Review

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A review of 3D bioprinting for organoids

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Abstract: Current two-dimensional (2D) cell models for effective drug screening suffer from significant limitations imposed by the lack of realism in the physiological environment. Three-dimensional (3D) organoids models hold immense potential in mimicking the key functions of human organs by overcoming the limitations of traditional 2D cell models. However, current techniques for preparation of 3D organoids models had limitations in reproducibility, scalability, and the ability to closely replicate the complex microenvironment found *in vivo*. Additionally, traditional 3D cell culture systems often involve lengthy and labor-intensive processes that hinder high-throughput applications necessary for a large-scale drug screening. Advancements in 3D bioprinting technologies offer promising solutions to these challenges by enabling precise spatial control over cell placement and material composition, thereby facilitating the creation of more physiologically relevant organoids than current techniques. This review provides a comprehensive summary of recent advances in 3D bioprinting technologies for creating organoids models, which begins with an introduction to different types of 3D bioprinting techniques (especially focus on volumetric bioprinting (VBP) technique), followed by an overview of bioinks utilized for organoids bioprinting. Moreover, we also introduce the applications of 3D bioprinting organoids in disease models, drug efficiency evaluation and regenerative medicine. Finally, the challenges and possible strategies for the development and clinical translation of 3D bioprinting organoids are concluded.

Keywords: 3D bioprinting; volumetric bioprinting; organoids; bioinks; regenerative medicine

Introduction

Three-dimensional (3D) printing technology has revolutionized a wide range of industries, including manufacturing [1], architecture [2] and healthcare [3], by enabling the precise fabrication of complex structures with high customization capabilities. Its ability to control the spatial arrangement of materials in a layer-by-layer fashion allows for the creation of intricate geometries, providing significant advantages in terms of flexibility, cost-efficiency, and rapid prototyping [4, 5]. In the medical field, for example, 3D printing enables the creation of personalized implants, dental devices, and surgical models that are tailored to the patient's anatomy, improving surgical outcomes and treatment effectiveness [6]. Furthermore, it plays a pivotal role in tissue engineering, where its ability to control the spatial organization of biomaterials allows for the precise construction of biomimetic tissues [7, 8]. These potentials of 3D printing open up new avenues for the development of more effective therapeutic and diagnostic tools, particularly in the construction of sophisticated models of human tissues and organs, which are increasingly being used in biomedical research and therapeutic applications [9, 10].

In recent years, 3D printing incorporating living cells or other active biomaterials (called 3D bioprinting) have accelerated the evolution of numerous research areas in the health sciences that traditionally uses cells as a starting

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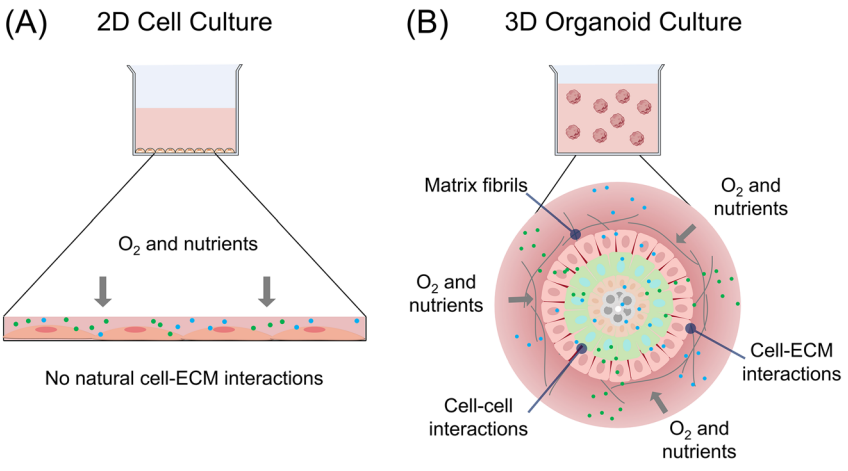
material, particularly for regenerative medicine, and the design of more relevant bioanalyses for drug discovery and development. This advancement has paved the way for the development of sophisticated 3D models, such as organoids, which are essential for improving the accuracy of biological research. Organoids represent a breakthrough in biomedical research because they could mimic the key functions of native tissues much more accurately than traditional two-dimensional (2D) cell cultures [11] (Figure 1), which has helped organoids to serve as a model for drug screening instead of animal models in preclinical studies. Therefore, the efficiency and the reproducibility of organoids are critical for drug research. Moreover, the methods commonly used to produce organoids could be classified into three types: conventional 3D culture, organoid-on-a-chip, and 3D bioprinting (Figure 2). Conventional 3D culture of organoids is simple and easy to use, however, it lacks precise control and has a low reproducibility. Organoid-on-a-chip technology provides a reproducible and controllable method for organoids, but the complexity of the microenvironment of organoids is limited by the design of the chip, which prevents scaling up. Compared to the previous two technologies, the integration of 3D bioprinting technology into organoids fabrication enhances the precision with which these structures can be created, allowing for better spatial organization of cells, more complex microenvironments, and the ability to replicate specific physiological features than the other methods, such as vascularization and organ compartmentalization [11, 12] (Table 1).

Currently, the primary types of 3D bioprinting technologies used in organoids preparation are inkjet-based bioprinting [13], extrusion-based bioprinting [14], photo-curing bioprinting [15] and volumetric bioprinting (VBP) [16–18]. Inkjet-based bioprinting is capable of high-precision droplet deposition, making it suitable for applications that require delicate and accurate placement of cells and biomaterials [13]. Extrusion-based bioprinting, on the other hand, involves pushing bioink through a nozzle to form layers, which is especially useful for printing with high-viscosity materials and building scaffolds with high mechanical strength [14]. Conventional photo-curing technology employs a nozzle-less approach to pattern and form layers of photosensitive materials by light projection, often using a laser for high-precision printing [15]. Despite its effectiveness in achieving fine resolutions, this layer-by-layer printing technique suffers from low printing speeds, which limit its capacity to produce scaffolds with high cell viability. An advanced layer-



Comparation	2D Cell Culture	3D Organoid Culture
Physiologic representation	Limited	Paraphysiology
Vascularization and immune system	No	No
Manipulability	Excellent	Good, but limited by experimental variability
Dynamic stimuli	No	No
Modelling organ formation	Poor	Suitable for basic research on cell-cell communication
Recapitulate the functions of human organs	Poor, due to simple non-physiological conditions	Yes

Figure 1: Schematic of difference between 2D cell culture and 3D organoids culture. (A) The traditional 2D culture, in which cells grow at the bottom of the dish, has fewer cell-to-cell interactions and lacks interactions between cells and the extracellular matrix; (B) 3D organoids culture system in which cells are clumped together with increased cell-cell and cell-extracellular matrix interactions, but limited access to O_2 and nutrients close to the core of the cell clump, recapitulating the cellular microenvironment *in vivo*. 2D, two-dimensional; O_2, oxygen; cell-ECM, cell extracellular matrix; 3D, three-dimensional.

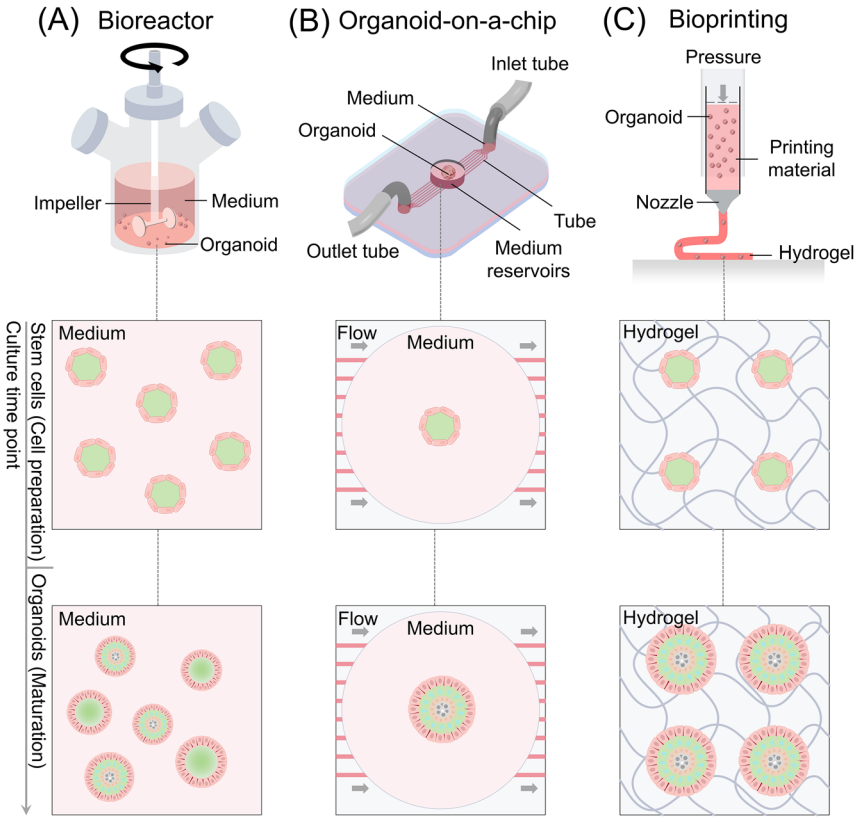


Figure 2: Schematic of various methods for organoids fabrication and cultivation. The common methods for organoids fabrication are (A) bioreactor, (B) organoid-on-a-chip and (C) extrusion-based bioprinting.

Table 1: Comparison of classic organoids, organoid-on-a-chip, 3D bioprinting organoids.

Feature	Classic organoids	Organoid-on-a-chip	3D bioprinting organoids
Structure	Self-organizing 3D tissue-like aggregates	Microfluidic systems with channels to mimic organ-like structures	Layered, complex structures with cellular organization
Complexity	Simpler, relying on natural self-organization of cells	Medium complexity, controlled environment with simulated flow	High complexity, precise control over shape and microenvironment
Cell types	Multiple stem cells (e.g., pluripotent stem cells)	Varies; typically includes endothelial, epithelial, and stromal cells	Various cell types, often more diverse, mixed with bioinks
Customization	Limited in spatial configuration	Limited by microfluidic chip design	Highly customizable, with control over cell arrangement and bioinks
Scalability	Difficult to scale for large quantities	Can be scaled with multiple chips, but still has limitations	Potentially scalable for large quantities through bioprinting
Applications	Disease modelling, drug testing, tissue regeneration	Disease modeling, drug screening, mechanical stress simulations	Drug testing, tissue engineering, disease modeling, regenerative medicine
Challenges	1) Limited by the natural self-organization capacity 2) Lack of vascular network	1) Microfluidic complexity and cost 2) Scaling issues 3) Limited capacity to meet the broad range of needs	1) Material compatibility 2) Complexity of design
Advantages	1) Simple to culture 2) Less expensive 3) High biological relevance	1) Mimics organ-level interactions 2) Dynamic environments	1) Precise control 2) Potential for more realistic models 3) Scalable
References	[109, 110]	[111–113]	[82–85]

3D, three-dimensional.

less photo-curing technology is VBP [16], which leverages photopolymerization to rapidly create entire 3D structures within tens of seconds. VBP stands out for its ability to fabricate organ-like structures with high fidelity and cellular resolution, opening new avenues for developing functional organoids that closely replicate native tissues [16–18].

In addition, 3D bioprinting allows cells to be homogeneously dispersed within the scaffolds formed by bioinks, which serve as carriers for cells and play a critical role in their proliferation and differentiation. The selection of bioinks tailored for organoids culture is therefore a pivotal factor in the successful 3D bioprinting of organoids [19, 20]. Despite the significant progress has been made in bioink development in recent years, several challenges remain in selecting bioinks for organoids bioprinting, as there is a need to balance biocompatibility, mechanical strength, and degradation rates, while also support cell viability and differentiation [21, 22]. Various biomaterials have been developed for specific applications, such as Matrigel [14], decellularized extracellular matrix (dECM) [23], silk [18] and gelatin methacryloyl (GelMA) [24]. Each of these biomaterials offers unique advantages for constructing organoids, allowing researchers to tailor the mechanical and biological properties of 3D bioprinting organoids. By ingeniously designing and optimizing these bioinks, 3D bioprinting can closely replicate the complexity of native tissues, yielding organoids suitable for a wide range of biomedical applications [14].

Herein, we aim to discuss current advances in 3D bioprinting technology for organoids applications. Starting with an overview of different types of 3D bioprinting technologies, with a particular focus on VBP. Then we summarize their specific advantages and limitations. Subsequently, we describe the application of different biomaterials in 3D bioprinting to inspire and guide researchers in designing and formulating bioinks that achieve desirable organoids structures with fine printability and high bioactivity. In particular, we provide representative examples of 3D bioprinting organoids for disease models, drug research, and regenerative medicine. Finally, we discuss the challenges and prospects in this field.

3D bioprinting technologies

The process of creating 3D (bio)structures by integrating 3D printing technique with cell-containing biomaterials is referred to as 3D bioprinting [25]. This innovative technology is gaining significant attention in the biomedical field due to its ability to precisely control the spatial arrangement of cells, making it an essential tool for disease research and regenerative medicine [26]. Based on fundamental printing principles, 3D bioprinting strategies are generally classified into three categories: inkjet-based bioprinting [27, 28], extrusion-based bioprinting [29], photo-curing bioprinting [30], and VBP (one of cutting-edge photo-curing bioprinting technique) [17, 18]. Table 2 lists the specific advantages and limitations of the above bioprinting technologies, and we will introduce these 3D bioprinting techniques in the following parts, especially focus on VBP technique.

Inkjet-based bioprinting

Inkjet-based bioprinting, which utilizes thermally, piezoelectrically, acoustically, or electrostatically power-driven nozzles to eject material [27, 28] (Figure 3A). This technique involves depositing cell suspensions or biomaterials onto the substrate by ejecting a series of droplets layer by layer through a nozzle, allowing for the construction of complex patterns. The advantages of this method include fast printing speed and high resolution [31]. This method is suitable for encapsulating organoids in materials for printing a stable and independent space to promote organoids self-assembly. However, inkjet-based bioprinting relies heavily on low-concentration bioinks which have limited ability to effectively encapsulate cells, while using high-concentration or high-viscosity bioinks may cause clogging of the nozzles due to insufficient driving force. Therefore, inkjet-based bioprinting has been limited by high-concentration bioinks or high-cell density bioinks [32]. Overall, it is essential to balance material concentration, nozzle size, and jetting frequency to optimize the creation of desirable constructs using inkjet-based bioprinting [33].

Extrusion-based bioprinting

Extrusion-based bioprinting is a widely utilized technology in 3D bioprinting, involving the extrusion of material through a nozzle using either air pressure or mechanical stress [29]. The extrusion-based bioprinting process consists of a power source, bioink, and an appropriate nozzle (Figure 3B). A key advantage of extrusion-based bioprinting is its capability to print materials with high concentrations or viscosities, allowing for a diverse array of biocompatible materials [34]. In addition, high-density cell suspensions could be incorporated into biocompatible materials for extrusion-based bioprinting. Therefore, high-density cell-incorporated bioinks can be used for extrusion-based bioprinting with close cell-to-cell connections, which promote organoids self-assembly polymerization and improve functionalization of organoids. However, excessively high cell densities may decrease cell viability due to shear stress effects [35]. The ratio of shear stress to shear rate determines the viscosity of the material. Therefore, it is essential to strike a balance between cell concentration and material viscosity [36]. By optimizing nozzle size, applying appropriate squeeze pressure, and utilizing highly biocompatible materials with favorable shear properties, it is possible

Table 2: Advantages and disadvantages of different types of 3D bioprinting techniques.

Type	Principle	Advantage	Disadvantage	Reference
Inkjet-based bioprinting	Thermal, piezoelectric, acoustic and electrostatic forces	1) High resolution (~5 μm) 2) High print speeds with a rate of up to 1000 droplets per second 3) Low cost and wide availability 4) High cell viability (>82 %) 5) Multiple nozzles	1) Restricted viscosity of bioink 2) Inefficient cell encapsulation due to the low concentration of the ink 3) Nozzle clogging	[27, 28, 31]
Extrusion-based bioprinting	Pressure-driven technology with pneumatic or mechanical dispensing system	1) Print with high cell densities (2×10^7 cells/mL) 2) Resolution between 50 μm and 200 μm 3) Enable to print in stable form	1) Shear-stress during printing 2) Low speed 3) Nozzle clogging 4) Nozzle can compromise cell viability	[11, 14, 34, 37]
DLP-based bioprinting	Use UV or visible light to cure photosensitive polymers in a layer-by-layer fashion	1) No negative effects of shear pressure 2) High resolution (50 μm)	1) Cytotoxic effects of the photoinitiators 2) Damage caused by UV and near UV light to cell DNA 3) Limited choice of photosensitive biomaterials 4) Non-ideal density and uniformity of printed cells	[15, 29, 42]
Volumetric bioprinting	Tomographic projection combines with light dose accumulation to form a 3D (bio) construct at same time	1) High cell viabilities (90–95 %) 2) Fast-speed printing (~30 s) 3) Contact free 4) Low bioink concentration 5) The layer-less enables smooth surfaces of prints 6) Print without supporting materials	1) Limited resolution 2) Cytotoxic effects of the photoinitiators 3) Limited types of transparent and photo-curing bioink	[17, 18, 45, 47]

DLP, digital light process; UV, ultraviolet; 3D, three-dimensional.

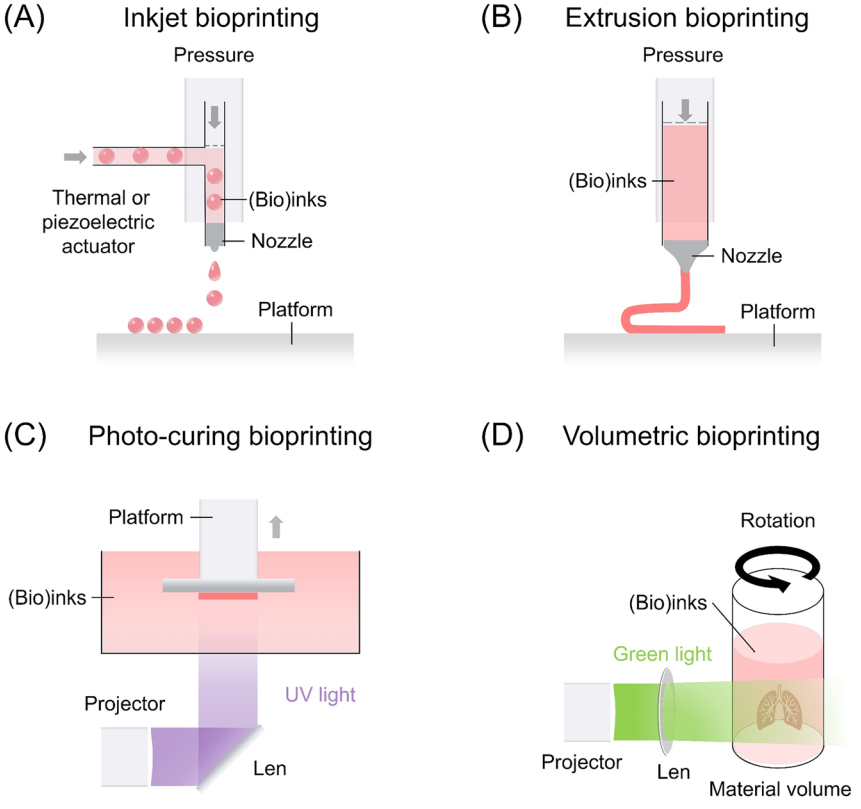


Figure 3: Schematic of the most common fabrication techniques used within 3D bioprinting organoids, including (A) Inkjet-based bioprinting, (B) Extrusion-based bioprinting, (C) Photo-curing bioprinting and (D) VBP. UV, Ultraviolet; VBP, volumetric bioprinting.

to efficiently create 3D structures with high cellular activity [37].

Photo-curing bioprinting

Photo-curing bioprinting, which utilizes surface projection technology, involves the solidification of photosensitive liquids through ultraviolet (UV) or visible light and has gained significant attention in recent years [30] (Figure 3C). Photo-curing bioprinting techniques are primarily categorized into stereolithography (SLA) [38] and digital light processing (DLP) [39]. Both technologies primarily exploit UV cross-linking of photosensitive materials. Additionally, DLP has evolved to employ visible light for cross-linking, aiming to minimize cell damage caused by the light source during long-term printing, while also expanding the range of available photosensitive materials [40]. Regarding printing principles, SLA operates through a vat-polymerization dot method, constructing patterns on a platform by point-to-point polymerization. In contrast, DLP irradiates the material on the platform with a patterned light source, generating patterns based on the irradiated area. Notably, with specific photosensitive materials, SLA can achieve a resolution of up to 10 μm through vat-polymerization [41], whereas DLP can reach up to 50 μm using surface polymerization techniques. However, both high and low materials concentrations may lead to a loss of resolution [42]. Compared to extrusion-based bioprinting, photo-curing bioprinting offers enhanced precision and speed, which provides a higher degree of scalability for organoids fabrication and guarantees organoids activity in the case of simulated tissue structures. Additionally, since the photo-curing bioprinting process is independent of nozzles and shear forces, it enhances cellular viability and facilitates the rapid fabrication of organoids.

VBP

VBP, a novel light-based bioprinting method, has recently garnered considerable attention in the field of additive manufacturing and holds great potential for organoids research [17, 43] (Figure 3D). The technical principle of VBP is initially introduced by Shusteff et al. group [44] in 2017, drawing inspiration from holography and multi-angle projection techniques. This technology is refined further in 2019 when Kelly et al. group [45] develops a computed axial lithography system that utilizes UV light, with applications spanning machine building, military, and medical fields. However, exposure to UV light poses risks to cell viability due to potential DNA structural breaks. In response, our group [18] proposes a strategy that utilizes a green light (525-nm wavelength) as the light source for VBP, significantly enhancing cell viability and overcoming the limitations associated with traditional UV light source.

The advancement of VBP technology has resulted in substantial breakthroughs in the temporal and spatial dimensions of additive manufacturing. Temporally, VBP has evolved from 2D planar layer-by-layer construction to 3D volumetric construction, dramatically improving molding efficiency. Spatially, VBP has transitioned from conventional 3D coordinates (X, Y, Z) to incorporating rotational angles (θ). This innovation enables the rapid construction of centimeter-scale 3D sophisticated structures within tens of seconds. In addition, the VBP process involves continuous light absorption, enabling the creation of layer-less 3D structure [17, 43, 45]. Specifically, VBP employs computed tomography (CT)-reconstructed backward projection algorithms and filtered projection algorithms to pattern each angle of a computer-aided design model prior to printing. The 3D model is then constructed by projecting the patterned angles from a digital light projector onto the photosensitive bioink [45]. Additionally, the VBP system comprises a digital light projector and a rotatable bioink container [18]. This configuration ensures a non-direct contact between the device and the cells, which provides a stable environment for organoids printing, therefore, maintaining high cell viability during the VBP process, which plays a key role in the initial stages of organoids culture. Moreover, VBP allows for a broad concentrations range of (bio)inks [18, 46], which increases the scalability of VBP organoids. It is noteworthy that this contact-free, highly efficient printing method surpasses the capabilities of traditional bioprinting techniques. Overall, regardless of the model complexity, VBP could complete the printing process in tens of seconds without requiring additional support, while achieving high accuracy. With its advantages of printing speed, contactless operation, and enhanced cellular activity, VBP opens new avenues for rapid additive manufacturing in the field of organoids [47].

In summary, the unique advantages such as high-precision modulation of physical microstructures and good reproducibility make 3D bioprinting a perfect tool for organoids fabrication. Moreover, the properties of (bio)inks, which serve as carriers for 3D bioprinting, are critical for organoids fabrication and cultivation [21, 22] (Figures 4 and 5). Various formulations of biomaterials and cells (or organoids) can be used for organoids fabrication and culture, which can be broadly categorised into five types: (1) cells are seeded on the surface of biomaterials, which is similar to 2D cultures; (2) cells are mixed with biomaterials, which is commonly used for 3D bioprinting; (3) biomaterials encapsulate cells, which allows for the

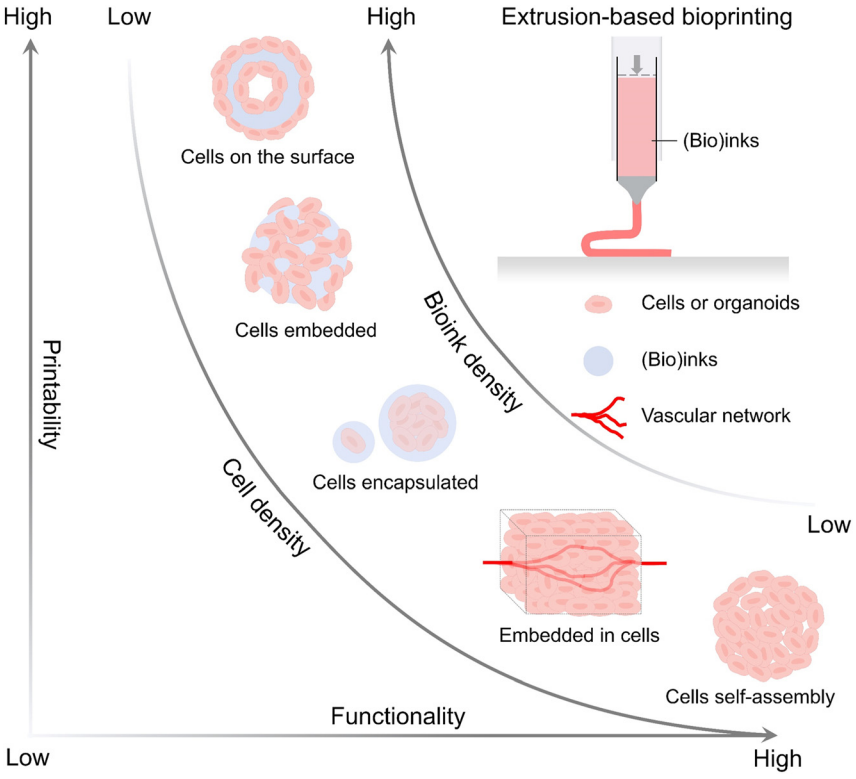


Figure 4: Schematic of the relationship between bioink density, cell density and function and printability of organoids by using extrusion-based bioprinting technology.

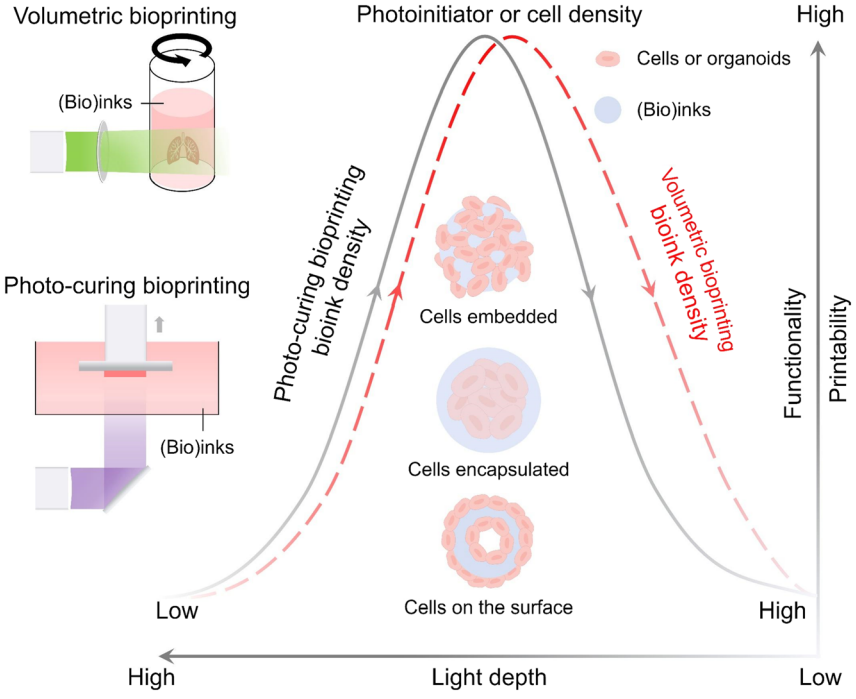


Figure 5: Schematic of the relationship between bioink density, light penetration depth and function and printability of organoids by using light-based bioprinting technology.

bioprinting of single cells; (4) sacrificial biomaterials are used to prepare a vascular network for the cells (or organoids) aggregates to provide nutrients; (5) pure cells can be used as bioinks for bioprinting, with cell-cell connections close to those of the *in vivo* microenvironment. The variety of combinations and associations between biomaterials and cells (or organoids) provides a wide range of model construction options for 3D bioprinting organoids-like models (Figure 4).

In contrast, light-based bioprinting relies on the cross-linking of photosensitive materials with photoinitiators

under specific light irradiation, which prevents them from achieving dematerialised bioprinting or cellular bioprinting. Furthermore, the printability of light-based bioprinting is influenced by multiple factors, including material density, photoinitiator density, and cells density, which are the main influencing factors that determine the light penetration and the printability [18]. Therefore, a balance between the density of various solutes is critical for light-based bioprinting. Particularly, VBP enables low-concentration material bioprinting, which broadens the printable bioink range of traditional light-cured bioprinting and aids in improving the scalability of 3D bioprinting organoids. Overall, the quality of 3D bioprinting organoids can be improved by choosing a reasonable bioink density as well as the printing method (Figure 5). The following section will introduce the major bioinks currently used for 3D bioprinting organoids, with a particular focus on those compatible with VBP.

3D bioprinting bioinks for organoids

(Bio)ink, composed of biocompatible materials and/or cells, serves as an engineered raw material for bioprinting [48]. Various biomaterials can be used as *in vitro* models in tissue engineering, enabling the replication of *in vivo* tissue structures through bioprinting and facilitating the investigation of cell-environment interactions [49]. The primary component of tissue structures *in vivo* is extracellular matrix (ECM), which is abundant in growth factors, nutrients, and adequate hydration that contribute to the proliferation of cells or organoids and can serve as a (bio)ink [50]. Nevertheless, the suboptimal mechanical properties of ECM render it unsuitable for direct bioprinting, therefore, the printability of (bio)inks that can replicate ECM structure is a critical consideration [51]. Current (bio)inks, with established processes, can be categorized into two groups: natural polymeric biomaterials such

Table 3: Bioink used for 3D bioprinting organoids.

Bioink	Advantage	Disadvantage	Organoids	References
Gelatin	1) Consists of natural ECM components 2) Thermo-responsive property 3) Good cytocompatibility 4) Support cell adhesion	1) Low mechanical stability 2) Not stable at physiological temperature	Tumor Islet Vascular networks Spinal cord	[34] [53] [97] [102]
GelMA	1) Derived from modification of gelatin, and consists of natural ECM component 2) Compatible with many cell types	1) Low mechanical strength 2) Cell viability depends on the photo-crosslinking time, the intensity of the light and photoinitiator concentration	Islet Liver Heart	[42] [47] [114]
Sodium alginate	1) Viscosity can be easily manipulated by changing the concentration 2) Compatible with a lot of cell types 3) Non-toxic and biologically inert to mammalian cells	1) Mechanically unstable for prolonged culture 2) Low degradation rate 3) Do not support cell proliferation	Bone Neuron Lung cancer	[29] [58] [90]
Matrigel	1) Natural biomimetic ECM 2) Thermal-crosslinking at a higher temperature	1) Uncertain biosecurity and tumorigenicity 2) Low homogeneity and reproducibility	Muscle Intestinal	[11] [14, 62]
PEG	1) Good mechanical stability 2) Multiple bioactive sites	1) Do not support for cell proliferation 2) High molecular weight result in high cytotoxicity	Intestinal Breast cancer	[15] [115]
SF	1) Good cytocompatibility 2) Large range of adjustable mechanical strength	1) Poor stability and short storage time 2) High concentration can easily precipitate precipitation 3) The solution quality is affected by the ambient temperature	Kidney Cartilage	[116] [117]
HA	1) A natural polysaccharide whose main component is ECM 2) Act as a growth factor for cell migration and proliferation	1) Low mechanical stability 2) Pure hyaluronic acid lacks printability	Lung cancer Tumor Brain tumor	[90] [118] [119]
dECM	1) Retain ECM components 2) Outstanding tissue specificity	1) Poor shape fidelity 2) Possible immune responses 3) Slow gelation kinetics	Kidney Glands	[100] [103]

ECM, extracellular matrix; GelMA, gelatin methacryloyl; PEG, polyethylene glycol; SF, silk fibroin; HA, hyaluronic acid; dECM, decellularized extracellular matrix.

as Matrigel [14], dECM [46], and silk [18], and synthetic polymeric biomaterials, including GelMA [52], hyaluronic acid methacrylate (HAMA) [53] and polyethylene glycol (PEG) [15]. These materials exhibit properties analogous to those of ECM, including high water content, favourable biocompatibility, and biodegradability. Particularly, many of these (bio) inks are compatible with VBP, allowing VBP with low-concentration bioinks to improve cell viability of printed organoids. However, differences in biocompatibility and printability exist among various biomaterials, making the proper selection of materials essential for the success of cell bioprinting. Table 3 summarizes the respective advantages and limitations of different (bio)inks.

Natural polymers

Matrigel

Matrigel, derived from the basement membrane matrix of Engelbreth-Holm-Swarm mouse tumors, has become a critical component frequently utilized in organoids technology in recent years [14]. This natural biopolymer comprises laminin, type IV collagen, heparan sulfate proteoglycans, and a variety of growth factors that promote the growth and differentiation of cultured cells [54]. Additionally, it exhibits thermal reversibility, rapidly transitioning from liquid to gel upon heating; however, reverting to a liquid state necessitates prolonged immersion in an ice bath, which helps maintain the gel's stability at 37 °C, making it more favorable for cell culture [55]. Moreover, since the mechanical properties of the ECM are determined by the biochemical characteristics of the corresponding tissues, the limited mechanical modulation capabilities of Matrigel restrict its applications *in vitro* [56]. Although increasing the concentration of Matrigel enhances its printing performance, it also causes higher costs than other biomaterials. Thus, Matrigel is frequently combined with other biomaterials to form composite (bio)inks for extrusion-based (bio)printing to promote organoids growth [57, 58]. In addition, there are no report on photo-curing bioprinting utilizing Matrigel-based (bio)inks.

dECM

dECM refers to biomaterials derived from organs or tissues from which cells have been removed [23], and it is widely used in tissue engineering and organoids preparation technologies [59]. The dECM hydrogel retains the original complex components of the ECM corresponding to the source organ or tissue, thereby replicating the cytocompatible microenvironment necessary for enhancing cellular functionality, tissue morphogenesis, and the development of homologous organoids [60, 61]. Compared to tumor-derived Matrigel, dECM is considered more suitable for human applications and is increasingly regarded as a viable replacement for Matrigel in organoids tissue culture [62]. However, pure dECM-based (bio)inks exhibit poor mechanical stability, making it challenging to construct stable structures, similar to the limitations seen with Matrigel [63]. Notably, VBP is a technique that allows low concentrations of (bio) inks to overcome the restrictions of inadequate mechanical properties towards the construction of stable 3D (bio)structures. For instance, our group [46] reports a strategy utilizing VBP with pristine dECM. The unmodified dECM derived from porcine heart and meniscus tissues is successfully reconstructed into functional heart and meniscus-like structures via VBP.

Silk

Silk, derived from the domestic silkworm (*Bombyx mori*), has garnered significant attention in biomedical applications due to its unique properties [64]. It is primarily composed of silk fibroin (SF) and silk sericin (SS), with SS serving to protect the surface of SF and secure it within the cocoon [18, 65]. Pure SF is obtained through the degumming process, which is mainly composed of glycine, alanine, serine, and tyrosine. In addition, SF spontaneously forms crystalline β-sheets which enhance the mechanical strength of SF [66]. These structural protein chains organize to maintain the stability of the SF network without the need for chemical crosslinking. Moreover, the remarkable properties of SF, such as high biocompatibility, safety, and controlled degradation rates have facilitated its extensive use in a variety of biomedical applications [67].

In addition, SS is extracted from cocoons and often discarded as a waste. However, recent advancements have highlighted its beneficial properties, including high hydrophilicity, anti-inflammatory, antioxidant, and antibacterial effects, suggesting significant potentials in the biomedical fields [68]. SS is frequently utilized as an effective additive in extrusion-based (bio)printing, while limited studies report on the direct application of pure SS in bioprinting [69]. Notably, SS contains abundant tyrosine bonds, similar to those found in SF, which makes it suitable for photo-curing (bio)printing (Figure 6). For example, our group [18] employs photochemical reactions between the tyrosine groups in silk-based (bio) inks (both SF and SS) with the visible-light photoinitiator tris-bipyridylruthenium (II) hexahydrate (Ru)/sodium persulfate

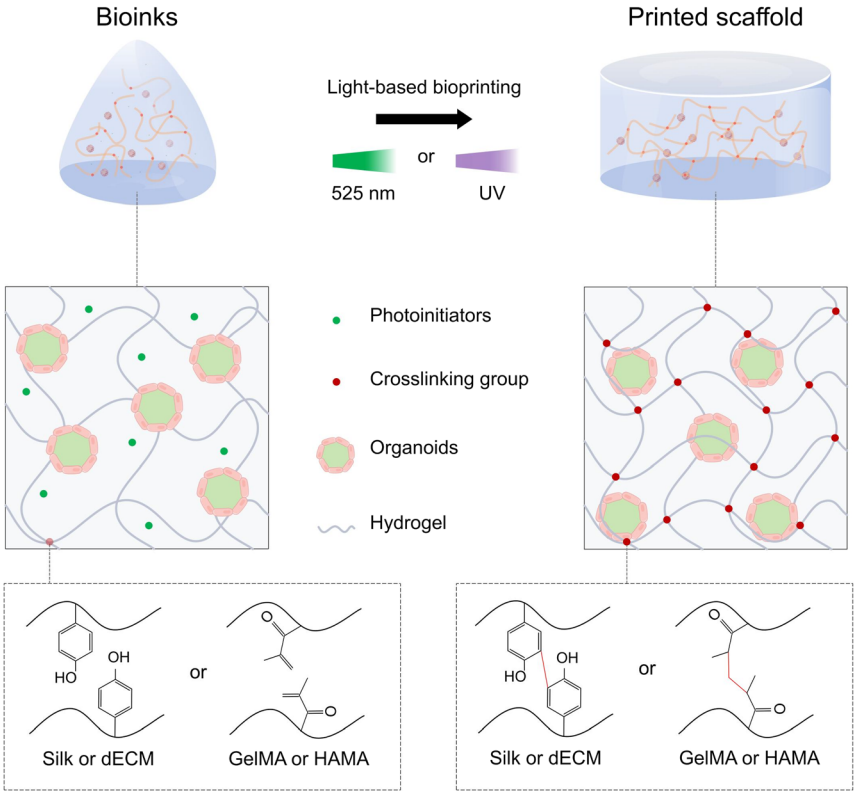


Figure 6: Bioink structure and gelation mechanism of light-curing polymers commonly used for 3D bioprinting organoids. The common visible light-curing biomaterials, including silk and dECM, which are under visible photoinitiator system, the crosslinking group on the polymer chains would form a gel via 525-nm green light; The common UV light photo-curing biomaterials, including GelMA and HAMA, which are under the UV photo-initiator system, and the methacrylic acid bonds on the polymer chains would form a gel via UV light. dECM, decellularized extracellular matrix; GelMA, gelatin methacryloyl; HAMA, hyaluronic acid methacrylate; UV, ultraviolet.

(SPS) to construct scaffolds for biomedical applications using VBP. This method overcomes the restriction that pristine silk proteins cannot be photo-crosslinked. The unmodified silk-based (bio)inks exhibit inherent biocompatibility and exceptionally high cellular activity, offering promising prospects for advancing organoids cultures.

Synthetic polymers

GelMA

GelMA is a modified gelatin produced through the reaction of gelatin with methacrylic anhydride [70] (Figure 6). It exhibits several advantageous characteristics, including photo-crosslinking capabilities, favorable biocompatibility and excellent printability, making it widely applicable in tissue engineering and regenerative medicine [71]. GelMA retains the biological properties of gelatin, featuring a highly hydrated polymer network and biophysical properties similar to those of natural tissues, which supports cell growth [72]. In addition, non-crosslinked GelMA maintains thermal reversibility akin to gelatin, transitioning from a solid colloid at room temperature to a liquid state at 35–40 °C [71]. This thermal sensitivity of GelMA has been widely used in (bio) printing, for example, the rheological properties of GelMA could be tuned for extrusion-based bioprinting by modulating the temperature of GelMA-based (bio)ink to fabricate scaffolds with good physical stability [49]. Furthermore, GelMA is irradiated by UV or visible light and combines with photo-initiators to form photocrosslinks, and after photochemical cross-linking GelMA does not have thermo-reversible properties, allowing for structural stability [52, 70]. Consequently, light-cured GelMA could provide 3D (bio)structures with favorable mechanical properties, resulting in the cultivation of high-quality 3D bioprinting organoids. In addition, Bernal et al. group [47] used GelMA for VBP to rapidly fabricate porous biological scaffolds, which have good shape fidelity and good biocompatibility to culture liver organoids. Compared to the other methods of culturing organoids, the centimeter scale porous scaffold of VBP, which is embedded organoids, shows a high degree of liver organoids differentiation, indicating that VBP has a promising future in the construction of organoids models.

HAMA

HAMA is a semi-synthetic, photo-crosslinked hydrogel that has gained widespread use in tissue engineering due to its exceptional biocompatibility and ease of crosslinking [53]. Derived from hyaluronic acid, HAMA supports cell migration and proliferation. It retains the bioactive properties of

hyaluronic acid (HA), including high viscosity, which enhances tissue dispersion and plays a key role in protecting cells [73]. Additionally, HAMA exhibits functionalities such as lubricating joints and regulating the permeability of blood vessel walls. Compared to pristine HA, modified HAMA offers improved crosslinking and stability, effectively addressing the limitations of poor mechanical properties associated with pristine HA [74]. However, non-crosslinked, low-concentration HAMA displays rheological properties that render it unsuitable for filament extrusion and layer-by-layer assembly, whereas, after photocrosslinking with UV light, it can be utilized for various biological applications [74]. For example, Wang et al. group [53] reports a method for preparing islet organoids by photo-curing bioprinting using HAMA/pancreatic extracellular matrix (pECM) bioink to explore the development of pancreatic islet organ tissue transplants. With its superior characteristics, HAMA presents promising prospects in 3D bioprinting organoids.

PEG

PEG is a synthetic polymer composed of polyethylene oxide (PEO) and water, widely employed in pharmaceutical and biomedical applications [75]. Its high hydrophilicity, water solubility, and excellent safety profile have led to approval from the U.S. Food and Drug Administration (FDA) for use in certain drugs and medical devices [75]. Additionally, PEG-based hydrogels can be synthesized in various structural forms, such as linear [76], Y-shaped [77], and multi-arm configurations [78], making them suitable for applications in nanocarriers, drug delivery, and (bio)printing [79]. Specifically, the surface of PEG features multiple bioactive sites, enabling the grafting of other materials onto its chains. PEG can be functionalized with "clickable" groups, UV cross-linking agents, or bioactive and cell adhesion moieties, enhancing its utility in bioprinting [80]. Commonly, these modifications involve incorporating photopolymerizing groups, such as acrylates or methacrylates, to form hydrogels through photoinitiated radical polymerization. For example, Hockaday et al. group [80] utilizes porcine cell-loaded polyethylene glycol diacrylate (PEGDA) in photo-curing bioprinting to produce aortic valves of infant and adult sizes, which demonstrated mechanical heterogeneity and cytocompatibility suitable for anatomical studies. Another approach involves the combination of polyethylene glycol dimethacrylate (PEGDMA) and GelMA to encapsulate human mesenchymal stem cells (hMSCs) for the biological construction of bone and cartilage tissues [81]. Furthermore, Carberry et al. group [15] reports an innovative method to reconstruct Matrigel with a complementary structure of intestinal villi using a desborenylated PEG-based hydrogel as a sacrificial model for intestinal organoids culture.

Overall, the bioinks helps to maintain structural stability and integrity during the entire bioprinting and culturing process of organoids, and further improves the reproducibility and scalability. In addition, the multiple 3D bioprinting methods and adapted bioinks provide a solid foundation for customization of high-quality complex structural organoids, which shows promising applications in a broad biomedical field.

Applications of 3D bioprinting organoids

3D bioprinting organoids plays a critical role in disease, drug research and regenerative medicine [22, 82]. Cells derived from patients are bioprinted to organoids models to construct disease models *in vitro*, which reproduce the *in vivo* disease microenvironment for promoting the understanding of disease genes [83]. Furthermore, these homogeneous 3D bioprinting disease organoids are used in drug research to construct disease models for high-throughput drug screening and verification of drug efficacy, thus providing access to drugs to treat diseases [84]. In addition, 3D bioprinting organoids can be used to rebuild human organ tissue cells and aid in human organ or tissue

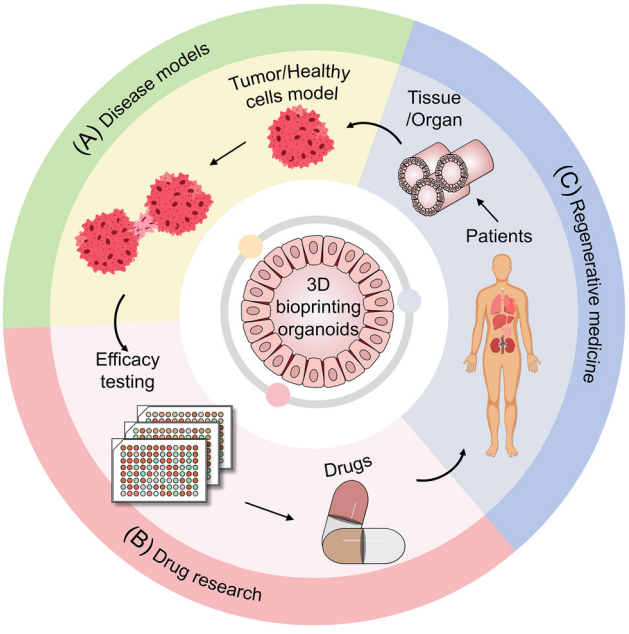


Figure 7: Various applications of 3D bioprinting organoids technology. (A) 3D bioprinting organoids technology provides disease models derived from tumor or healthy cells to mimic human genetic diseases or cell to cell interactions *in vitro*; (B) 3D bioprinting organoids technology constructs the disease models for drug research, for example make drug efficacy testing quickly before drugs; (C) 3D bioprinting organoids technology provides ideal models for regenerative medicine. 3D, three-dimensional.

repair, which is widely used in regenerative medicine [85] (Figure 7). Here, we present the current advances in 3D bioprinting organoids, especially VBP organoids models.

Disease modeling

3D bioprinting has become a powerful tool for organoids to study genetic diseases or human disease models [82]. The ability of organoids to replicate cellular functionality to reproduce histological, genomic and phenotypic features of primary tumors for studying their genetic composition and predicting treatment options, plays an important role in disease research [86]. Bioprinting technology provides organoids with physiological geometry that mimics ECM to effectively replicate the *in vivo* tumor microenvironment (TME) [87]. In addition, the bioprinted scaffolds facilitates the tumor organoids to replicate the ECM and cell-cell interactions and improves the efficiency and success rate of replicating genetic profiles. Especially, Shi et al. group [86] successfully constructs a lung tumor model *in vitro* to culture transgenic mouse-derived tumor organoids using low concentrations of collagen with the assistance of embedded bioprinting. The 3D bioprinting tumor organoids exhibit morphology similar to primary breast tumors and reconstructed multiple features of TME, including angiogenesis, epithelial-mesenchymal transition, and invasion, contributing to the advancement of the understanding of tumor biology. Additionally, Chen et al. group [88] proposes an acoustic bioprinting organoids protocol for observing the interaction between tumor cells and healthy cells to predict treatment response and assist in determining the optimal cancer treatment for an individual patient. The acoustic bioprinting model is structurally similar to the *in vivo* microenvironment and contains tumor cells and healthy cell-derived organoids, which enables to simulate the process of tumor cell invasion on healthy cells in human body. In short, homogeneous *in vitro* disease models have been efficiently constructed by 3D bioprinting organoids, which contribute to advance the understanding of diseases such as angiogenesis, tumor invasion, metastasis, and the TME environment, and provide a solid foundation for further drug research [89].

Drug discovery

One of the critical applications of 3D bioprinting organoids is drug research. In the process of cancer treatment, cancer cells from patients are extracted and cultured for drug screening. However, there are differences between patients in tumor cells, and the drugs used have varying efficacy, necessitating the rapid screening of suitable drugs for patients [84]. The 3D bioprinting organoids show promise to establish the disease model of patients quickly, thus mimicking patient responses to confirm personalized treatment options for suitable patients [11, 89]. 3D bioprinting organoids provide patients with a personalized therapeutic tool to rapidly build a large number of disease models for drug screening via printing of disease-relevant organoids [90]. For example, Tebon et al. group [91] reports a method for producing organoids using bioprinting technology, which is combined with high-speed live cell interferometry to obtain data for 3D models of cancer and allows high-throughput, label-free biomass spectrometry for drug screening. They suggest that the 3D bioprinting tumor organoids share the same histological characteristics as manually seeded 3D organoids and are molecularly indistinguishable from manually seeded organoids. Through combining bioprinting with the cultural method of inducing pluripotent stem cells, joint culture of thousands of organoids and high-throughput manufacturing for drug research can be achieved.

Furthermore, disease models have been prepared by modulating genes in organ tissues, which leads to the construction of various disease models for drug research. For instance, Tran et al. group [92] reports a kidney organoids system that generates thousands of similar organoids which are controlled by genes for therapeutic drug screening. In addition, 3D bioprinting organoids allow for the evaluation of drug safety, including drug toxicity testing and drug metabolism for further therapeutic options [93]. Overall, 3D bioprinting organoids provide effective culture protocols for the numerous experimental subjects required for drug screening.

Regenerative medicine

3D bioprinting organoids hold a significant potential for regenerative medicine. Current research indicates that the organoids are promising in recapitulating the development of human organs, as *in vitro* organoids possess the ability to perform certain organ or tissue functions [85]. Besides, 3D bioprinting enables the rapid construction of a large number of homogeneous organoids, which could serve as organ models to improve organ repair efficiency [94]. Thus, combining 3D bioprinting with organoids technology can be used to improve the application of organoids in regenerative medicine. With the ongoing advancements in 3D bioprinting technology, various human tissues or organs such as heart, kidney, liver and intestine tract have been successfully

modeled using 3D bioprinting organoids technology, demonstrating favorable functional response [95]. This review presents the research progress of 3D bioprinting organoids in regenerative medicine from different 3D bioprinting approaches, especially focuses on VBP organoids. In general, the method of constructing complex organ models through bioprinting organoids have accelerated the development of regenerative medicine and provides new possibilities for organ transplantation.

Extrusion-based printed organoids

Extrusion-based bioprinting is characterized by precisely deposited and controllable extrusion volumes, providing a solid foundation for ensuring uniformly equal-sized organoids spheres. Cadena et al. group [96] fabricates a microchannel scaffold as a tray for organoids culturing with extrusion bioprinting and light curing techniques (Figure 8A). GelMA microchannel scaffold models are printed in a support bath and then be cured by UV light. After inoculation of human umbilical vein endothelial cell (HUVEC) on the wall of the scaffold tubes, the organoids are cultured in the center of the scaffold. After a period of co-culture, HUVEC in the printed channel walls successfully migrate toward and imbed into the organoids. Lawlor et al. group [89] obtains small-sized renal organoids from cultured and differentiated induced pluripotent stem cell-derived (iPSCs) cardiomyocytes and separates

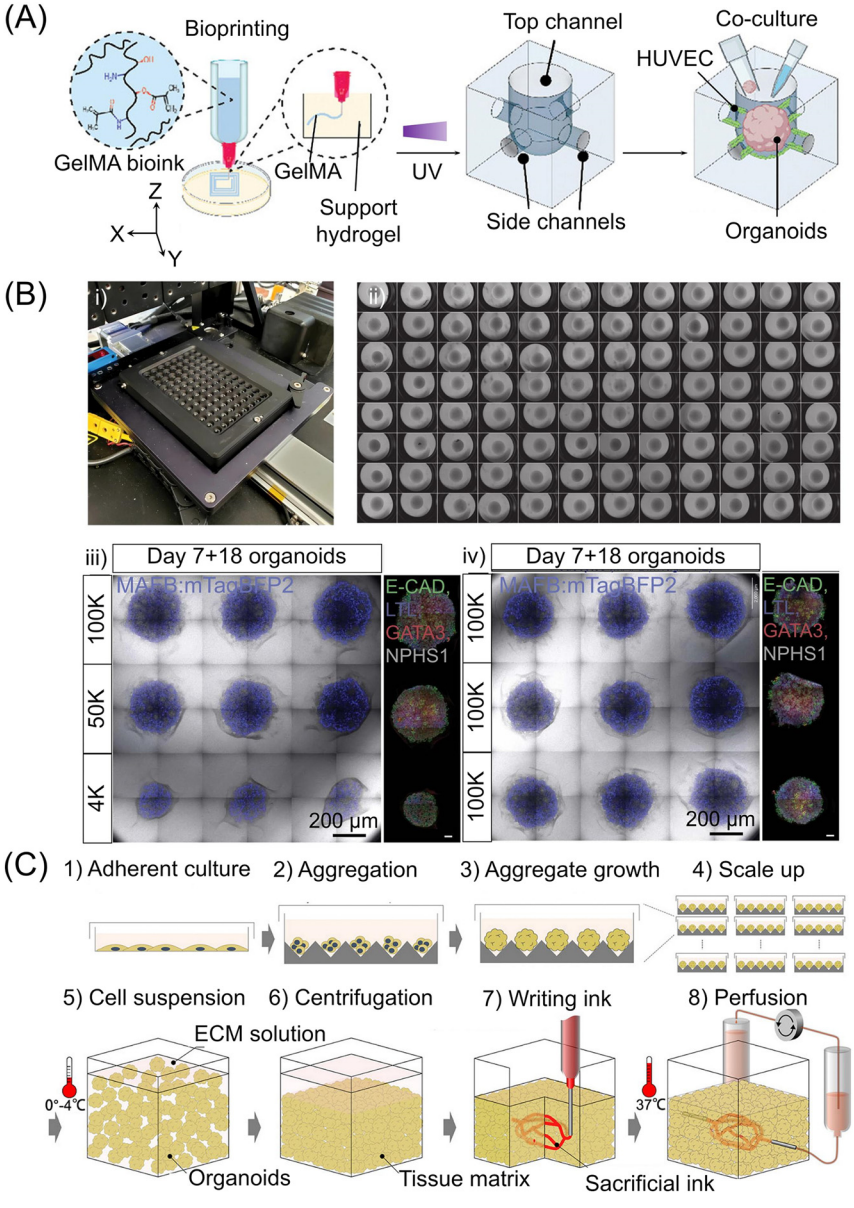


Figure 8: Preparation of extrusion-based bioprinting organoids. (A) Embedded bioprinting a GelMA scaffold with channels, which is seeded with HUVEC and organoids to form the co-culture system between HUVEC and organoids (adapted with permission from Ref. [96]); (B) The kidney organoids obtained from cultured and differentiated iPSCs were homogeneously printed on 96-well plates via extrusion-based bioprinting for drug screening and mechanistic of kidney research. (i) 96-Well plate within plate holder on print stage. (ii) Bioprinted day 7 + 18 organoids within a 96-well transwell format. (iii) $MAFB^{mTagBFP2}$ bioprinted organoids on the same transwell filter with 4 K, 50 K or 100 K of cells per organoid showing fluorescence reporter imaging. Scale bars=200 μm. (iv) $MAFB^{mTagBFP2}$ bioprinted organoids on the same transwell filter all generated using 100 K of cells per organoid showing live fluorescence imaging. Scale bars=200 μm (adapted with permission from Ref. [89]); (C) The sacrificial vessel structure was fabricated in the compacted organoids via embedded printing to deliver nutrients to the organoids (adapted with permission from Ref. [97]). GelMA, gelatin methacryloyl; HUVEC, human umbilical vein endothelial cell; EBs, embryoid bodies; ECM, extracellular matrix; iPSCs, induced pluripotent stem cells.

the organoids by centrifugation to obtain renal organoids solution (Figure 8B). The organoids solution is used as a bioink for extrusion-based bioprinting, and the organoids are homogeneously printed on 96-well plates for drug screening and mechanistic studies. Skylar-Scott et al. group [97] also uses the thermally reversible properties of gelatin to create sacrificial ductal structures via embedded printing among compacted organoids, which deliver nutrients to the organoids and promote organoids culture (Figure 8C). Brassard et al. group [11] uses an extrusion-based bioprinting system which mainly relies on a manual way to complete the power of whole printing to control the bioprinting speed and cell concentration. The bioink forms a stable continuous structure in the ECM solution and is ultimately cultured into 3D organoids in the ECM, opening new avenues for drug discovery, diagnostics and regenerative medicine.

Photo-curing-based printed organoids

Photo-curing bioprinting exhibits high-precision, reproducible capabilities with light polymerization to support high-throughput culturing of organoids. Zhang et al. group [98] uses GelMA as well as alginate to prepare pipe structures by extrusion-based bioprinting and photo-curing techniques, which are used to construct cardiovascular-like models after inoculation of cardiomyocytes on the pipe models (Figure 9A). Carberry et al. group [15] uses 3D bioprinting to construct the complementary crypt-villus structure (Figure 9B). They combine light curing bioprinting and the polyethanol sulfate with adjustable degradation rate. PEG-based bioinks are served as degradable sacrifices for constructing complementary structures of intestinal villi. The structure of intestinal villous in cell-loading Matrigel was gradually patterned by the degradation of the embedded PEG scaffold. Therefore, the

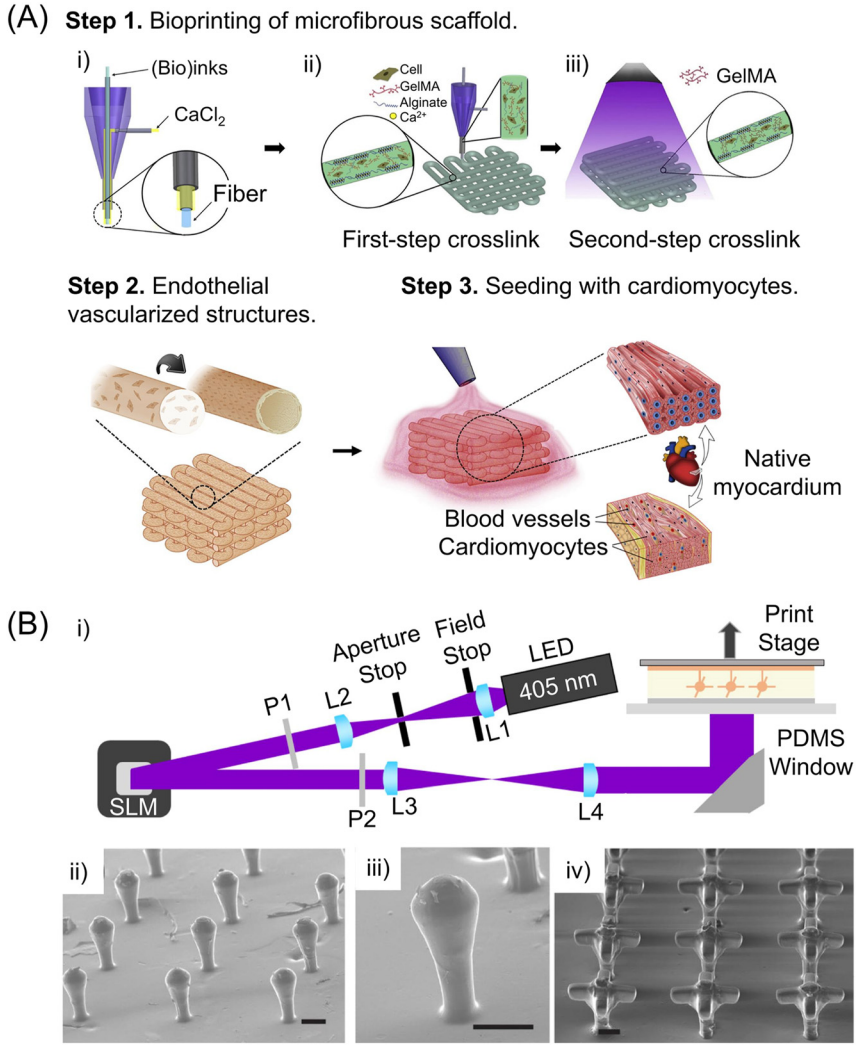


Figure 9: Preparation of photo-curing bioprinting organoids. (A) Combined extrusion-based bioprinting and photo-curing techniques were used to print cardiovascular-like models using GelMA and algin ate with four steps. (i) Schematic of the coaxial needle where the bioink is delivered from the core and the ionic crosslinking $CaCl_2$ solution is sheathed on the side. (ii) Schematic diagrams showing the two-step crosslinking process, where the alginate component is first physically crosslinked by the $CaCl_2$ followed by (iii) chemical crosslinking of the GelMA component using UV illumination (adapted with permission from Ref. [98]); (B) (i) Schematic of DLP 3D printing stage and process. (ii-iv) SEM was used to take images of 3D constructs containing overhang features including: arrays and crypt and branched crypt structures. Scale bars=100 μm (adapted with permission from Ref. [15]). GelMA, gelatin methacryloyl; $CaCl_2$, calcium chloride dihydrate; SLM, spatial light modulator; PDMS, polydimethylsiloxane; UV, ultraviolet; DLP, Digital light process; 3D, three-dimensional; SEM, scanning electron microscope.

stem cells could be guided to proliferation and differentiation from the 3D environment *in vitro*. Han et al. group [99] uses extrusion-based bioprinting with photo-crosslinking to fabricate the tubular intestinal construct. The bioink composed of the colon dECM is demonstrated that could promote the formation of mature cells. Ali et al. group [100] uses materials derived from the ECM of porcine kidneys, which are then methacrylated for fabricating organoids by 3D bioprinting. The bioink consisted of photoinitiators and modified materials and the structures are constructed by UV curing and eventually shows normal function like human kidney.

VBP organoids

VBP breaks through the limitations of traditional bioprinting and allows for high-throughput culturing of highly active organoids with ultra-fast, layer-less printing as well as contact-free features. Bernal et al. group [47] proposes a breakthrough strategy for VBP of complex, functional organoids-loaded structures (Figure 10A). For the first time, they combine VBP with liver-like organs to print centimeter-scale bioprinting constructs with designed structures for enhanced metabolite pathways which are embedded in human hepatic epithelial-like organs derived from primary cells found in intrahepatic bile ducts. Unlike the dense aggregation of normally differentiated hepatocytes, which commonly lack the inherent microstructural features of the liver, the VBP organoids form cyst-like structures with an internal hollow tubular lumen surrounded by a thin monolayer of epithelial cells, which promotes hepatocyte polarization that is critical for the function of the liver [47], indicating that VBP liver organoids improve the viability of printed organoids, and shows a higher

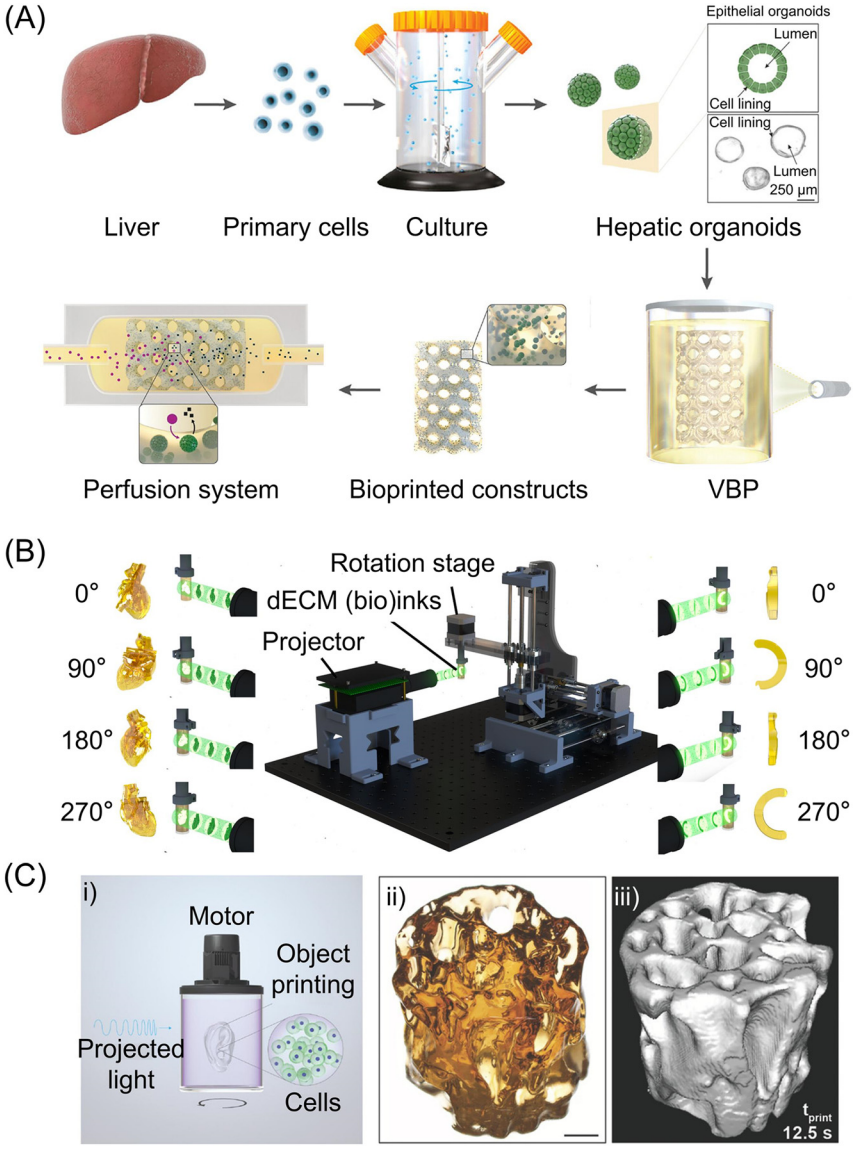


Figure 10: Preparation of volumetric bioprinting organoids. (A) Liver-derived organoids were mixed with GelMA to form bioinks, which were cultured into liver organoids with higher histomorphology and polarization via VBP of porous structures (adapted with permission from Ref. [47]). Scale bars=2 mm; (B) VBP process and the customized VBP system, which mainly includes a projector, a vial-rotation motor, and a (bio) ink-containing vial (adapted with permission from Ref. [46]). (C) (i) Showing the cell-laden gelRESIN reservoir connected to a rotating platform. (ii) The VBP-printed trabecular bone model exhibited a porosity that extended throughout the extended 3D construct. Scale bars=2 mm, (iii) as shown through μCT imaging (adapted with permission from Ref. [17]). dECM, decellularized extracellular matrix; GelMA, gelatin methacryloyl; VBP, volumetric bioprinting; 3D, three-dimensional; μCT, micro computed tomography.

degree of organoids morphology and polarization than other methods.

In addition, our group [46] presents a strategy of pristine, unmodified dECM for VBP (Figure 10B). The VBP myocardial structures of heart-derived dECM (h-dECM) containing human induced pluripotent stem cell (hiPSCs) show good cell proliferation, expansion, spreading, biomarker expression, and synchronized contraction, whereas the VBP meniscus structures of their meniscus-derived dECM (Ms-dECM) containing hMSCs present appropriate chondrogenic differentiation results. Furthermore, Bernal et al. group [17] uses VBP to print mesenchymal stromal cells (MSC)-laden GelMA bioink to construct a human trabecular bone-like structure with convoluted, interconnected porous network that exhibited expression of significant physiological functions after osteogenic differentiation in culture (Figure 10C). It has been shown that the control of microstructures greatly influences the final tissue morphology of organoids, while the ability of VBP to allow for the support-free construction of complex structures and to achieve refined microregulation, which advances the development of organoids technology and offers great potential for the development of advanced regenerative medicine methods.

In fact, beyond the important organs previously described, 3D bioprinting organoids are also used to prepare other tissues or organ-like models as listed in Table 4. In summary, 3D structure provides a suitable living environment that mimics the *in vivo* environment for organoids development, which is supported by the favorable properties of biomaterials, allowing 3D bioprinting organoids models to exhibit higher structural complexity, higher functionality and more plausible therapeutic efficacy than classic organoids in applications such as disease research, drug discovery, and regenerative medicine. Thus, 3D bioprinting organoids have a promising future in the biomedical field.

Challenges and outlook

Currently, 3D bioprinting organoids are utilized in various biomedical applications. These 3D bioprinting organoids could replicate various aspects of human organs, from the surface structures of skin to the intricate networks of blood vessels and nerves, showcasing the remarkable potential of 3D bioprinting organoids technology. However, there are limitations that must be addressed. Firstly, vascularization is

Table 4: Tissues or organs accomplished in 3D bioprinting organoids.

Tissue or organ	Bioprinting methods	Bioinks	Cell types	Application	Reference
Brain	Extrusion-based bioprinting	Alginate, gelatin	Patient-derived GBM cells	Disease model	[119]
	Embedded bioprinting	GelMA, pluronic F-127,	hiPSC	Regenerative medicine	[120]
Heart	Embedded bioprinting	GelMA, alginate, gelatin	hiPSC	Regenerative medicine	[121]
	Extrusion-based bioprinting	hiPSC	hiPSC	Regenerative medicine	[122]
Liver	VBP	GelMA	Liver biopsies	Regenerative medicine	[47]
	Extrusion-based bioprinting	Alginate, matrigel	PSC	Drug research	[123]
	Two-photon polymerization	Gelatin, PEG, matrigel	Human fetal livers	Disease model	[124]
Lung	Embedded bioprinting	Collagen, SF	MDA-MB-231 breast cancer cell	Disease model	[86]
	Inkjet-based bioprinting	SA, HA	A549	Drug research	[90]
	Extrusion-based bioprinting	dECM	Includes patient-derived lung cancer	Disease model	[125]
Kidney	Extrusion-based bioprinting	Differentiated iPSC	iPSC	Regenerative medicine	[89]
	DLP	dECM	Human primary kidney cells	Regenerative medicine	[100]
Islet or pancreas	DLP	GelMA, dECM	Mouse islet β cells	Regenerative medicine	[42]
	SLA	HAMA, pECM	Rat islet	Drug research	[53]
Intestines	Extrusion-based bioprinting	Collagen	hMSC	Regenerative medicine	[11]
	Extrusion-based bioprinting	dECM, GelMA	Small intestinal crypts from mice	Basic research	[126]
Spinal cord	Extrusion-based bioprinting	Gelatin	iPSC	Regenerative medicine	[102]
Bone	Extrusion-based bioprinting	Alginate, gelatin	hMSC	Regenerative medicine	[37]
	DLP	GelMA, AlgMA	BMSC	Regenerative medicine	[49]
Skin	Extrusion-based bioprinting	GelMA	Human keratinocytes	Regenerative medicine	[101]
Neural networks	Extrusion-based bioprinting	Alginate, gelatin	hiPSC	Basic research	[127]
	Extrusion-based bioprinting	Matrigel	Patient-derived cancerous tissues	Disease model	[128]
Glands	Extrusion-based bioprinting	dECM	MCF-7	Disease model	[103]
Vascular networks	Embedded bioprinting	Gelatin	iPSC	Regenerative medicine	[97]

GBM, glioblastoma; GelMA, gelatin methacryloyl; hiPSC, human induced pluripotent stem cell; VBP, volumetric bioprinting; PSC, pluripotent stem cell; SLA, stereolithography; PEG, polyethylene glycol; SF, silk fibroin; SA, sodium alginate; HA, hyaluronic acid; dECM, decellularized extracellular matrix; iPSC, induced pluripotent stem cell; DLP, digital light process; HAMA, hyaluronic acid methacrylate; pECM, pancreatic extracellular matrix; hMSC, human mesenchymal stem cell; AlgMA, alginate methacrylate; BMSC, bone mesenchymal stem cells.

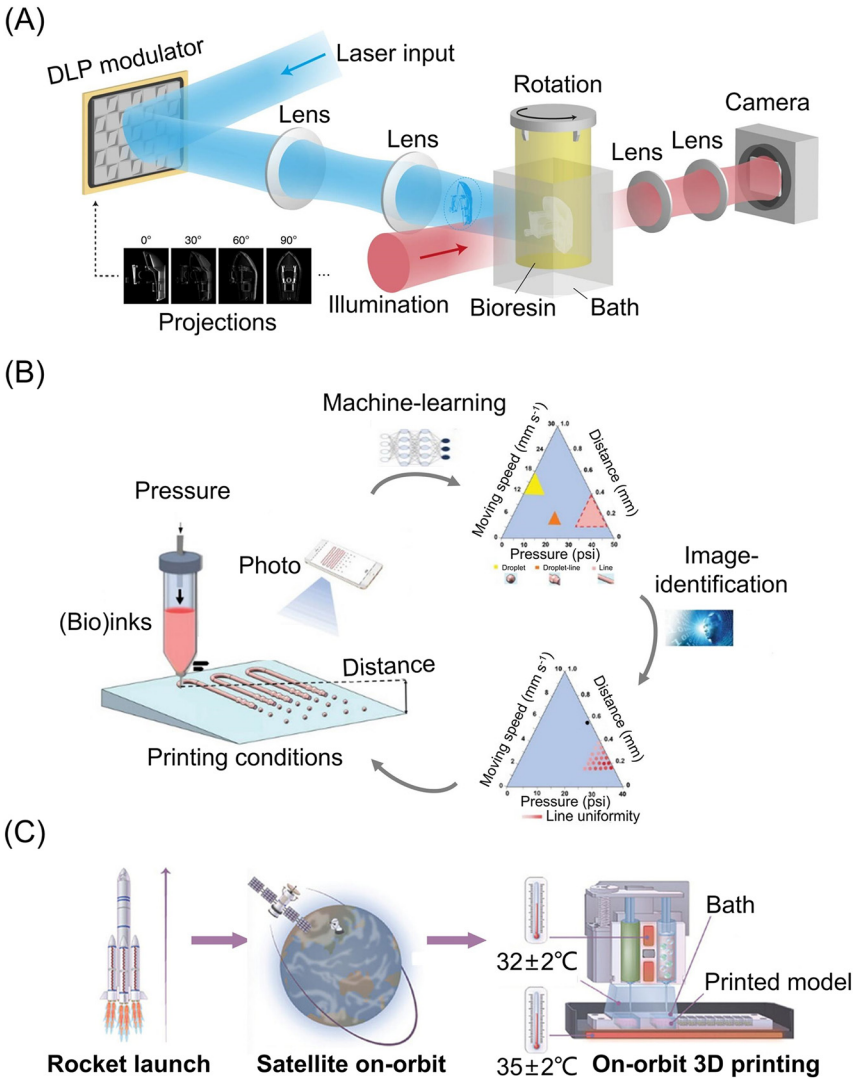


Figure 11: Schematic of potential technologies for promoting 3D bioprinting organoids. (A) Visualizing and recording for organoids culture and the feedback system for 3D bioprinting (Adapted with permission from Ref. [105]); (B) artificial intelligence technology to predict and evaluate the potential of 3D bioprinting organoids (adapted with permission from Ref. [107]); (C) Space 3D bioprinting unlock the potential of 3D bioprinting organoids (adapted with permission from Ref. [108]). DLP, digital light process; 3D, three-dimensional.

an essential process for tissue or organ expansion, however, classic organoids lack the vascular network for nutrient delivery, oxygen exchange and waste removal. As organoids size increases, cells in the center often suffer from inadequate oxygen and nutrient supply, resulting in diminished survival rates and stunted growth [104]. Overcoming these challenges is critical to the development of large and functional organoids. Based on the advances in 3D bioprinting technology, researchers have been able to fabricate *in vitro* platforms with network in which endothelial cells are cultured to construct vascular networks. Organoids are seeded on these vascular networks, and a co-culture system is constructed between the vascular network and the organoids, thus promising to address the challenge of vascularization of organoids [96]. Variability between organoids batches presents another challenge. Most organoids are derived from iPSCs, whose differentiation process is frequently heterogeneous, leading to discrepancies in organoids for drug screening or human organ disease research. While this issue could be ameliorated by 3D bioprinting technology, this variability complicates the establishment of a standardized gold standard for organoids applications and makes it difficult to measure the quality of high-throughput organoids preparations. Future efforts should aim to standardize differentiation protocols and improve the consistency of organoids fabrication [27]. Notably, the structure and microenvironment of different organs and tissues in the body are heterogeneous, and the classic organoids culturing environment is based on Matrigel, which offers simple and easily-used organoids but low scalability, thus it is difficult to scale up. In contrast, a variety of bioinks of 3D bioprinting can be used to dynamically adjust the culturing environment of organoids, and it is possible to create more types and complex structures of disease models to match the requirements of various disease research than Matrigel.

The future of organoids technology will focus on directional modification and the formation of biological structures that more closely resemble complete organs or tissues. For example, the development of technologies in other fields can be used to support research into 3D bioprinting of organoids, such as dynamic imaging. Visualization of the printing process and the dynamic growth process of organoids in 3D structures can also assist in the structural adaptation of 3D printed organoids [105, 106] (Figure 11A). Artificial intelligence technology is a popular assistive technology in recent years, which can be integrated into the 3D bioprinting organoids technology, through the integration of machine learning algorithms to build artificial intelligence models, predict and evaluate the potential of 3D bioprinting organoids for drug response [107] (Figure 11B). Space 3D bioprinting has made new advances with the successful in-orbit printing of complex tumor models on low-Earth orbit research satellites, with the model showing a stable shape and moderate cell viability [108] (Figure 11C). By overcoming the current challenges, researchers can unlock the full potential of 3D bioprinting organoids, paving the way for innovative solutions in personalized medicine, regenerative therapies, and advanced drug discovery. Continued interdisciplinary collaboration and innovation in materials science, cell biology, and engineering will be essential to advance this exciting field.

Research ethics: Not applicable.
Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
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Data availability: Not applicable.

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