Review Article

Narrative review of 3D bioprinting for the construction of *in vitro* tumor models: present and prospects

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Background and Objective: The conventional in vitro research on tumor mechanisms is typically based on two-dimensional (2D) culture of tumor cells, which has many limitations in replicating *in vivo* tumorigenesis processes. In contrast, the three-dimensional (3D) bioprinting has paved the way for the construction of more biomimetic in vitro tumor models. This article comprehensively elucidates the features of 3D bioprinting and meticulously summarizes its applications in several selected tumors, aiming to offer valuable insights for future relevant studies.

Methods: A literature search was conducted in the databases of PubMed and Web of Science for articles on 3D bioprinting for *in vitro* tumor model construction.

Key Content and Findings: This article introduces various 3D bioprinting technologies for *in vitro* tumor model construction, focusing on their pros and cons, principles, and protocols. Several *in vitro* tumor models are presented, detailing their utility in tumorigenesis research and their constraints. To date, 3D bioprinting has been widely applied in oncology, addressing the limitation of traditional 2D tumor cell culture in replicating tumor microenvironment (TME).

Conclusions: Advanced 3D bioprinting technology accurately replicates the complex TME and the heterogeneity of intratumor structures, enabling further *in vitro* tumor studies. It significantly fuels our understanding of tumor pathophysiology and offers new hope for cancer patients.

Keywords: Bioprinting; tumor microenvironment (TME); *in vitro* tumor models

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Introduction

Cancer is a major global health threat, being the leading cause of deaths among individuals under 70 years of age in 112 countries (1). Thus, how to systematically treat cancer has become a global concern. In the 21st century, the advent of molecularly-targeted therapies and immunotherapeutic agents has rendered the potential transformation of malignant tumors into chronic conditions or even curable diseases. Nonetheless, drug resistance in tumor cells remains a major obstacle to complete cancer elimination. Even more devastatingly, prolonged treatment regimens are associated with an incremental increase in drug resistance.

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Table 1 The search strategy summary

Items	Specification
Date of search	From 9 March to 25 September 2024
Databases and other sources searched	PubMed and Web of Science (secondary references cited in the searched articles were also reviewed)
Search terms used	("tumor model") and ("3D bioprinting" or "*in vitro*" or "TME" or "bioink")
Timeframe	2006–2024
Inclusion and exclusion criteria	Inclusion criteria: (I) original articles or reviews; (II) English language only
	Exclusion criteria: (I) letters to the editor; (II) non-English languages
Selection process	J.Y.T. and J.Z. independently screened studies included in this review; all the authors contributed to the final version of this manuscript

It has been estimated that multidrug resistance (MDR) is responsible for over 90% of cancer-related mortalities. Therefore, optimal biomimetic tumor models are urgently needed to elucidate drug resistance mechanisms.

The traditional tumor models mainly consist of two-dimensional (2D) cell cultures and animal models. Unfortunately, 2D cell culture systems are inadequate for replicating the intricate three-dimensional (3D) architecture and interactions of tumor cells, not to mention the complex tumor microenvironment (TME). Animal models, although informative, are characterized by their high time and cost requirements, challenges in recapitulating *in vivo* disease progression, and ethical concerns that limit their scalability and utility. The shortage of appropriate *in vitro* tumor models has prevented most anti-tumor drugs from advancing to later-stage studies (2,3). Currently, 3D *in vitro* tumor models have become premier research subjects due to their ideal physiological structures (e.g., biomimetic vasculature) (4) and their capabilities to mimic biochemical signaling (5) and cell-matrix interactions, which effectively recapitulate tumor heterogeneity and complexity, thereby facilitating studies on tumorigenesis and personalized cancer therapies. Techniques such as spheroids, organoids, cancer chip technology, tissue engineering scaffolds, and 3D bioprinting enable the creation of 3D tumor models (6-11).

The 3D bioprinting techniques encompass inkjet bioprinting, extrusion bioprinting, and stereolithography-based bioprinting. Inkjet bioprinting employs thermal, acoustic, or electrical methods to eject bioink microdroplets. Extrusion bioprinting deposits ink via electric, hydraulic, pneumatic, or mechanical tools. stereolithography-based bioprinting constructs 3D structures via laser-induced photopolymerization. The selection of bioinks and biomanufacturing materials is equally important. These biomaterials are either natural or synthetic. In contrast to conventional text-based printing methodologies, the process of 3D bioprinting for tissue fabrication necessitates an integration of cellular components, biomaterials, and growth factors to generate a 3D architecture. Subsequently, the model is combined with a bioreactor to promote tissue maturation and vascularization with appropriate mechanical and chemically stimuli in a necessary perfusion system.

This review article briefly elucidates the applications and potentials of 3D bioprinting for tumor model construction, focusing on the technical processes, success cases, technological challenges, and future prospects. We present this article in accordance with the Narrative Review reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-128/rc).

Methods

A non-systematic literature review was performed to explore recent advancements in 3D *in vitro* tumor models. The final literature search was conducted on 25 September 2024, utilizing the PubMed and Web of Science databases. The search strategy was ("tumor model") and ("3D bioprinting" or "*in vitro*" or "TME" or "bioink"). Secondary references cited in the searched articles were also reviewed (*Table 1*).

Key findings

Bioprinting stages

The three principal stages in bioprinting include: preparation for bioprinting, bioprinting, and post-

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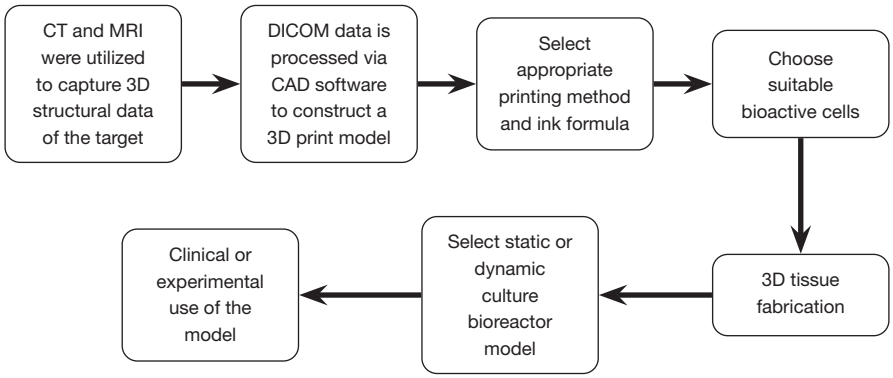

Figure 1 Bioprinting process diagram. 3D, three-dimensional; CAD, computer-aided diagnostic; CT, computed tomography; DICOM, digital imaging and communication in medicine; MRI, magnetic resonance imaging.

bioprinting processing (*Figure 1*).

Preparation for bioprinting

Bioprinting preparation involves two steps: readying the print material and acquiring 3D structural data of the target object. The core of the printing material is bioink, which includes bioactive molecules, living cells, and cell aggregates. These biologically active tissues require prior *in vitro* proliferation and mass storage. Primary ink types include hydrogel bioinks (12,13), cell aggregates, granular bioinks (14), and composite bioinks. Subsequently, imaging data derived from magnetic resonance imaging (MRI) and computed tomography (CT) scans are subjected to software-based processing, resulting in the transformation of these data into model files compatible with bioprinters and thus facilitating the acquisition of 3D structural information for the target object.

Bioprinting

The formal printing begins after the preprocessing. The subsequent step involves choosing bioink, printing method, and printing accuracy, thereby establishing the printing sequence and strategy. At present, the mainstream bioprinting methods include stereolithography-based bioprinting, inkjet bioprinting, and extrusion bioprinting, with the printing accuracy determined by 3D data, printer precision, and bioink properties. This step is pivotal to the whole bioprinting procedure, dictating the rheology (15), bioactivity, and degradation property of the printed outcomes (16). Bioprinted constructs intended for implantation within the body need to interface directly with bodily fluids and viable tissue, and any toxicity or immunogenicity incurred can be fatal. Thus, biocompatibility is a paramount consideration in the selection of bioinks (17).

Within the bioprinting process, the physical and chemical environment of bioinks undergoes substantial alterations. Consequently, it is imperative to maintain the biological viability of cells and tissues throughout the printing procedure. The printability of bioinks must be carefully considered during the selection of its composition, which includes hydrogels, cells, and growth factors. The printability of bioinks is primarily governed by their rheological characteristics and cross-linking kinetics (18). The viscosity of the bioink, which is defined as the resistance encountered by the fluid when subjected to an applied pressure, is influenced by the types, concentrations, and molecular weights of its constituents (19). Generally, printing accuracy is enhanced with higher bioink viscosity; however, an increase in viscosity corresponds to an elevation in shear stress during the printing process, which can be detrimental to the cells contained within the bioink. Crafting optimal bioinks and refining printing techniques, given the above-mentions numerous variables, poses a significant challenge to bioprinting.

Post-processing

After a target object is printed, post-bioprinting processing becomes a top priority to process the object to achieve physiological functionality and maturity. Although static culture is currently the primary technique for scaffold maturation, it has several limitations, including inefficient nutrient and oxygen delivery and difficulty in waste removal (20). Various bioreactors have been designed to address these issues, enabling dynamic culture of cells or tissues. Zhao *et al.* have utilized a bioreactor applying

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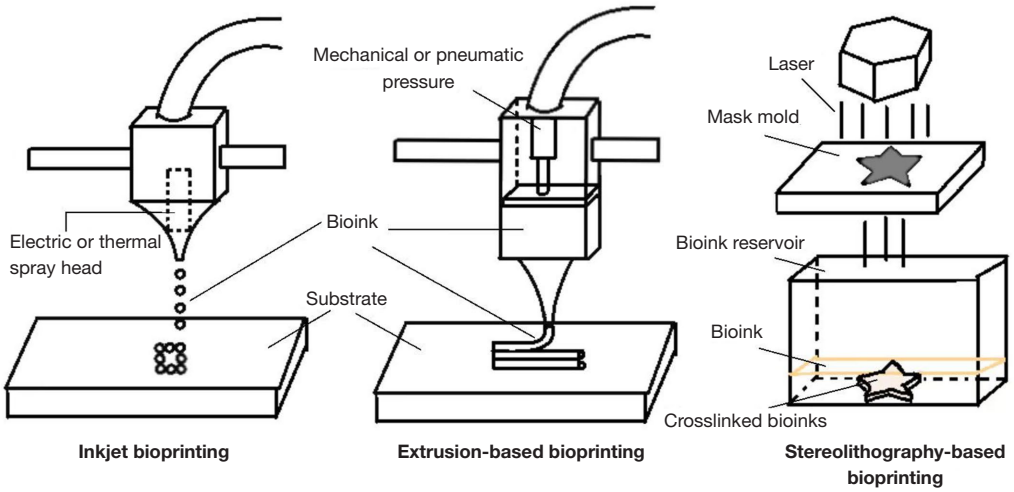

Figure 2 Overview of bioprinting technologies: inkjet, extrusion, and stereolithography-based bioprinting.

hydrostatic pressure to enhance nutrient transport and improve model maturation (21). The dynamic bioreactors can control and monitor pH, oxygen saturation, flow rate, and temperature of culture fluids and offer mechanical/electronic stimulation as required. However, bioreactor selection is based on the intended function and biomechanical setting of the target tissues. According to the Wolff law, bone strength increases in response to higher mechanical loading. Consequently, for the cultivation of bone tissues, bioreactors that simulate compressive forces, shear stresses, and perfusion environments are becoming increasingly prominent in the field (22).

3D bioprinting technologies

Current bioprinting methods primarily include inkjet bioprinting, stereolithography-based bioprinting, and extrusion bioprinting. Each printing method has its strengths and limitations (*Figure 2*).

Inkjet bioprinting

Inkjet bioprinting is an early, cost-effective technique that employs thermal, mechanical, or electromagnetic forces to jet droplets as needed (23). The initial attempt to print bioactive cells with a modified commercial printer resulted in immediate cell death upon substrate contact due to desiccation. Researchers have developed a method to encapsulate cells in highly hydrated polymers to address this issue. Hydrogels, beneficial as highly hydrated materials, typically necessitate milder production conditions. Furthermore, the hydrogel possesses superior ionic conductivity, thereby facilitating the replication of *in vivo* ion transport mechanisms. Most inkjet bioprinters can produce high-cell-viability tissues and spray viable-cell-containing microdroplets with 50–100 μm precision. In addition to high resolution, inkjet bioprinting has many strengths including rapid printing, low cost, and wide applicability; however, it also faces challenges such as low droplet viscosity, poor directional accuracy, low structural stability, and decreased cell encapsulation reliability due to low ink concentration.

Stereolithography-based bioprinting

Stereolithography-based bioprinting is a laser-assisted technique using photopolymerization for the construction of complex 3D structures with specialized bioinks. It can also be used to facilitate precise cell positioning utilizing techniques such as direct laser writing (DLW) and laser-induced forward transfer (LIFT) (24). Hoffmann *et al.* (25) developed a photopolymerization method without photoinitiators by leveraging the thiol-ene reaction between olefins and thiols, aiming to mitigate laser (in particular ultraviolet ray) damage and photoinitiator side effects. Stereolithography-based bioprinting is nozzle-size-independent and less harmful to cells, resulting in higher cell viability and unrestricted bioink viscosity. However, cell damage from direct exposure to ultraviolet ray or near ultraviolet ray remains a technical bottleneck for stereolithography-based bioprinting (26). Photoinitiator-free materials or visible-light photoinitiators may offer new

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solutions.

Extrusion bioprinting

Extrusion bioprinting involves the extrusion of bioinks using air pressure, pistons, or screws to create structures (27). The extruded materials include cell aggregates, cell-laden hydrogels, microcarriers, and decellularized matrices. Ink extrusion speed is typically controlled mechanically or pneumatically. This technology maintains the viability and physicochemical properties of cells, minimizes cell irritation, enables the printing of various cell types, and is cost-effective (28). The bioink's viscosity is crucial parameter in printing: low viscosity prevents the formation of stable 3D structures, whereas high viscosity can cause nozzle clogging, hinder cell-cell interactions, reduce cell viability, and suppress cell contraction (29). Other parameters can also influence the printing accuracy: (I) geometries (i.e., needle type and size) of nozzle opening; (II) printing velocity; (III) extrusion rate; (IV) printing temperature; (V) curing protocol/duration; and (VI) physicochemical properties of bioinks that interact with the substrate, including polymer concentration, shear modulus, and surface tension. Ongoing iteration and refinement of devices and bioinks will further expand the technology's potential and applications.

Tumor models

With the development of technology, the tumor models constructed by 3D printing are becoming increasingly sophisticated. Initially, single tumor cells were printed together with extracellular matrix (ECM) materials through corresponding bioprinting technologies to establish a single-cell culture system with a specific microenvironment, which helps to deeply understand the growth, proliferation and drug response characteristics of single tumor cells. However, single-cell tumor models have great limitations in simulating the complex tumor TME *in vivo*. Later, 3D models with complex cell-cell interactions were constructed by combining various cell types such as tumor cells, stromal cells and immune cells with biomaterials. For example, spheroids are tiny aggregates of cancer cells formed by 3D *in vitro* culture. Due to limited molecular transport, these tumor spheroids can generate oxygen and nutrient gradients similar to those *in vivo*, with a necrotic core, a quiescent intermediate region and a thick proliferative outer shell, which creates a unique opportunity for studying the impact of hypoxia on tumorigenesis, angiogenesis and drug efficacy (30,31). At present, tumor spheroids have been used as a conventional drug screening model to reduce the cost of drug development and unnecessary animal experiments. Due to the existence of tumor heterogeneity among patients, personalized tumor models have developed rapidly. A study has utilized normal endothelial cells and tumor-associated endothelial cells from patients with clear cell renal cell carcinoma to establish different microfluidic vascular models, and determined alternative treatments with two anti-angiogenic drugs, nintedanib and sirolimus. This indicates that microfluidic models can be used to select the most effective and suitable drugs clinically to restore normal vascular function (32).

TME

The TME is a complex architecture consisting of diverse immune cells, stromal components, ECM, cytokines, as well as blood and lymphatic vessels (33). The TME is pivotal in mediating bidirectional signaling that facilitates tumor metastasis and immune evasion, thereby enhancing tumor proliferation (34). The most widely used 2D culture models are only simplified versions of the *in vivo* physiological environment and cannot dynamically represent multiple physiological functions and cell evolutionary processes. In contrast, 3D tumor models can better mimic *in vivo* cell growth environments, simulate multiple cell-tissue interactions, and support *in vitro* cell growth and differentiation. For instance, in a study on the DNA mutations caused by TME, the mutation rate in solid tumors grown *in vivo* was markedly elevated compared to the same tumor cells cultured *in vitro* (35). 3D tumor models can effectively capture tumor heterogeneity, mutation profiles, and drug responses and replicate the complex metastasis and resistance mechanisms, aiding in drug screening. In animal models, human tumors are embedded into a microenvironment composed of animal cells, and such models can not accurately represent human physiological and pathological conditions. Evidence reveals a more than 92% failure rate for drug candidates transitioning from animals to human trials (36), with up to 97% of preclinical anti-cancer drugs failing in clinical phases (37). In contrast, 3D bioprinting will aid greatly in creating reproducible, precise, and biomimetic *in vitro* tumor models.

The metastasis of cancer cells to distant sites mainly occurs through the vascular network (38). Neovascularization can increase the nutrient supply for tumor cells, thus accelerating the growth rate of tumors. Tumor cells can engage in paracrine signaling with endothelial cells,

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prompting the latter to secrete vascular endothelial growth factor, ultimately promoting angiogenesis. After tumor cells invade the bloodstream, they can colonize in other tissues, forming secondary tumor colonies. Most of the tissues invaded and colonized by tumors have certain specificity. Therefore, it is of great significance to study the environmental characteristics of the secondary sites that promote or inhibit tumor proliferation. It is easy to see that hollow vascular-like structures are crucial for *in vitro* studies of tumor metastasis. The tumor model constructed by 3D bioprinting should be composed of a material that can simulate the ECM, and its mechanical properties and bioactivity can be adjusted to reproduce the interaction between cells and the ECM. Currently, light-based 3D printing, sacrificial template 3D printing, etc., can be used to create 3D scaffolds with hollow, perfusable networks. These scaffolds can mimic blood vessels and thus simulate tumor metastasis. Light-based printing technology can also pattern the mechanical stiffness in a gradient, which is used to study the impact of the mechanical properties of the ECM on the local invasion of cancer cells. In addition, the progress of multi-material 3D printing technology has further enhanced our ability to replicate the TME by patterning multiple bioinks. It can construct cell co-culture models with controllable spatial arrangements and simultaneously prepare scaffolds with complex ECM components.

Intrinsic strengths of 3D bioprinting for *in vitro* tumor model construction

The majority of chemotherapeutic agents exert their anticancer effects by directly or indirectly influencing the tumor cell cycle, such as by interfering with DNA replication or mitosis. However, most previous studies have relied on 2D culture models for such investigations. These models lack the spatial heterogeneity in the distribution of nutrients, drugs, and oxygen, and the interaction between tumor cells and drugs does not accurately replicate the *in vivo* conditions (39,40). In contrast, 3D models can more effectively recapitulate the physiological responses to drug treatments observed *in vivo*.

3D bioprinting allows for precise control of the physicochemical properties of tumor cells and ECM. The behaviors (including metastasis and differentiation) of tumor cells are influenced by a variety of factors including ECM stiffness and vessel thickness, which can be accurately manipulated using optimized printing protocols and various bioinks. Soman *et al.* developed a polyethylene glycol (PEG)-based 3D construct with tunable stiffness (0.9–5.5 MPa) for cell migration studies (41). Furthermore, the most recent freeform reversible embedding of suspended hydrogels (FRESH) technology enables more precise control over the biomolecule distribution within hydrogel inks (42). This technique has successfully been applied for the reconstruction of some components of the human heart (43). Furthermore, vascularization is essential for replicating the physiological functions of native tissues *in vitro* (44), and 3D bioprinting along with the microfluid cancer-on-a-chip enable the implantation of blood vessels into the model during the initial printing process. Cancer-on-a-chip platforms are engineered using optically transparent substrates, including polymers, glass, and polydimethylsiloxane (PDMS), and feature integrated microfluidic networks with channel dimensions spanning from tens to hundreds of micrometers. These microscale architectures facilitate precise spatial and temporal regulation of the physicochemical microenvironment, while also recapitulating key aspects of *in vivo* vascular dynamics. Furthermore, these systems can be equipped with biosensing modalities to enable high-resolution, real-time monitoring and quantification of biochemical and metabolic parameters. This advanced functionality allows for the acquisition of critical quantitative data, such as fluid shear stress profiles, interstitial pressure gradients, and spatiotemporal distributions of chemical species within the engineered tissue microenvironment. Hence, to fully harness the synergistic potential of 3D printing and cancer-on-a-chip platforms, it is important to precisely pre-define the orientation and distribution of blood vessels before the initiation of bioprinting process and tailor the vascular network configuration to investigate tumor cell metastasis. Vascularized tumor models using stromal cells and growth factors will greatly enhance tumor migration research.

Utilization of 3D tumor models

3D lung cancer models

Lung cancer is a prevalent and highly fatal malignancy globally (45). Primary treatments for lung cancer include surgery, chemotherapy, and targeted therapy. One of the current research priorities is how to inhibit lung cancer growth and metastasis. Previous research, primarily in 2D culture, failed to replicate *in vivo* TME-cell interactions effectively. In contrast, 3D bioprinting offers a novel, promising method for constructing *in vitro* lung cancer models.

Wang *et al.* employed A549 and 95-D human lung cancer

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cells in an extrusion printing process to fabricate a 3D cell-laden hydrogel multilayer grid scaffold structure (46). The structure comprises eight layers, each 12 mm × 12 mm. Upon the completion of printing, the structure was soaked in a 3% calcium chloride solution for 3 minutes to cross-link sodium alginate with calcium ions, thus reinforcing its strength. In the early stage of the cultures, the 3D lung cancer model exhibited growth rates to the 2D model. After day 8, however, the tumor cell proliferation surged in the 3D model, which was quite different from the slowing proliferation rate in the 2D model. The study revealed that the 3D lung cancer model created by extrusion bioprinting had consistently higher cell density than the 2D model, attributed mainly to the increased space, which delayed contact inhibition and therefore extended the proliferation cycles. The authors also employed matrix metalloproteinases 2 (*MMP-2*) and *MMP-9*, genes associated with invasive migration, as targets for quantitative polymerase chain reaction (qPCR). Additionally, cell scratch assays were conducted to assess the *in vitro* migratory capacity of cells from various sources across different culture systems. The findings indicated a significantly enhanced migratory potential of lung cancer cells in the 3D model compared to the 2D environment.

Mei *et al.* created a lung cancer model utilizing methacrylated gelatin to form a cross-linking network (47). The model was robust enough to integrate a full perfusion system during printing, thus nourishing tissues and cells while clearing metabolic waste. Generally, immunohistochemical analysis of some signaling factors in the apoptosis signaling pathways can be valuable for assessing the cytotoxicity of anticancer drugs on tumor cells. The study tracked gemcitabine-induced apoptosis of H358 lung cancer cells by detecting the levels of caspase-3 and PARP-1 (48), key players in DNA damage and repair. It was found that the 3D model necessitated a gemcitabine concentration 1,000-fold greater than that for the 2D cultured tissues in the dose-response assay, exhibiting IC_{50} values down to 2.5 nM. The dense hydrogel-cell network preserves most tumor cells, even at peak gemcitabine concentrations. Similar findings were reported in many other studies. For instance, the 3D head and neck squamous cell carcinoma models showed reduced sensitivity to cisplatin and cetuximab versus 2D models. Similarly, DU145 prostate cancer cells and U87 glioblastoma (GBM) cells exhibited increased resistance to dasatinib toxicity in 3D models (49). Agena *et al.* (50) also compared the effects of *Caulerpa sertularioides* (CSE) in 2D and 3D SKLU-1 lung cancer culture models. The results demonstrated that the IC_{50} in 2D and 3D culture models were 80.28 and 530 µg/mL, respectively, indicating that tumor cells in the 3D model exhibited significantly stronger drug resistance. The SKLU-1 spheroid tumor model used in this study also provided an excellent platform for investigating the mechanisms and pathways by which CSE triggers apoptosis. Researchers observed that cells treated with 800 and 1,000 µg/mL CSE accumulated in the S and G2/M phases of the cell cycle compared to the control group. Additionally, CSE reduced ATP levels in the 3D culture model, leading to a loss of mitochondrial membrane potential and an increase in apoptosis-related cysteine proteases (caspase-3, -7, -8, and -9), ultimately inducing apoptosis in SKLU-1 spheroids after 24 hours.

3D *in vitro* breast cancer models

Breast cancer is the most common malignant tumor in women worldwide and has remained a leading cause of cancer-related mortality in women due to its high invasion and metastasis potentials. Although lymphatic metastasis is the prevalent form, hematogenous metastasis has garnered significant interest lately (51) as 25% of breast cancer patients initially present with hematogenous metastases. 3D printing for the construction of breast cancer models allows precise reflection of tumor cell proliferation/apoptosis, neovascularization, and signaling pathways. Lugo-Cintrón *et al.* employed a microfluidic device integrated with a 3D hydrogel matrix to investigate the effects of different ECM components on the migration distance of the highly aggressive human breast cancer cell line MDA-MB-231 (52). The results demonstrated that, in the presence of human mammary fibroblasts (HMFs), a fibronectin (FN)-enriched ECM induced greater cancer cell migration and concurrently drove increased secretion of matrix metalloproteinases (MMPs) by HMFs (53-55), thereby promoting tumor progression and metastasis.

3D tumor models can reliably predict the prognoses of estrogen receptor (ER)-positive and ER-negative breast cancers and identify the prognostic genes. Prognostic genes include *AURKA*, *CEP55*, *RRM2*, *EPHA2*, *FGFBP1*, and *VRK1* in ER^+ patients and *ACTB*, *F0XM1*, and *SERPINE2* in ER^- patients (56). Treatments can be selected according to specific prognostic genes for optimal outcomes. Januškevičienė *et al.* constructed a 3D-printed breast cancer model to assess doxorubicin (DOX) resistance relative to 2D cultures and found some cancer cells (e.g., MDA-MB-231 phenotypic sublines) exhibited enhanced DOX resistance in

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3D-printed spheroids. A side-by-side comparison with the model in the 2D medium revealed that hypoxic conditions significantly reduced DOX permeation, which might involve efflux pumps such as hypoxia-inducible factor (HIF) and P-glycoprotein (57). It is therefore speculated that combining DOX with HIF inhibitors or P-glycoprotein blockers might help to combat drug resistance in breast cancer.

3D bioprinting creates *in vitro* breast tumor models such as spheroids, magnetic rings, and coaxial constructs to replicate tumors. Mollica *et al.* pioneered the creation of organoids/tumoroids by using both ECM hydrogels from decellularized breast tissue and 3D bioprinting (58). It is expected that 3D bioprinting will revolutionize breast cancer research.

3D *in vitro* glioma models

GBM is the most prevalent and aggressive form of brain tumor, particularly in individuals aged 65 years and older. According to the World Health Organization (WHO) classification, GBM is categorized as a grade IV tumor, the most severe among brain neoplasms. Typical molecular changes in GBM include mutations in receptors related to signal transduction pathways, such as receptor tyrosine kinases (RTK), phosphatidylinositol 3-kinase (PI3K), p53 and retinoblastoma protein (RB). Despite advances in treatment protocols including surgery, chemotherapy, and radiotherapy, the prognosis of GBM remains extremely poor. Therefore, *in vitro* models are urgently needed to aid research on GBM treatment.

Traditional brain tumor models lack a blood-brain barrier (BBB) (59-61), and therefore fail to mimic *in vivo* drug penetration and are difficult to apply in assessing the final treatment response in an objective and effective way. Currently, the 3D *in vitro* GBM models are predominantly constructed by employing extrusion bioprinting. Yi *et al.* created a compartmentalized cancer-stroma concentric-ring structure using reconstituted GBM tumors consisting of patient-derived tumor cells, vascular endothelial cells, and decellularized ECM from brain tissue via extrusion bioprinting to mimic *in vivo* oxygen gradient in the internal tumor environment (62). This model mimicked the main features of GBM TME *in vivo* and recapitulated patient-specific radiotherapy and temozolomide (TMZ) resistance *in vitro*. This model can be widely used in drug screening and research on the mechanism of GBM metastasis, thus informing the selection of second- and even third-line drugs in patients experiencing drug-resistance to first-line chemotherapy.

The tumor-stromal cell interaction remains a key focus in the applications of *in vitro* GBM models. Heinrich *et al.* employed extruded gelatin bioinks for creating a mini-brain model to study GBM-macrophage interactions in mice (63). Briefly, parts of the mini-brain were printed using mouse GBM-associated macrophages (GAMs), followed by the infusion of mouse GBM cells into the luminal space. Compared with the 2D model, this model had significantly higher expression of interleukin 1β (Il-1β), ECM-remodeling enzymes (Mmp2 and Mmp9), and GBM-specific marker (64), which indicates that 3D microenvironment provides low stiffness, high cell-to-cell contact in 3D space. The model offered a reliable, reproducible, and accessible platform for screening drugs for targeted therapy, immunotherapy, and chemotherapy in preclinical setting. Truong *et al.* developed a 3D microfluidic tumor model featuring a microvascular network by co-culturing patient-derived glioma stem cells (GSCs) with human umbilical vein endothelial cells (HUVECs). In subsequent studies, they found that the microvascular network enhanced the migration of GSCs within the 3D hydrogel, exhibiting invasive patterns highly consistent with those observed in animal models. This model not only facilitates the investigation of GSC metastasis and drug screening but also provides a platform for studying the interactions between GSCs and the TME (65).

Discussion: limitations and prospects

The past decades have witnessed rapid advancements in 3D printing. However, the wide application of this technique still faces many challenges including the technical limitations of bioprinting, material- and cell-related constraints, and problems associated with the long-term viability and clinical use of printed tissues. At present, bioprinting is based on a fundamental logic: layer-by-layer modeling and printing, which hinders the creation of hollow models. Printing hollow models risks the partial loss of structural support from previous layers, resulting in partial or total sag of the pattern, rendering the final product far from what was expected. A common solution involves using sacrificial materials (e.g., gelatin, Pluronic F-127, and carbohydrate glass) for internal scaffold support in hollow structures, and these materials are removed post-printing. Naturally, the adding of novel additional materials makes the printing process even more complex. First, the printing materials or nozzles must be readily replaceable; second, the

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products can be decomposed into biocompatible substances in the subsequent printing stages to prevent varying levels of cytotoxicity, which could impair the utility of the model.

Minimizing print duration is another major challenge. The printing large organs remains slow and can last for up to several days. For the purpose of ensuring the viability and metabolism of the initial printed parts and maintaining the structural stability of the final product, a potential solution may lie in developing bioprinters with increased printing speed and larger nozzle count. Optimizing the geometry of the models to enhance tissue integration may be another promising strategy.

The choice of inks available for bioprinting is still limited and remains a key bottleneck and challenge in this field. A variety of factors must be considered during ink selection, including printability and battery compatibility. Therefore, only a small number of materials (e.g., alginate, gelatin, collagen, fibroin, chitosan, and agarose) have been investigated in published articles (66).

For highly mimicked *in vitro* tumor tissues, research on the heterogeneity of ECM often requires the use of a variety of materials and methods, which requires higher distribution gradients of growth factors and intercellular signaling molecules in tissues and urges the development and use of smarter biomaterials. Artificial intelligence (AI), machine learning (ML), and other technologies can achieve the precise control and optimization of the printing process, thereby boosting model quality and repeatability.

Vascularization is a crucial step for *in vitro* tumor model establishment, and detailed pre-print tissue modeling is required to intricately arrange complete vascular trees for true vascularization (67-69). Culturing a layer of vascular epithelial cells on the inner surface of these prefabricated vascular trees may allow for the formation of more biomimetic vascular tissue. In addition to vascularization, the lymphatic system plays a critical role in tumor nutrient supply, anticancer drug recirculation, and tumor immune escape. However, the incorporation of a functional lymphatic system into *in vitro* tumor models remains challenging. Although many studies have attempted to introduce lymphatic cells to self-assemble into primitive vessel-like structures, the spatial distribution, diameter, length, and tortuosity of these lymphatic structures are difficult to control. This lack of uniformity across different batches of tumor models hinders the observation of the lymphatic system's role in tumor progression. To address this limitation, Cao *et al.* developed an improved tumor model called tumor-on-a-chip with bioprinted blood and lymphatic vessel pairs (TOC-BBL) (70). The TOC-BBL consists of four layers: two inner PDMS layers sandwiched between two outer poly (methyl methacrylate) (PMMA) layers. This model features perfusable curved hollow blood vessels and blind-ended lymphatic vessels. By utilizing a multi-layered, concentric, and coaxial nozzle, different bioinks and crosslinkers for each layer can be co-delivered, ensuring the precision and reproducibility of the printed structures while providing a more physiologically relevant TME model.

The integration of 3D printing and cancer-on-a-chip technologies holds significant promise for advancing the study of tumor proliferation and metastasis. Such *in vitro* tumor models, by enabling precise adjustments to the spatial architecture, mechanical properties, chemical composition, cellular constituents, and the structure and distribution of vascular and lymphatic networks within the ECM, provide a parameterizable platform for investigating cell-cell interactions and cell-ECM interactions. However, a key limitation lies in the acquisition of these parameterized data, which necessitates high-throughput *in vitro* model construction and extensive experimental validation.

The development of various tumor models is advancing swiftly, with many reaching maturity and moving towards commercialization, tremendously aiding oncology progress and anti-cancer drug research and development (R&D). However, the use of models is hindered by the lack of mature analytical methods. Future development of more visual tumor models and quantitative standards will expand the applications of *in vitro* tumor models. Concurrently, its combination with technologies in other fields (e.g., regenerative medicine and tissue engineering) will drive new advancements in cancer research and anti-cancer therapy. *Table 2* lists some of the popular therapies targeting the TME.

Conclusions

This review centers on 3D-printed *in vitro* tumor models, aiming to recreate the *in vivo* TME in an *in vitro* manner. We first outlined the 3D printing process, followed by the description of three most prevalent and commercially viable bioprinting technologies—inkjet, extrusion, and stereolithography-based bioprinting—highlighting their pros and cons. Next, we introduced the concept of TME, emphasizing the huge strengths of 3D bioprinting in replicating TME. Several successful 3D-printed *in vitro* tumor models are described. Finally, we concluded by listing

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Table 2 Current popular therapeutic approaches according to the research of TME

Therapy	Action mechanism	Associated targets
Immune checkpoint therapies (ICTs) (71)	Reactivate T cell cytotoxicity	PD-1, PD-L1, CTLA-4
HSPs (72)	Trigger cellular heat shock response	HSP70/HSP90
Chimeric antigen receptor T-cell therapy (73)	Enhances T-cell function	CD19, CD20, CD22, CD23, CD30, ROR1 (74), etc.
CIK cell therapy (75)	Enhances T cell cytotoxicity	IFN, IL-2, etc.
Blocks tumor metabolism (76)	Inhibits cancer cell metabolism	AMPK, PKM2, etc.
Blocks tumor blood vessel growth (77)	Suppresses angiogenesis	VEGF, HIF-1a, c-MET
Facilitates the regulation of apoptosis (78)	Induces tumor cell apoptosis	Bax/Bcl-2, TNF, Fas/FasL, STAT3, etc.
Cancer therapy (79)	Efficacy via cancer cell cytotoxicity	Induces DNA damage and mitotic arrest
Bacteriotherapy (80)	Modulates immunity or targets cancer cells via live bacteria or microbial agents	Bacteria, viruses, and other microorganisms
Photodynamic therapy (PDT) (81)	Eliminates cancer cells using reactive oxygen species	Light-activatable compounds

AMPK, adenosine monophosphate-activated protein kinase; Bcl-2, B-cell lymphoma-2; CIK, cytokine-induced killer; c-MET, cellular-mesenchymal epithelial transition factor; CTLA-4, cytotoxic t-lymphocyte associated protein-4; DNA, deoxyribonucleic acid; Fas, first apoptosis signal; HIF-1a, hypoxia-inducible factor 1 Alpha; HSPs, heat shock proteins; IFN, interferon; IL-2, Interleukin-2; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; PKM, pyruvate kinase muscle; ROR, receptor tyrosine kinase-like orphan receptor; STAT3, signal transducer and activator of transcription 3.

current bioprinting limitations and future prospects for 3D bioprinting. Overall, 3D bioprinting is pivotal in oncology research and anti-cancer drug R&D. Despite the current limitations, it is expected to yield significant advancements in the coming decades.

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