multiple organs.[100,101] The use of 3D bioprinting technology is expected to facilitate the design of integrated platforms for multiple organ types, which can serve as a platform for studying communication between organs. Furthermore, 3D bioprinting may enable the interconnection of multiple types of organs, providing an effective solution for studying tissue-tissue interfaces; (vi) At present, most organoids developed are primarily used for disease modeling and drug screening, whereas 3D bioprinting has seen widespread application in tissue regeneration research. Therefore, combining organoids with 3D bioprinting can further advance their application in tissue regeneration. 3D bioprinting can transform miniature organoid models into tissue engineering scaffolds, making them highly suitable for promoting tissue regeneration. The functional effects of organoids and their regulation of the microenvironment differ from traditional multicells constructs, which warrants further exploration.

In this review, we have focused on summarizing the application of 3D bioprinting in the development and functionalization of organoids. However, the primary focus has been on current research progress, without a comprehensive overview of the design concepts and requirements for different 3D bioprinting equipment. Future reviews can address the role of different 3D bioprinting technologies in organoid development and application.

In conclusion, combining 3D bioprinting with the development and application of organoids is a highly effective and promising strategy. Future research can focus on developing appropriate 3D bioprinting strategies to further promote the development and application of organoids.

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Conflict of interest

Yin Xiao and Chengtie Wu are members of the Editorial Board of this journal but were not involved in the editorial and peer-review process conducted for this paper, either directly or indirectly. Separately, the other authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Writing-original draft: Wenping Ma
Writing-review & editing: Hongxu Lu, Yin Xiao, Chengtie Wu

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Availability of data

Not applicable.

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