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REVIEW

The prospects for bioprinting tumor models: recent advances in their applications

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Abstract

Three-dimensional (3D) tumor models prepared from patient-derived cells have been reported to imitate some of the biological development processes of in situ tumors in vitro. These 3D tumor models share several important characteristics with their in vivo tumor counterparts. Accordingly, their applications in tumor modeling, drug screening, and precision-targeted treatment are promising. However, the establishment of tumor models is subject to several challenges, including advancements in scale size, repeatability, structural precision in time and space, vascularization, and the tumor microenvironment. Recently, bioprinting technologies enabling the editorial arrangement of cells, factors, and materials have improved the simulation of tumor models in vitro. Among the 3D bioprinted tumor models, the organoid model has been widely appreciated for its advantages of maintaining high heterogeneity and capacity for simulating the developmental process of tumor tissues. In this review, we outline approaches and potential prospects for tumor model bioprinting and discuss the existing bioprinting technologies and bioinks in tumor model construction. The multidisciplinary combination of tumor pathology, molecular biology, material science, and additive manufacturing will help overcome the barriers to tumor model construction by allowing consideration of the structural and functional characteristics of in vitro models and promoting the development of heterogeneous tumor precision therapies.

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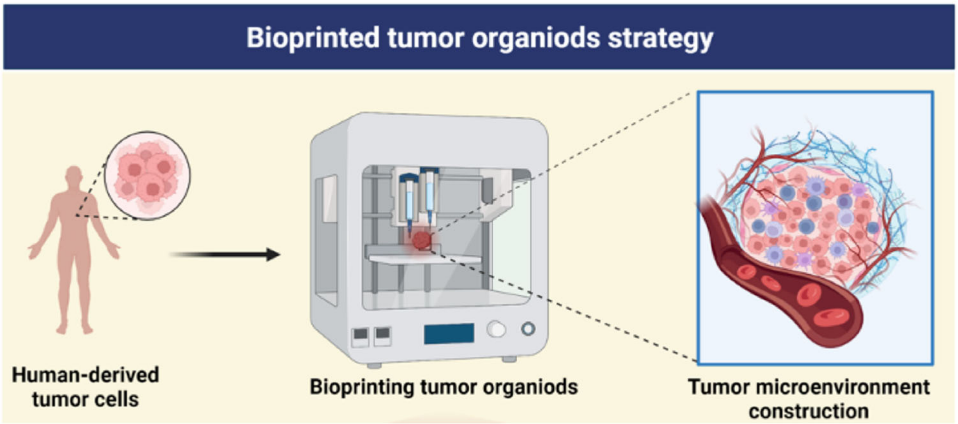
Graphic abstract



Keywords Bioprinting · Tissue engineering · Tumor organoid

Introduction

Tumor models are three-dimensional (3D) models of in vitro cell culture systems that resemble source tissues or organs in vivo [1–3]. Compared with traditional two-dimensional (2D) culture, the in vitro model of 3D culture is more conducive to representing the self-organization of cells, as well as tissue development and maturation [4] (Fig. 1). In tumor models, tumor organoids are of increasing interest to researchers because they maintain a high degree of heterogeneity [5]. A tumor organoid mimics a patient's tumor microstructure in vitro through the use of a sample source of primary tumor tissues [6–8]. The tumor structure contains various specific cell types, forming a spatial structure similar to the corresponding tumor tissues. Organoid culture protocols have been established for several types of tumors, including ovarian [9], lung [10], liver [11], pancreatic [12], gastric [13], breast [14], and colorectal tumors [15]. Moreover, these organoid culture protocols have become increasingly advanced [16, 17]. Animal models are widely employed for tissue regeneration, drug screening, and other research; however, the available animal models fail to comprehensively reflect the pathological characteristics of human tumors [18–20]. The unique in vitro tumor model bridges the difference between human and animal models by precisely mimicking the composition and activity of tumor cells, endothelial cells, and other stromal cells in normal organisms, along with the physiological structure of human tumor tissues.

Tumor models have the potential to be used for gene editing, rapid modeling, high-throughput drug or gene screening, and individualization [21–23]. In addition, they serve as a crucial experimental basis for examining fundamental biological processes in humans by narrowing the gap between simple cellular models and intricate animal tumor models. Given their considerable theoretical relevance and potential application in fundamental biological research, drug detection, molecular medicine, and tumor models have emerged as a contemporary research hotspot [21]. However, the drawbacks of tumor models must be addressed, including the inability to completely replicate the microenvironment in vivo, lack of vascularization and immunization, a slight difference in the size of self-organization between tumor models and normal tumor tissues, lack of coculture systems with other cell types, and an absence of fine spatial ordering [24]. Researchers have resolved some of these concerns by employing bioprinting strategies.

By employing the additive manufacturing technique known as bioprinting, it is possible to generate 3D live tumor tissues by designing and distributing tumor cells where they are needed [25–27]. Prior to the emergence of biomanufacturing technologies, cell self-organization and developmental biology provided a new paradigm for enhanced tissue engineering. A crucial stage in this technology is the molding of self-organizing multicellular modules. One key obstacle is the capacity to precisely combine intermediate modules into larger tissue units [27]. Therefore, the functionalization of tumor tissues is facilitated by the cooperative advancement of cellular self-organization and bioprinting technologies. Bioprinting techniques can be applied to design specific spatial structures similar to actual tumor tissues, enabling high-throughput and precise, rapid printing of tumor cells, vascular endothelial cells, and stromal cells into defined tumor tissue architectures. These "printed" cells may self-organize rapidly

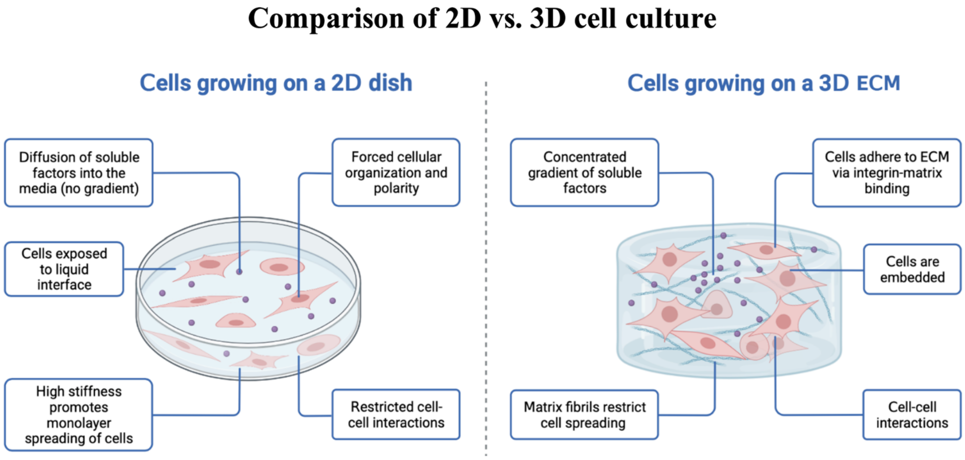


Fig. 1 Comparison of 2D and 3D cell culture methods (created using BioRender.com). ECM: extracellular matrix; 2D: two-dimensional; 3D: three-dimensional

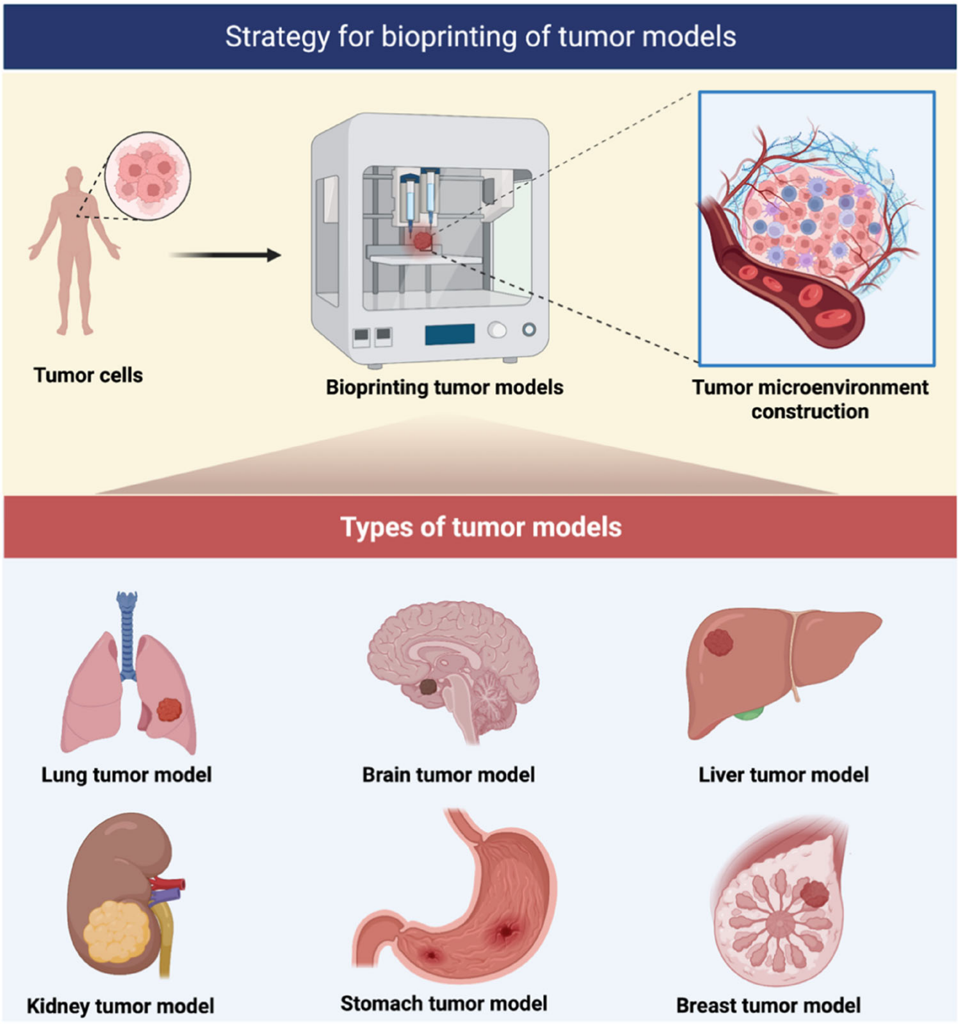


Fig. 2 Schematic diagram illustrating the types of tumor models and their bioprinting methods. Tumor cells of patient origin and related cells are bioprinted to precisely control the spatial and temporal distribution and construct the tumor microenvironment. Created using BioRender.com

and more effectively to create organisms (Fig. 2). Furthermore, stem cell suspensions can self-organize into structures of modest complexity at the millimeter scale, which could be subsequently printed into more complicated tumor tissues. Herein, we examine existing tumor model bioprinting methods and bioinks, highlight current applications of tumor model bioprinting that have been successful, and list plans and future prospects for tumor model bioprinting.

Technology for bioprinting tumor models

Layer-by-layer stacking and additive manufacturing techniques are alternate terms for 3D printing, whereas 3D bioprinting implies the printing process used for printing cells [25, 28, 29]. Existing bioprinting techniques include extrusion bioprinting which is dependent on the molding principles and printing materials (including piston-driven, pneumatic, and spiral) [30], inkjet bioprinting (piezoelectric and temperature-controlled) [31], laser-assisted bioprinting [32], and light-cured bioprinting [33]. Human tissues and organs can be created with biological 3D printing methods using cells, biomaterials, and/or cytokines as bioinks (Table 1). However, to date, no bioprinting technology has successfully generated tumor tissues of various sizes and complexities.

Inkjet bioprinting

The first bioprinting technique used an inkjet printer for cell printing [34, 35]. This technique involved a noncontact printing procedure using a conventional inkjet printing technique that employed piezoelectric or thermally driven nozzles to create a string of droplets in accordance with the bioink-specified 3D structure (a combination of cells and hydrogel) [36]. The advantages of inkjet-based bioprinting include high cellular activity, rapid printing, low cost, and high resolution [37, 38]. Additionally, it allows the simultaneous use of several nozzles for printing with different bioactive materials, cytokines, or cells [39]. Major advances have been achieved in the mapping of tumor cells, proteins, and organoids using inkjet-based bioprinting. Researchers have shown how to create curved structures that can suspend the vasculature without the need for scaffolds using fibroblasts and inkjet-based bioprinting [40]. Using high-throughput inkjet printing, which regulates cell adhesion and proliferation, collagen has also been precisely and automatically deposited [41].

Inkjet bioprinting is unable to print highly viscous materials or dense cell populations owing to the low driving pressure. Therefore, the challenges facing inkjet technology-based bioprinting at physiological tumor cell densities limit the printing of complex tumor tissue structures at high cell densities. Low-viscosity biomaterials render bioprinted objects that are less mechanically stable and fail to provide cells with a similar or comparable tumor microenvironment for successful in vivo and in vitro cultures [42, 43]. Risks include the potential for nozzles to catch during inkjet bioprinting and experience wear and the likelihood of mechanical or thermal harm to tumor cells, placing further restrictions on the broad adoption of inkjet-based bioprinting techniques.

Laser-assisted bioprinting

Laser direct writing and laser-induced transfer are two techniques used in laser-assisted bioprinting [44]. While using these techniques, the ribbon-absorbing layer is exposed to concentrated laser pulses that create high-pressure bubbles. After that, the cross-linked suspended bioink is compelled onto the receiving substrate. Contrary to traditional printing methods, non-nozzle printing techniques such as laser-assisted bioprinting may avoid direct nozzle contact with bioink, decreasing nozzle clogging and mechanical damage to cells and biomaterials [45]. Therefore, laser-assisted bioprinting can produce both high-cell-density and high-viscosity biomaterials. The tumor organoids generated with this method displayed high cell activity and density. Laser bioprinting can be used to create 3D designs for spinal cord healing that have long, axon-like extensions and a high degree of cellular activity [46]. Additionally, with a method utilizing a straightforward crossover procedure with laser-assisted bioprinting, human umbilical vein endothelial cells (HUVECs) were applied to a biopaper surface. To create a network of vascularized tissues, these cells were stretched and differentiated [47].

Laser-assisted bioprinting has several drawbacks. First, it is difficult to commercialize laser-assisted bioprinting devices given their relatively high cost, the complexity of running laser printing systems, and the dearth of hydrogel materials suitable for the process. Second, owing to poor printing efficiency, it is necessary to repeatedly apply each ink layer. However, there is no assurance of uniformity, and the procedure is time-consuming and difficult. Thus, the application of this method for printing intricate structures is challenging. Furthermore, given that the negative effects of laser irradiation on cells remain poorly understood, the application of this approach has been widely restricted.

Extrusion bioprinting

Currently, the most popular bioprinting technique is extrusion-based, which regulates bioink extrusion via a nozzle using pneumatic or mechanical stress [48]. High-density tumor cell suspensions and viscous biomaterials can be printed using this technology [49]. The most prominent advantage of this strategy is the large selection of

Table 1 Comparison of the performance of four bioprinting methods: inkjet, laser-assisted, extrusion, and light-cured bioprinting

Method	Printing speed	Resolution (μm)	Cell density (cells/mL)	Cell viability	Nozzle	Cost	Features
Inkjet bioprinting	High	75	10^6–10^7	High	Based	Low	Equipped with multiple nozzles that are piezoelectric or thermally driven; unfit for substances with high viscosity or cell concentration; printed biomaterial must be in liquid form
Laser-assisted bioprinting	Medium	10–100	$>10^8$	High	Free	High	Few hydrogel materials are laser-bioprintable; poor printing performance; high cell viability (typically >95%) without mechanical damage to cells; the negative impact of laser exposure on cells is unknown; complex laser printing control system
Extrusion bioprinting	Low	100–500	10^8–10^9	Low	Based	Medium	Various printed biocompatible materials; high shear and mechanical stresses; minimal cell viability; printable, highly viscous biomaterials
Light-cured bioprinting	High	50	$>10^7$	High	Free	Low	High efficiency; easy to control; layer-by-layer coagulation and light-based selective cross-linking of bioinks; ultraviolet radiation and its initiating agent may harm cells

printed biocompatible materials, such as hydrogels with shear attenuating and quick cross-linking capabilities, ranging in viscosity from 30 to 6×10^7 mPa·s [50, 51]. Compared with the two methods described above, continuous extrusion pressure provides more shear tension and mechanical load on biomaterials or tumor cell-containing solutions, resulting in the continuous deposition of filamentous strands rather than isolated droplets [51]. The survival rate of printed cells has been shown to be reduced by this technique, which is particularly evident when using bioinks that have a high tumor cell density. Currently, extrusion bioprinting is a widely employed technique for organ generation, and other bioprinting techniques have been developed based on it. An extrusion bioprinter with two nozzles and a motorized *X*–*Z* robot has been created by researchers. It has been reported that using gelatin methacryloyl (GelMA) hydrogels loaded with hepatocytes and fibroblasts could allow for the bioprinting of organs or cell aggregates while maintaining a certain level of sustained biological activity [52]. Additionally, an innovative printing method called bioprinting-assisted tissue emergence has been proposed. For real-time monitoring and accurate control of tissue growth in space and time, this system combines an extrusion printing system with a microscope system to produce a printing system with its own microscopy image [53].

The challenges of oxygen transport and nutrient permeation in seeded cells of hydrogel materials seriously affect the survival, proliferation, differentiation, and other life activities of cells, but the construction of vascular networks is expected to solve these challenges. A research team developed an aqueous-phase embedded extrusion bioprinting method to generate endothelial vascular networks of free-form structures with perfusable interconnected lumens using an interfacial cohesion strategy [54]. Lian et al. [6] developed a uniaxial or coaxial vertically embedded extrusion bioprinting strategy that utilizes a double-layer coaxial nozzle to simultaneously print two bioinks, precisely regulating the diameter and length of the thread-like structures printed in the support base and successfully constructing extremely complex hair follicle tissue structures. The coaxial bioprinting of functionalized monolayer and bilayer hollow vascular tissues combined with high-throughput microfluidics to mimic natural veins and arteries, respectively, achieves anatomical structure, mechanical properties, and important vascular functions [55]. Innovative printing technologies such as embedded bioprinting and coaxial bioprinting also have significant advantages in the construction of complex internal pores and fine structures, and they have broad application prospects in the construction of tumor models and organ-like tissues, addressing the problems of difficulty with print size adjustment and low fidelity of printed structures.

Light-cured bioprinting

Surface-based light-cured bioprinting, e.g., stereolithography (SLA) and digital light processing (DLP), allows for the fabrication of biological 3D models [56, 57]. Light-induced photopolymer molding is used in both procedures. While DLP uses a projector to irradiate and photocure the photopolymer layer by layer, SLA uses a laser to travel from point to point and from line to surface [58]. Digital light projectors are used in light-curing printing technology to efficiently cure the entire bioink surface. Irrespective of the intricate nature of the monolayer structure, printing requires the same amount of time, and the precision is superb [59]. A platform that moves vertically is necessary for the printer in this method. The device is reasonably straightforward to use when compared with previous approaches, and the nozzle-free printing mode circumvents drawbacks such as nozzle clogging and shear pressures that interfere with cell activity [60]. Owing to its capacity for direct self-organization and reasonable regulation of the differentiation of tumor-associated cells, light-cured bioprinting is a potential printing strategy for generating tumors, fibroblasts, stromal cells, other cell assemblies, and tumor organoids. For example, Creff et al. [61] designed intestinal epithelial structures using the SLA method with intestinal cell lines and photosensitive hydrogels (polyethylene glycol diacrylate/acrylic acid polymers). These structures maintained small intestine epithelial cell proliferation and differentiation for up to three weeks. One disadvantage is that ultraviolet (UV) radiation (and agents capable of causing it) may harm tumor cells [62]. As an important benchmark for future biological 3D printing, light-cured bioprinting is predicted to replace extrusion bioprinting, representing an increasingly crucial component of the cell printing approach.

Several new light-curing printing methods have been developed. Bernal et al. [63] molded gelatin hydrogels containing organoids into complex centimeter-scale 3D structures in 20 s by using volumetric bioprinting, a layer-free, nozzle-free technique that does not cause harmful mechanical stress to the organoids, resulting in superior viability and morphological preservation after printing. The bioprinted organoids underwent hepatocyte differentiation, showing albumin synthesis, liver-specific enzyme activity, and significantly acquired natural-like polarization, making it a technology that opens up new possibilities for regenerative medicine and personalized drug testing. Chen et al. [64] developed a 3D printing technique based on digital near-infrared (NIR) photopolymerization (DNP), in which subcutaneously injected bioink is noninvasively printed in situ into customized tissue structures by ex vivo irradiation with NIR light. A personalized ear-like tissue construct with cartilage and a cell-loaded conformal scaffold that can repair muscle tissue were obtained in vivo without surgical implantation. This technology enables noninvasive in vivo 3D bioprinting of tissue constructs, breaking through the limitations of traditional surgical implantation or exposure to trauma. Complex heterogeneous tissues and organs have macromicro-nano-composite topology, and it is difficult for a single printing technology to perfectly render the fine structure at all levels. Therefore, the combination of multiple biological 3D printing means the fabrication of multiple materials, and bioink is headed in an important direction for development.

Bioinks for bioprinting tumor models

The construction of bioprinted tumor models requires the application of bioinks. An ideal bioink would exhibit suitable mechanical properties, biodegradability, biocompatibility, and tumor microenvironment bionic properties [65, 66]. The selection of bioinks for bioprinted tumor models is based on the rheological properties of the bioink, its effect on cell behavior, and its ability to restore the tumor microenvironment in vitro. The tumor microenvironment is a complex environment for the survival and development of tumor cells, which mainly includes peripheral blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, various signaling molecules, and the extracellular matrix (ECM). At present, microenvironment simulation is mainly used to construct tumor model 3D structures by selecting bioink materials for component simulation and adding patient-derived tumor cells and other relevant cells [67]. In addition, various bioinks generate different tumor microenvironments that affect the growth, migration, and self-organization of cells associated with tumor models [60]. Currently, bioinks are based on both natural and synthetic materials (Fig. 3).

For bioprinting, natural raw materials for bioinks include agarose, collagen, aldose, alginate, hyaluronic acid (HA), fibrin, silk protein, cellulose, and ECM [50, 68]. Among the synthetic polymer-based bioinks, polyethylene glycol (PEG), polylactic acid (PLA), and poly(lactic-co-glycolic acid) (PLGA) are the most common [69–73]. Each bioink affords particular benefits and drawbacks and can be adapted to different tumor microenvironments. Agarose, a marine polymer made from seaweed, displays the right mechanical properties but has a low capacity to maintain cell proliferation, which hampers the development of tumor models. It is often necessary to combine agarose with other biomaterials to increase biocompatibility. The first bioprinting method for blood vasculature reported by Norotte et al. [74] employed blood veins and 300–500-μm-diameter supporting cell spheres. Using an agarose-printed mold, spheres were allowed to adhere to each other to create a single vessel. A hydrogel may be generated by rapidly cross-linking the negatively charged polysaccharide agarose with divalent cations. However, no cell attachment sites were observed [75]. Alginate is often combined with other polymers, such as polycaprolactone (PCL) and gelatin, to construct diverse shapes for bioprinted tissues. Collagen, the major component of the ECM, is biocompatible and can be cross-linked by adjusting the pH or temperature. Compared with alginate ink alone, alginate ink combined with collagen and other bioinks yields superior outcomes. The stabilization of polymer systems and tumor model structures will be facilitated by using bioinks containing two or even three biomaterials, making them more receptive to cell division, proliferation, and self-organization. HA is another example of a naturally existing ECM. After gel formation, HA gels are more viscous and exhibit poor mechanical capabilities, and double cross-linking or chemical alterations improve their mechanical properties. To print primary liver spheroids, Skardal et al. [76] developed a flexible hydrogel method based on HA and gelatin. Carboxymethyl cellulose (CMC) is a semiflexible polymer made from cellulose. By appropriately changing the concentration and molecular weight, CMC may be converted into hydrogels that are sensitive to the environment. Markstedt et al. [77] mixed chondrocytes and nanocellulose-alginate complexes to create scaffolds, which appeared as bent moons and ears. A pro-coagulant protein is fibrin. The creation of hydrogels with acceptable biocompatibility and biodegradability using enzymatic coagulation has been shown to be possible. Gruene et al. [78] used natural hydrogels made of fibrin precursors and HA as cellular transporters and environmental materials to create durable vascular networks utilizing laser-assisted bioprinting. Decellularized ECM (dECM) is derived from natural tissues and is a potential bioink with tissue-specific components. To overcome the mechanical limitations of a single ECM bioink, a composite bioink comprising natural polymer alginate and dECM was formulated. The dECM facilitated tissue-specific cell differentiation, encouraged full-layer vascularization of implants in vivo, and improved primary human progenitor cell survival during 3D bioprinting [79]. Natural polymeric materials exhibit excellent biocompatibility and facilitate adherent cell growth and proliferation. However, the stability and scalability of experimental results are frequently challenged by the instability between sources and batches. For example, type I collagen from bovine, porcine, and murine sources exhibits different polymerization, mechanical, and degradation properties [80].

The immunogenicity of animal-derived materials limits their clinical applications. Synthetic polymers have the potential to overcome these concerns by ensuring stable product quality through raw material control and appropriate design of the synthesis process. Polyethylene glycol hydrogels are typical synthetic biomaterials that are frequently utilized in 3D bioprinting because of their excellent biocompatibility. Like most soft biomaterials, the printing accuracy

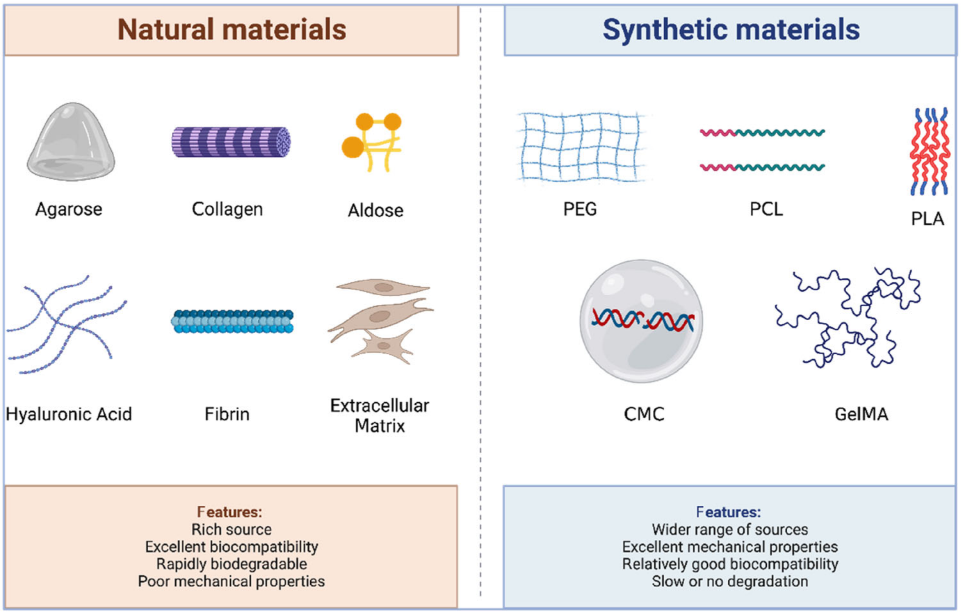


Fig. 3 Bioprinted tumor organ matrix materials. Bioink materials may be derived from natural or synthetic materials. Natural materials tend to have excellent biocompatibility and facilitate cell adhesion and proliferation, while synthetic materials typically afford controllable quality, better mechanical properties, and adjustable degradation rates. Created using BioRender.com.. CMC: carboxymethyl cellulose; GelMA: gelatin methacryloyl; PCL: polycaprolactone; PEG: polyethylene glycol; PLA: polylactic acid

must be improved to correct for the low viscosity before cross-linking. The nonspreadability of bioink, as represented by thiol PEG hydrogel particles, has significant advantages for 3D printing of heterogeneous biological structures, including in vitro tumor models, with a cell survival rate of >90% [81]. Mechanical strength and elasticity are often lacking in traditional hydrogel polymers. A biodegradable tri-block polymer (PCL-PEG-PCL) modified with acryloyl chloride at both ends was designed to address this issue. For cell printing, a variety of human cells, including HUVECs, human aortic smooth muscle cells, and newborn human lung fibroblasts, were combined with polyethylene (glycol)-PCL-diacrylate (PEG-PCL-DA) hydrogels. The cell/material blended system maintained high cell activity after seven days of in vitro culture. This biodegradable hydrogel with a single network structure is extremely elastic and maintains good cell viability [82]. Hull et al. [83] grafted bioorthogonal chemical groups onto the backbone of a hydrogel material via modification of the complementary double orthogonal chemical groups on cross-linker molecules through the strain-promoted azide-alkyne cycloaddition (SPAAC) process. The authors confirmed that corneal stromal cells and neural precursor cells maintained good viability and functional expression within the bioink material, potentially enabling multimaterial and multicellular bioprinting that mimics in vivo structures; this would improve 3D bioprinting to create tumor tissues in vitro and provide therapeutic advantages. PCL is an ester-bonded polymer created via the ring-opening polymerization of cyclohexyl lactone monomers, whereas PLA is an aliphatic polyester. As easy-to-print thermoplastic materials with glass-transition temperatures above 60 °C, PLA and PCL are widely employed in 3D bioprinting. They are primarily utilized as support structures or templates for bioprinting as they cannot be used to enclose cells. Zhou et al. [84] applied a dual extrusion head and temperature-controlled system to construct a novel meniscal bionic scaffold with good biocompatibility and excellent mechanical and biological properties using PCL as the frame support and GelMA/meniscus extracellular matrix/meniscal fibrochondrocytes (GelMA/MECM/MFCs) bioink as the active ingredient. In addition, Ke et al. [85] used human mesenchymal stem cell-loaded hydrogels and PCL for bioprinting tracheal tissue, exhibiting high postprint cell viability (>95%), which could be induced by rapid proliferation and early diffusion of smooth muscle and cartilage hydrogels.

Construction methods of tumor models

Given the advances in precision medicine and organoid cultivation methods, research into tumor organoid models has gained considerable momentum [86–88]. For instance, Drost et al. [89] created a technique for producing 3D prostate organoid cultures of normal mouse and human prostate cells (bulk or fluorescence-activated-cell-sorted single-lumen and basal cells), metastatic prostate cancer cells, and circulating tumor cells. This approach could promote the growth of advanced prostate cancer and intraductal and basal prostate epithelial cell lines. Fujii et al. [90] constructed a model library of 55 colorectal tumor organoids encompassing various types of tumors that may provide light on the genetic and pathogenic pathways behind colorectal cancers and offer

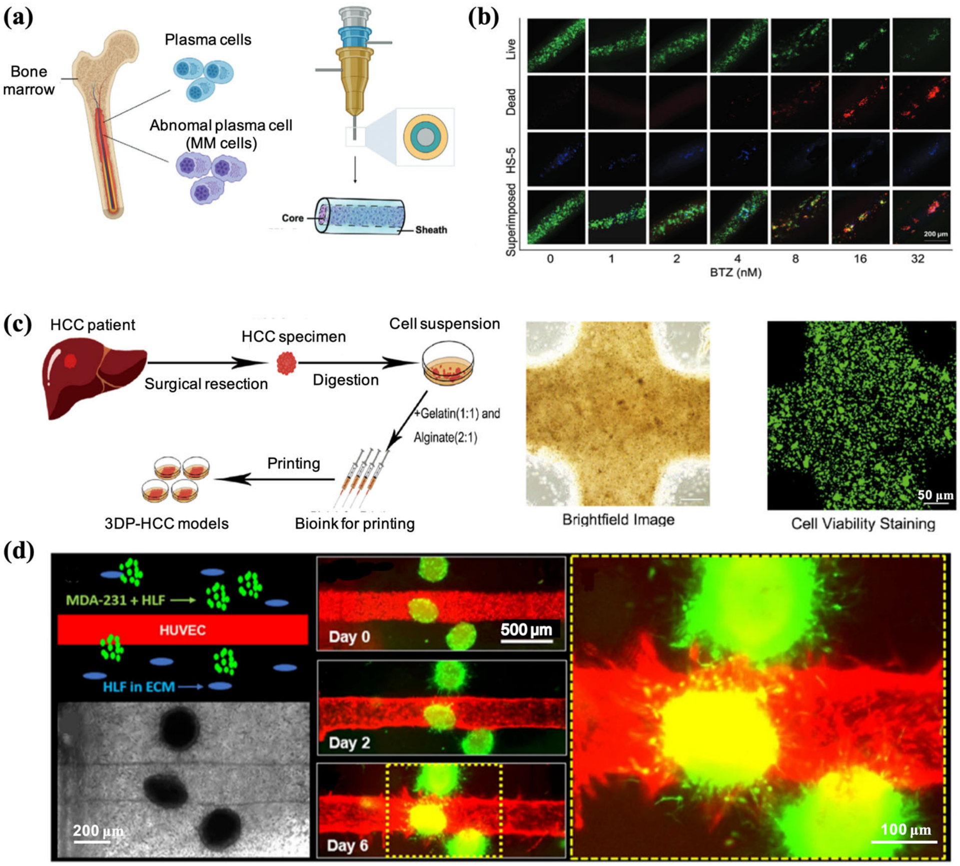


Fig. 4 **a** Schematics of MM in vivo and the use of coaxial bioprinting for highly detailed in vitro modeling of MM. **b** Live and dead staining of MM1S 3D mono-culture treated with various BTZ concentrations. Adapted from [95], Copyright 2021, with permission from Wiley-VCH GmbH. **c** Diagram of patient-derived, 3D bioprinted HCC (3DP-HCC) models (adapted from [97], Copyright 2021, with permission from Elsevier). **d** Schematic representation of HUVEC vascular channels and tumor spheres on a microfluidic chip and tumor globulin-induced angiogenesis (adapted from [99], Copyright 2020, with permission from the authors, licensed under CC BY 4.0). 3D: three-dimensional; BTZ: bortezomib; HCC: hepatocellular carcinoma; HUVEC: human umbilical vein endothelial cell; MM: multiple myeloma

suggestions for patient-centered treatment. However, studies examining the association between tumor organoids and the stroma, as well as that between tumor organoids and the microenvironment, remain limited [91, 92]. By accurately controlling the mix of tumor-associated cells, ECM components, and particular tumor microenvironments and organizing them into exact geographic distributions, tumor microenvironments have been created by bioprinting.

Bioprinting of tumor models

Over the last decade, bioprinting has gained momentum for constructing in vitro tumor models, given its considerable advantages, such as the application of various cytokines and tumor microenvironments, along with its capacity to fabricate complex structures efficiently [86]. Zhao et al. [93] adopted bioprinting technology to construct an in vitro 3D tumor model using Hela cells, a subset of cervical cancer cells. This tumor model was shown to more accurately represent tumor formation and evolution in vivo, providing an accurate representation of the lesion characteristics of cancer cells in vivo. The construction of a tumor microenvironment for a tumor model is crucial. To more accurately mimic the multifaceted tumor microenvironment, Cao et al. [94] constructed a tumor model utilizing a coaxial bioprinting technique that included vascular channels with perfusable lymphatic capillaries encased at one end and combined gelatin hydrogels containing breast cancer cells to create a pair of tumor organoids with vascular and lymphatic organs.

Multiple myeloma (MM) is one of the most common malignant hematologic diseases, and the tumor microenvironment is an important factor in MM biology, affording a potential therapeutic target. Traditional 2D cell cultures may lead to inaccurate testing of medication responses because they are unable to accurately imitate the physiological characteristics of the tumor microenvironment. Wu et al. [95] used a coaxial 3D printing system to simulate the hollow structure of the bone marrow cavity, in which MM cells were cultured to achieve a high in vitro simulation of MM and allow antitumour drug screening (Figs. 4a and 4b). In addition, establishing physical and chemical property gradients is important in simulating the tumor microenvironment. To create localized structures of the vascular matrix around the tumor tissue and oxygen gradients inside the tumor tissue, researchers printed tumor cells, vascular endothelial cells, and the ECM of pig brain tissue into concentric cancer-colored rings [96].

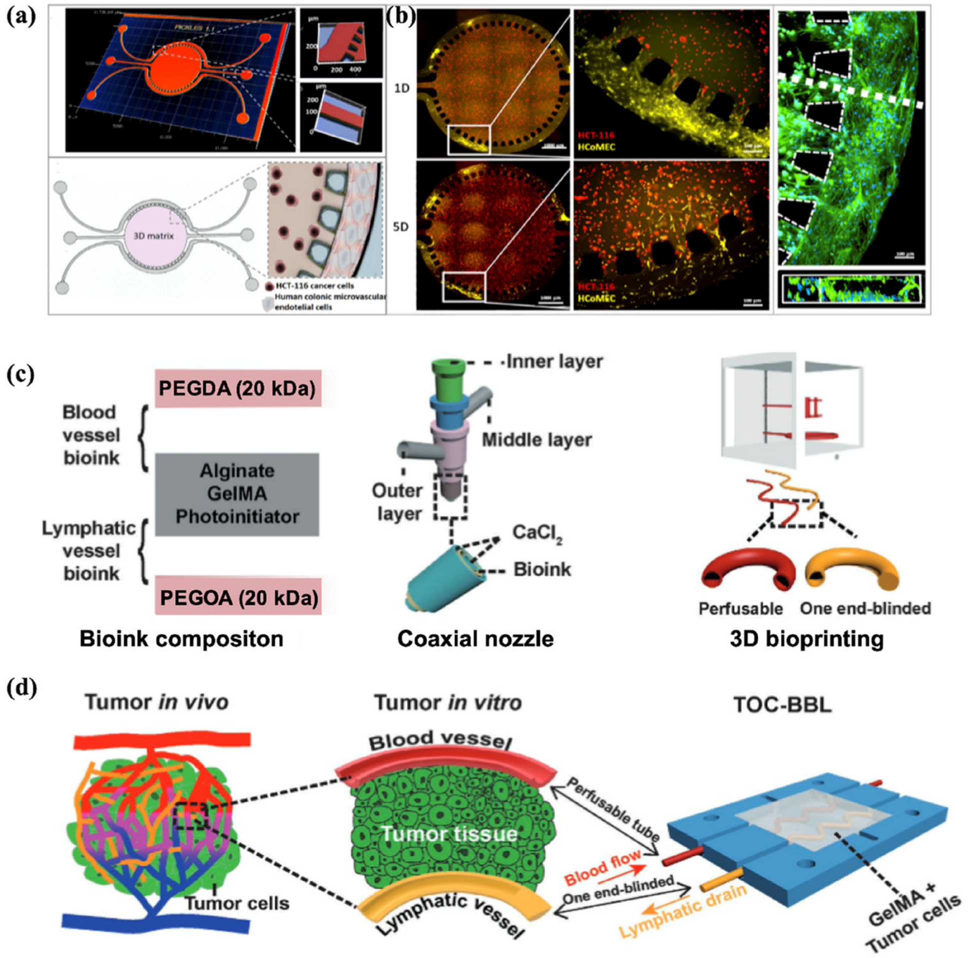


Fig. 5 **a** Microfluidic tumor microarray three-dimensional (3D) structure. **b** Establishment of a microvascular 3D microenvironment for colorectal tumor microarrays. Adapted from [107], Copyright 2019, with permission from the authors, licensed under CC BY-NC. **c** Biological inks for bioprinting blood and lymphatic vessels and the composition of coaxial nozzles. **d** Schematic diagram of a complex tumor structure termed as the tumor-on-a-chip with a bioprinted blood and a lymphatic vessel pair (TOC-BBL). Adapted from [94], Copyright 2019, with permission from WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. PEGDA: polyethylene glycol-diacrylate; PEGOA: polyethylene glycol-octaacrylate

Intrinsic tumor heterogeneity is one challenge encountered during solid tumor therapy, and different genotypes or subtypes of cells can exist within the same tumor. Accordingly, tumors with the same tissue origin can exhibit distinct therapeutic effects or prognoses in different individuals, while individuals can also present markedly different tumor cells. Xie et al. [97] successfully constructed the first personalized model of hepatocellular carcinoma (HCC) by mixing primary HCC tumor cells with a gelatin sodium alginate bioink using a biological 3D printing device, subsequently performing in vitro individualized screening of four common hepatocellular carcinoma-targeting drugs with unexpected results (Fig. 4c). Jiang et al. [98] used microfluidic droplet technology and sheared cell-containing Matrigel into homogenized microspheres, approximately 500 μm in diameter; this was used as a structural template for cellular activity. This platform was used to successfully culture normal mouse liver, lung, and kidney organoids, as well as tumor organoids of different human tissue origins. Whole-gene exon sequencing revealed that the organoid maintained 97% genetic similarity to its original tissue and was representative of tumor tissue gene mutations. Molecular interactions between intratumoral tumor cells and blood vessels are crucial in carcinogenesis and metastasis. Engineered blood vessels lined with HUVECs and tumor spheroids embedded in the ECM were used to develop a solid tumor–vessel interface microfluidic model. This model can provide a framework for drug discovery and mechanistic research on the interplay between solid tumors and functioning circulatory systems (Fig. 4d) [99].

The immune microenvironment of the tumor should be considered in the construction of tumor models, and bioprinting technology enables the embedding of immune cells in tumor models to mimic tumor immunity, which is currently difficult to simulate in vitro. Previous studies have used various strategies to mimic tumor immunity in vivo, such as whole block culture [100] and coculture of epithelial organs and immune cells. Jin et al. [101], for the first time, organically combined 3D bioprinting with immune cells to simulate the environmental characteristics of blood vessels and lymphatic vessels in vivo through the 3D space provided by 3D bioprinting to achieve better simulation of tumor immunity.

It is worth noting that the relevance of the constructed organoid to in vivo disease remains controversial, as this model system fails to consider numerous factors, including native tissue structure, microenvironment, and stroma, with characteristics of most cells altered upon removal from the tissue's native environment. Therefore, constructing organoids containing stroma, immune cells, and multicellular structural mimics remains an unmet challenge. A recent study used a bioprinting method to construct bladder assemblies from stromal fibroblasts, endothelial cells, and muscle layers to develop patient-specific bladder tumor assemblies, employing invasive urothelial carcinoma patient-derived tumor cells to precisely replicate the pathogenic characteristics of in vivo malignancies. The authors revealed a novel mechanism through which signals from the tumor microenvironment determine tumor cell plasticity [102]. These results imply that tumor plasticity is regulated by signal feedback between stromal and tumor cells.

Bioprinting synergizes organ-on-a-chip preparation of tumor models

Organ-on-a-chip methods can effectively control fluid flow for 3D cell culture platforms, enabling researchers to simulate crucial processes such as shear stress, interstitial pressure, and chemical (e.g., cytokines) complex gradients and examine their effects on 3D tumor models in real time, providing an advanced preclinical platform for cancer diagnosis as well as discovery and screening of new therapies [103–106]. Carvalho et al. [107] developed a sophisticated in vitro model using a 3D microfluidic chip to mimic the microenvironment of human colorectal tumors. This model reproduced the physiological function of microvascular tissue and helped assess the efficient delivery of nanoparticle-containing anticancer drugs via a dynamic controlled gradient in the central chamber of the microfluidic chip. This gradient was established and sustained by side channels of the modeled perfusable microvessels. These results confirmed the suitability of the constructed 3D platform for efficacy and toxicity screening in an environment closely resembling the physiological environment (Figs. 5a and 5b). A disruptive strategy for improving cancer-on-a-chip systems into dynamic in vitro tumor organoid models at the tissue scale is the combination of organ-on-a-chip platforms with additive manufacturing technologies. With the help of this technological convergence, it may be possible to create truly bionic 3D models that more accurately replicate the components of the biological environment surrounding tumors as well as their responses to multidimensional fluid dynamics [108]. In a recent study, 3D bioprinting technology was used to reconstruct the in vivo structure of a glioblastoma by printing cancer-stroma concentric ring structures from porcine-derived brain tissue ECM, vascular endothelial cells, and patient-derived tumor cells to better mimic biochemical and biophysical properties and radial oxygen gradients [96]. However, currently available in vitro antitumor drug screening models often lack a true perfusion and drainage microcirculatory system to comprehensively replicate the drug transport and action processes.

Tumor microcirculation should consist of lymphatic vessels in addition to blood vessels. Cao et al. [94] placed 3D bioprinted lymphatic vessels and blood vessels in a hydrogel matrix to simulate the 3D tumor microenvironment. To recreate the intricate transport processes of specific medicinal molecules in the tumor microenvironment, the authors created an in vitro tumor model. A microfluidic system was used to regulate the tumor chip platform, which had blood and lymphatic arteries. This provided a dynamic milieu for the 3D growth of MCF-7 breast cancer cells in a GelMA hydrogel matrix (Figs. 5c and 5d). The recent inclusion of tumor-on-a-chip sensors may also present new avenues to further enhance the potential of real-time high-throughput drug screening on 3D bioprinted cancer platforms through continuous monitoring to accelerate the drug screening process and facilitate clinical decision-making [109].

Conclusions and outlook

Cancer is a globally significant life-threatening disease and is well-known to exhibit intratumor heterogeneity. Tumor heterogeneity is associated with metastasis, recurrence, and drug resistance, potentially resulting in treatment failure or poor prognosis, all of which pose substantial challenges in clinical oncology. Precision medicine requires the urgent development of personalized tumor models to accurately analyze the tumor heterogeneity of individual patients. In recent years, 3D bioprinting technology has improved, and techniques to precisely and simultaneously print multiple materials and cells have been developed. We reviewed the successful construction of tumor organoids using 3D bioprinting technology for tumor models of lung, breast, gastric, liver, pancreatic, colorectal, kidney, bladder, and prostate cancers. We also discussed the simulation modeling of internal vascularization and the immune microenvironment of tumor models to accurately reproduce the structure, specific functions, molecular features, and microenvironment of primary tumors. Three-dimensional bioprinted tumor models have shown considerable potential for reconstructing cellular functions, drug screening, heterogeneous targeting studies, precision therapeutic strategies, and developing novel drugs. There is great synergy among bioprinting and technologies involving single-cell and spatial transcriptomics. scRNA-seq can capture the transcriptome in tumor-associated cell assemblies at single-cell resolution, while spatial transcriptomics allows for analysis of the geographical distribution of transcriptome states. By integrating these technologies and bioprinting based on spatial transcriptomics, various cell types, different cell states, and types of tumor structures can be accurately remapped. With interdisciplinary advances such as material science, engineering technology, and tumor molecular biology, the intersection and integration of various innovative technologies related to bioprinting will allow for the construction of tumor models and organs-on-a-chip similar or identical to actual tumor tissues. The successful construction of bioprinted tumor models will greatly accelerate the screening and development of antitumor drugs by examining tumor tissue structure, molecular mechanisms, and tumor microenvironment interactions.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

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