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Advanced tumor organoid bioprinting strategy for oncology research

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ABSTRACT

Bioprinting is a groundbreaking technology that enables precise distribution of cell-containing bioinks to construct organoid models that accurately reflect the characteristics of tumors *in vivo*. By incorporating different types of tumor cells into the bioink, the heterogeneity of tumors can be replicated, enabling studies to simulate real-life situations closely. Precise reproduction of the arrangement and interactions of tumor cells using bioprinting methods provides a more realistic representation of the tumor microenvironment. By mimicking the complexity of the tumor microenvironment, the growth patterns and diffusion of tumors can be demonstrated. This approach can also be used to evaluate the response of tumors to drugs, including drug permeability and cytotoxicity, and other characteristics. Therefore, organoid models can provide a more accurate oncology research and treatment simulation platform. This review summarizes the latest advancements in bioprinting to construct tumor organoid models. First, we describe the bioink used for tumor organoid model construction, followed by an introduction to various bioprinting methods for tumor model formation. Subsequently, we provide an overview of existing bioprinted tumor organoid models.

1. Introduction

Malignant tumors are a significant public health problem and one of the leading causes of mortality globally [1]. It was estimated that tumors will impact approximately 28.4 million people globally in 2024, posing a significant risk to human health [2,3]. Owing to the complex heterogeneity of tumors and insufficient understanding of tumor development and invasion mechanisms [4,5], it is essential to increase the knowledge of tumor development and explore effective treatment methods.

Various *in vivo* and *in vitro* complex tumor models have been developed to advance the study of tumor pathology and promote progress in anti-tumor therapy. The 2D cell culture model provides hypothetical results related to the pathogenesis of tumors. However, the *in vivo* microenvironment is far more complex than that in 2D model, where the behavior of tumor cells is regulated by interactions between cells, cell-extracellular matrix interactions, and chemotaxis [6]. This can sometimes lead to contradictory results [7,8]. A large body of evidence suggests that three-dimensional cell culture models (3D models) are more physiologically relevant than 2D cell culture models. This has led to widespread adoption of 3D culture techniques to establish more reliable and complex tumor models [8,9]. 3D models allow for the replication of tumor migration and proliferation *in vivo* [10], and more accurately reflect tumor responses to anti-tumor drugs [11–14]. Conversely, xenograft models in mice exhibit significant potential but fall short in simulating tumor-specific microenvironments as the tumor stroma is typically replaced by host stroma. Additionally, tumor model constructed in immune-deficient mice cannot simulate interactions between tumors and immune cells, and issues such as ethical concerns, high costs, and technical differences make it a great challenge [15]. The extensive use of *in vitro* models has demonstrated their potential for application in medical tissue engineering [16–18].

Traditional 3D printing technology, also known as additive manufacturing or rapid prototyping, forms 3D geometric shapes by depositing inert materials layer by layer using computer-aided design [6,19]. The materials typically used in traditional 3D printing are geared towards non-biological substances such as plastics, metals, and ceramics. High temperatures and pressures are required during the printing process for material modification, catering to the needs of industries within the engineering and design sectors. As an extension of tradition 3D printing, bioprinting offers many advantages over

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traditional 3D printing [20,21]. Bioprinting is the process of manipulating cell-containing bioinks to create living structures [22–24]. By selecting appropriate printing methods, biologically active materials are printed layer by layer onto a receiving substrate or liquid reservoir. This enables efficient, cost-effective, consistent, and high-throughput creation of tumor organoid models containing complex geometric structures [25–27]. As bioprinting technology continues to mature, 3D scaffolds produced through bioprinting allow for the precise distribution and positioning of cells, active molecules, and biomaterials, enabling control over the shape and size of tumor organoids constructed using bioinks [28]. Furthermore, the complex structures created through bioprinting simulate the heterogeneous characteristics of the complex 3D tumor microenvironment, including cell arrangement, morphology, migration, and invasion, as well as cell-cell and cell-matrix interactions [29,30]. Consequently, tumor organoid models with different configurations and complexities have been studied for oncology research and drug discovery. Hence, bioprinting has become an ideal method for constructing *in vitro* tumor organoid models with batch-to-batch consistency and replicability [7,31] (Fig. 1).

The bioactive substances used for bioprinting can be called "bioink," and is composed of biomaterials and biological units [32]. Bioinks are crucial for developing functional tissue or organoid structures via bioprinting [33]. Bioinks have the following characteristics: printability, biocompatibility, favorable mechanical properties, and biological stability [34–36]. Therefore, to successfully construct tumor organoid models, an appropriate bioink should be selected based on the structure of the tumor tissue to be printed and the specific tumor biology behaviors intended to be investigated.

Although bioprinting for constructing *in vitro* tumor organoid models encompasses various methods, it is mainly based on three core techniques: extrusion, inkjet, and light-curing bioprinting. Extrusion-based bioprinting is the most commonly used printing method [37]. This method forms continuous filaments through extrusion and stacking [38], whereas inkjet-based bioprinting forms tumor organoid structures by printing discrete droplets [39]. Light-curing bioprinting can enhance the resolution of printed structures by solidifying them layer by layer [40]. Appropriate biomaterials, referred to as bioinks, are selected based on the tumor tissue structure and the expected printing method. The precursor structures of tumor organs can be established through layer-by-layer stacking, and stable 3D scaffolds can be created using suitable crosslinking methods. As each printing method has specific applications, the desired hardness, spatial structure, and cellular composition of the tumor model should be considered when selecting the appropriate printing method.

Bioprinting is deemed as a novel and promising technology in constructing tumor organoids [41]. Due to the precise and controlling character of bioprinting, a variety of cell types and ECM can be set at pre-designed location. This technology enables the construction of organoid models with cellular diversity and complexity, allowing for more realistic organoid model development by controlling the layers and composition of bioprinting. Additionally, bioprinting technology can build 3D structures by layer-by-layer stacking cells and scaffold materials, thus enhancing the long-term stability and manipulability of organoid models. Most importantly, bioprinting technology enables the customized construction of organoid models such as utilizing patient's autologous cells to build individual organoid that can recurrent personal disease mechanisms and, drug response. In conclusion, researchers can leverage bioprinting technology to construct intricate organoid models, leading to a better understanding of the structure and function of biological systems and advancing the development of biomedical research and applications [42].

In general, 3D bioprinting is an innovative technology that is leading the way for conventional *in vitro* and *in vivo* cultivation models. Unlike 2D cell culture models, 3D bioprinting can precisely construct structures composed of cells and biological materials in a three-dimensional space, allowing for a more realistic simulation of the biological environment. Unlike traditional 3D models that typically use non-biological materials like plastics or metals, bioprinting technology utilizes biological materials and cells to create models, resulting in more biologically similar tissue structures and thus a more realistic biological environment. Bioprinting technology can avoid animal experiments, reduce the use of animals and related ethical issues, and provide a more sustainable, stable, and controllable experimental environment. Compared to traditional co-culture organ models where tumor cells are directly co-cultured with other cell types, bioprinting allows for precise control of cell positioning and distribution during the model construction process. The advantages of traditional co-culture models lie in their simplicity and cost-effectiveness, requiring no special equipment or complex operations. However, the unpredictable cell interactions lead to variability in experimental results. Additionally, for biological entities with complex geometric shapes and microstructures, the inability to control interactions limits their ability to simulate real biological scenarios,

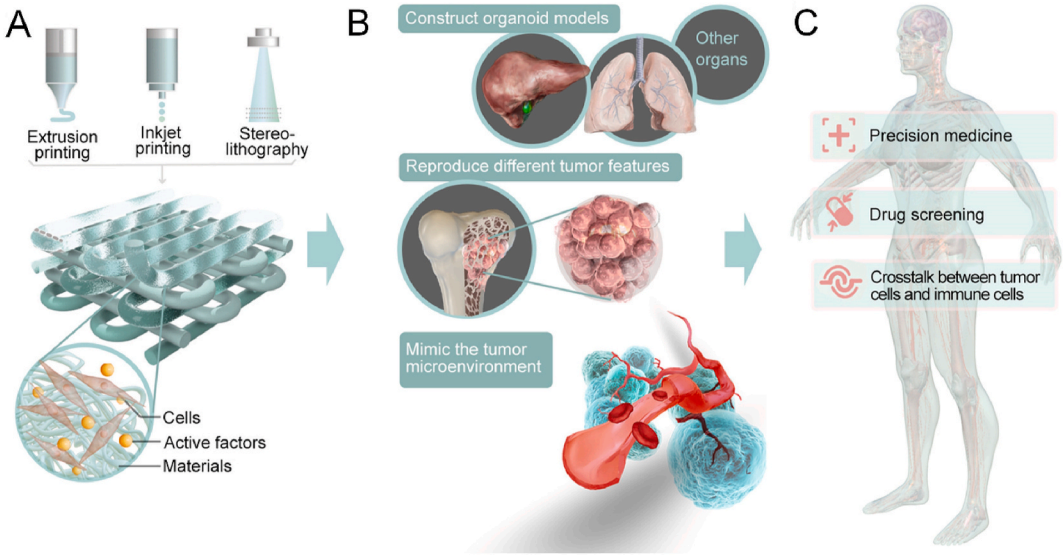

Fig. 1. Schematic illustration of the characteristics and applications of bioprinting to construct tumor organoid models. (A) Deposition of bioink using different bioprinting methods. (B) Prominent advantages of bioprinting methods in constructing tumor organoid models. (C) Potential application value of tumor organoid models.

highlighting the advantages of 3D bioprinting [43,44].

In this review, we summarize the research on constructing tumor organoid models using bioprinting technology over the years. Literature on the construction of *in vitro* tumor organoid models by bioprinting is listed in Table 1. Finally, we provide prospects for the future development of bioprinting technology to motivate further oncology research.

2. Materials and strategies for bioprinting

2.1. Bioinks

2.1.1. Alginate-based bioinks

Alginate is a polysaccharide mainly derived from brown algae and bacteria and is, widely used in bioprinting because of its excellent biocompatibility, low cost, rapid gelation, good printability, and versatility [84,85]. Alginate-based bioinks typically refer to bioinks containing alginate, which can be prepared by adding components such as alginate, crosslinkers, and cell suspensions. This type of bioink is commonly used in 3D printing systems. During the preparation process, alginate can serve as the scaffold material for the bioink, providing structural support and a conducive environment for cell growth. A low concentration of alginate-based bioink promotes cell activity and proliferation, but significantly reduces the mechanical strength of the 3D printed structure, leading to structural collapse. Conversely, a high concentration of alginate-based bioink decreases cell viability [86], limiting its application in simulating tumor organ formation. Moreover, alginate forms chemical crosslinks with divalent cations such as calcium (Ca^{2+}), strontium (Sr^{2+}), and barium (Ba^{2+}), resulting in immediate gelation, with the sol-gel transition temperature being below 0 °C. Therefore, using alginate as a standalone bioactive material for bioprinting is difficult. Typically, substances such as gelatin are added as physical crosslinking agents to enhance the stability of printed structures. Bioinks that form a fixed structural network through crosslinking to enhance stability and shape retention prior to printing are referred to as pre-crosslinked bioinks. Pre-crosslinked bioinks form a structurally stable scaffold during the printing process and provide a conducive environment for cell growth [49].

Owing to their excellent biocompatibility, rapid biodegradability, and chemical gelation properties, pre-crosslinked alginate-based bioinks can be bioprinted using extrusion-based methods to construct soft-tissue tumor organoid models, such as breast tumors, glioblastomas, and lung tumors. Alternatively, 3D vascularized tissue models with controllable vessel wall thicknesses can be printed using coaxial nozzle-assisted crosslinking [87,88].

2.1.2. Gelatin-based bioinks

Gelatin is a biologically sourced material obtained through the acidic or alkaline hydrolysis of collagen. It is a readily available water-soluble and highly biodegradable polypeptide that exhibits good biocompatibility [89]. At 28 °C, gelatin demonstrates unique thermally reversible gelation behavior, enabling the temperature or concentration of cell-loaded gelatin solutions to be conveniently adjusted to achieve the desired 3D printing structure [90,91], making it particularly attractive as a bioink. Therefore, gelatin-based hydrogels with specific thermo-responsive properties enable cells and bioactive substances to be extruded through the nozzle or needle of a 3D bioprinter. In this way, they can be stacked into layers in a relatively mild environment to form predefined 3D structures that support cell growth while maintaining extremely high cell viability. The versatility, biocompatibility, and high bioactivity of gelatin-based bioinks are widely utilized in high-throughput drug screening and the creation of organotypic tumor models with specific tissue structures.

However, the poor mechanical properties of gelatin limit its application as a bioink. The stability of printed structures can be improved by adding alginate and fibronectin and forming chemical crosslinks in gelatin-based bioinks [92]. By adding modifiers, gelatin-based bioinks can serve as both a support structure and a source of RGD peptides [93,94], providing the necessary biological signals for tumor cell migration. As a cell adhesion sequence, RGD peptide can bind with integrins on the cell surface, thereby enhancing cell adhesion and interactions within the biological scaffold. This simulated cell-matrix interaction contributes to better understanding and studying the mechanisms of tumor cell migration and invasion, providing crucial guidance and insights to unravel the process of tumor metastasis [95,96].

The amino groups in gelatin can be chemically modified with methacrylamide groups (such as chloro-methacrylate, glycerol methacrylate, and methacrylic anhydride) to form a hybrid gelatin-methacrylate hydrogel [97], which enhances the adhesion and printability of bioinks under physiological conditions [98]. Owing to the presence of an Arg-Gly-Asp (RGD) sequence and matrix metalloproteinase (MMP) degradable motifs in the polymer chain, GelMA demonstrates strong cell adhesion and migration capabilities. The cross-linking of functional groups added to the gelatin backbone by photocrosslinking or enzymatic cross-linking, along with temporal and spatial control of the cross-linking process, enables the manipulation of the GelMA-based bioink design and properties. This significantly improves the mechanical performance and shape fidelity of 3D-printed structures [99,100]. The GelMA bioink is often combined with photopolymerization-based bioprinting methods such that is rapidly solidify into finely structured microchannels with high shape fidelity in specific regions [101].

2.1.3. Collagen-based bioinks

Collagen is an abundant component in animals and a primary component of connective tissue with a triple helix structure. Various types of collagens, including Types I, II, III, IV, and V, are used in tissue engineering research. Type I collagen is widely used in bioprinting because of its ability to self-assemble. However, Type I collagen crosslinks slowly at 37 °C, which may result in insufficient structural stability in the later stages of bioprinting and lead to uneven cell distribution. In addition, the low viscosity and rapid degradation of pure collagen bioinks severely limit their application as "bioinks" in bioprinting tumor organoid models. Other compounds, such as alginate and hyaluronic acid, have been incorporated into collagen hydrogels to enhance viscosity, reduce degradation rate, and improve the printability of natural collagen.

Natural collagen molecules contain the same RGD peptide domain as gelatin [102], contributing to cell adhesion, proliferation, and differentiation. In addition, tumor-related modifications and remodeling of collagen proteins are key factors that enhance tumor invasion and metastasis [103,104]. Therefore, Type I collagen is typically used as an internal cell carrier. In contrast, compounds, such as alginate, are used as external support structures or combined with different polymers through extrusion to create consistent, high-throughput tumor organoid models [105].

2.1.4. Hyaluronic acid-based bioinks (HA)

HA is a polysaccharide present in organisms. As an important ECM component, hyaluronic acid possesses excellent rheological properties, biocompatibility, and biodegradability [106]. Furthermore, HA can promote cell proliferation and angiogenesis, mediate receptor interactions [107], and modulate cell behavior and function through physical or chemical cross-linking. These unique properties make it an ideal polymer for creating a 3D microenvironment that supports tumor cell growth. In addition, it is a lubricious hydrophilic polymer that can form highly viscous gels at low concentrations. It is commonly used as an additive to enhance the viscosity of gelatin and collagen-based bioinks, to maintain the stability of the printing structure.

However, the drawback of HA is its low shape fidelity during the bioprinting process. This can be addressed by utilizing photocrosslinking-based bioprinting methods with methacrylate to form methacrylated hyaluronic acid (HAMA) [108,109], or further

Table 1
Summary of methods and materials for constructing *in vitro* tumor models using 3D bioprinting.

Tumor type	3D bioprinting methods	Cell type	Hydrogel type	Research summary	Refs
Breast tumor	3D bioprinting based on light	MDA-MB-231, MCF7	GelMA hydrogel	The developed micro-patterned breast tumor microenvironment model can analyze different patterns of breast tumor cell migration and cytoskeletal organization within various regions	[45]
	3D bioprinting based on light	MDA-MB-231	PEGDA hydrogel	The interaction between breast tumor cells and osteoblasts in a novel 3D-printed bone matrix was investigated regarding proliferation, morphology, and cytokine secretion.	[46]
	3D bioprinting based on light	MDA-MB-231, MCF-7	Hydroxyapatite nanoparticles suspended in hydrogel	The interaction between human bone marrow mesenchymal stem cells and breast tumor cells was investigated within the biomimetic 3D bone matrix.	[47]
	3D bioprinting based on extrusion	MDA-MB-231	Alginate-gelatin hydrogel	A biomimetic *in vitro* model was created by co-culturing breast tumor cells with fibroblasts, resulting in multicellular tumor spheroids (MCTS) that could be maintained for several weeks.	[48]
	3D bioprinting based on extrusion	MDA-MB-231	Alginate-gelatin hydrogel	By optimizing the ratio of salt and gelatin bioinks, multicellular tumor spheroids (MCTS) were generated with controlled growth rates, frequencies, and sizes.	[49]
	3D bioprinting based on extrusion	MCF-7	GelMA hydrogel	Successful generation of high-fidelity ductal-like structures in an extracellular matrix (ECM) -like microenvironment was achieved, with tumor cells exhibiting similar characteristics to ductal carcinoma.	[50]
	3D bioprinting based on extrusion	MDA-MB-231, MCF-7	GelMA-collagen hydrogel	A hybrid hydrogel system composed of GelMA and hydrolyzed collagen simulated the tumor microenvironment and exhibited potential as an alternative to Matrigel for studying tumor invasiveness.	[51]
	3D bioprinting based on extrusion	MDA-MB-231	LAM-PBA-alginate hydrogel	A dual-network polysaccharide-based hydrogel bioink was designed for cell encapsulation and long-term cultivation.	[52]
	3D bioprinting based on extrusion	MDA-MB-231	Alginate-gelatin-Matrigel hydrogels	A hydrogel bioink composed of alginate (A), gelatin (G), and Matrigel (M) (AxGyMz) was used to successfully recover tumor spheroids, enabling cell expansion and the development of multi-generational tumor models.	[53]
	3D bioprinting based on extrusion	MDA-MB-231	Hyaluronic acid-based hydrogels	Co-culturing adipospheres with breast tumor cells led to decreased lipid content and alterations in ECM deposition within the adipose tissue, demonstrating the heterotypic interaction between breast tumor cells and adipose tissue.	[54]
	3D bioprinting based on inkjet	MDA-MB-231, MCF-7	PEG-4MAL bioink	A real-time monitoring, tracking, and measurement platform for cell movement within 3D structures was developed, which can be used for high-throughput screening of anti-tumor drugs.	[55]
Tumor type	3D bioprinting methods	Cell type	Hydrogel type	Research summary	Refs
	3D bioprinting based on extrusion	MCF-7	Alginate-gelatin hydrogel	Heterogeneous photodynamic therapy (PDT) responses of individual MCF-7 tumor cells within single tumor spheroids were observed through 3D imaging of irregular cell apoptosis within individual spheroids.	[56]
	3D bioprinting based on extrusion	MCF-7	Alginate-gelatin hydrogel	The growth of drug-resistant tumor spheroids was successfully maintained, and the EC50 values of the drug-resistant spheroids against anti-tumor drugs were measured based on *in situ* fluorescence within the embedded hydrogel.	[57]
Glioblastoma	3D bioprinting based on extrusion	U87MG	Alginate hydrogel	A glioma model was constructed using alginate hydrogels. The crosslinked alginate hydrogel maintained its structure and high cell viability for 11 days.	[58]
	3D bioprinting based on light	U87MG	PEGDA-hydrogel	Applying 3D micropatterning systems to glioblastoma cells using photolithography techniques enabled the formation of uniform GBM spheroids in 3D. The shape, size, and thickness of the cell spheroids could be controlled by adjusting the dimensions of the micropores.	[59]
	3D bioprinting based on extrusion	SU3, U87MG	Gelatin-alginate-fibrinogen (GAF) hydrogel	The 3D bioprinting glioblastoma stem cell model provided a novel platform, successfully mimicking the brain tumor microenvironment with high cell viability and intrinsic features.	[60]
	3D bioprinting based on extrusion	GSC23	Alginate-gelatin hydrogel	A self-assembled multicellular heterogeneous brain tumor fiber was manufactured using a coaxial extrusion 3D bioprinting system, providing an effective 3D model for *in vitro* research of the tumor microenvironment, particularly tumor-stroma interactions.	[61]
	3D bioprinting based on extrusion	U87MG	Fibrin-based hydrogel	A novel 3D-printed fibroblast-based glioblastoma model was generated using the RX1 bioprinter. It allowed a unique microfluidic printing head to print fragile neural tissue, making it an ideal choice for glioblastoma modeling.	[62]
	3D bioprinting based on extrusion	U118	Gelatin-alginate-fibrinogen (GAF) hydrogel	A gelatin-alginate-fibrinogen (GAF) hydrogel scaffold loaded with the U118 glioblastoma cell line successfully enriched GSCs, providing a new method for studying CSCs in tumor recurrence and other aspects.	[63]
	3D bioprinting based on extrusion	GL261	GelMA-gelatin hydrogel	The created 3D mini-brain could reproduce the phenotypic characteristics of *in vivo* GBM cells, and tumor cells could attract macrophages to their location and educate them on how to support their own survival and growth.	[64]
	3D bioprinting based on extrusion	U118, GSC23	Alginate-gelatin hydrogel	Both 3D-U118 and 3D-GSC23 were involved in *in vivo* tumor angiogenesis; however, 3D-GSC23 cells exhibited a stronger ability to form cell spheroids, secrete VEGFA, form tubular structures, and exhibited a higher cell proliferation rate *in vitro*.	[65]

(*continued on next page*)

Table 1 (*continued*)

Tumor type	3D bioprinting methods	Cell type	Hydrogel type	Research summary	Refs
Tumor type	3D bioprinting methods	Cell type	Hydrogel type	Research summary	Refs
	3D bioprinting based on extrusion	patient-derived GBM	Coll-MA-HA hydrogel	A method of immersion bioprinting is described, which enables the consistent and high-throughput manufacturing of PTO and provides a valuable *in vitro* model.	[66]
	FRESH 3D-printing	SH-SY5Y	Alginate-gelatin hydrogel	The newly developed conductive bioink promoted the differentiation and maturation of glioma cells and facilitated the generation of neural networks.	[67]
	3D bioprinting based on extrusion	SU3	Gelatin-alginate-fibrinogen (GAF) hydrogel	Cell fusion of GSCs and MSCs was achieved in the 3D bioprinting glioma model. The fused cells co-expressed biological markers of both GSCs and MSCs, demonstrating stronger proliferation, clonogenic, and invasive capabilities compared to GSCs and MSCs.	[68]
	3D bioprinting based on inkjet	STA-NB15	GelMA hydrogel	A vascularized tumor microenvironment was designed by combining 3D bioprinting and microfluidic chip technology. Patient-derived neuroblastoma spheroids attracted micro-vessels, thereby simulating *in vitro* tumor angiogenesis.	[69]
Lung tumor	3D bioprinting based on extrusion	A549,95-D	Alginate-gelatin hydrogel	The invasive and migratory abilities of lung tumor cells in a 3D *in vitro* model constructed with gelatin-alginate hydrogel were evaluated, supporting the feasibility of using 3D bioprinting to construct tumor-like lung tumor models.	[70]
	3D bioprinting based on extrusion	patient-derived xenograft	Alginate-gelatin hydrogel	An *in vitro* tumor co-culture spheroid was developed by co-culturing fibroblasts and patient-derived lung tumor cells to simulate the tumor microenvironment by mimicking tumor-stroma interactions.	[71]
	3D bioprinting based on extrusion	A549	Hphil-CNF hydrogel	An open culture platform was developed to observe cell morphology, response to external stimuli, and chemical flow within channels. This open platform was utilized to assess the impact of cisplatin on lung tumor cell death and determine the lethal dose of anti-tumor drugs.	[72]
	3D bioprinting based on extrusion	NCI–H23	GelMA-collagen hydrogel	A mixed hydrogel system composed of GelMA and hydrolyzed collagen simulated the tumor microenvironment and demonstrated potential as an alternative to Matrigel in studying tumor invasiveness.	[51]
	3D bioprinting based on extrusion	A549	Alginate-gelatin hydrogel	A 3D lung tumor *in vitro* model was constructed for screening eight anti-tumor drugs. The results indicated that this 3D-printed *in vitro* model could further be used for tissue-level anti-tumor drug screening.	[73]
	3D bioprinting based on extrusion	NSCLC-PDX, HCC-827, A549,	Ink H4, Ink H4-RGD	A tumor scaffold with *in vivo* matrix characteristics was developed using H4-RGD bioink. The scaffold maintained good stability, and the loaded NSCLC PDX cells exhibited spheroid formation within seven days.	[74]
	Stereolithography-based 3D printing	A549，PC9 NCI–H1395, NCI–H1650,	GelMA-PEGDA	Various rigid and hydrogel-based 3D scaffolds were successfully printed and used for the *in vitro* growth of lung CSCs. In addition, the hydrogel-based scaffolds appeared to be most suitable for the 3D culture of NSCLC primary cultures.	[75]
Tumor type	3D bioprinting methods	Cell type	Hydrogel type	Research summary	Refs
Cervical tumor	Stereolithography-based 3D printing	HeLa	PEGDA	A 3D *in vitro* microfluidic chip mimicking vascular morphology was constructed. It was observed that the migration of HeLa tumor cells increased as the channel width decreased, indicating that the size of the blood vessels influences the metastatic and invasive properties of tumor cells.	[76]
	3D bioprinting based on extrusion	HeLa	Gelatin-alginate-Matrigel hydrogel	An *in vitro* cervical tumor model was established by 3D printing HeLa cells, which rapidly formed spheroids representing tumorigenic features. The induction of TGF-β successfully achieved and tracked the EMT process within the HeLa/hydrogel 3D constructs.	[77]
Liver tumor	3D bioprinting based on extrusion	patient-derived ICC	Gelatin-alginate-Matrigel hydrogel	A personalized *in vitro* tumor model was developed using a bioink composite hydrogel system. The ICC cells from patients maintained continuous cell proliferation and colony-forming ability and exhibited stem cell-like characteristics.	[78]
Ovarian tumor	3D bioprinting based on inkjet	OVCAR-5	Matrigel™	Micro-patterning of ovarian tumor cells and fibroblasts was achieved through spatial control, co-cultivating to form 3D follicle-like structures, and similarly recapitulating the characteristics of *in vivo* ovarian tumor micro-nodules.	[79]
Melanoma	3D bioprinting based on light	A375	GelMA-PEGDA	A hydrogel scaffold mimicking the melanoma cell growth microenvironment was prepared using 3D bioprinting with appropriate concentrations of GelMA and PEGDA as materials. Compared to the 2D culture, the 3D bioprinting hydrogel scaffold was more suitable for the proliferation and differentiation of tumor cells.	[80]
Multiple myeloma	3D bioprinting based on extrusion	MM1S, RPMI-8226	GelMA-alginate PEGDA-nHA	A multiple myeloma (MM) model that simulates the human bone marrow niche was established. Co-culturing stromal cells with multiple myeloma cells promoted the proliferation and aggregation of MM cells.	[81]
Osteosarcoma	3D bioprinting based on inkjet	U–2OS, U2OS/CDDP	Collagen-Based hydrogel	A novel collagen-based hydrogel was used to construct osteosarcoma (OS) 3D *in vitro* model, which better mimicked the biological characteristics and chemical sensitivity of OS cells compared to 2D platforms, making it a promising tool for studying the biology of osteosarcoma cells.	[82]
Chronic lymphocytic leukemia	3D bioprinting based on extrusion	MEC1	CELLINK Bioink hydrogel	The first long-term 3D culture model of leukemia cells was established, which can better simulate the physiological 3D *in vivo* environment of leukemia cells and a wider range of immune cells.	[83]

incorporating GelMA to form HAMA-GelMA "bioink" for constructing highly authentic vascularized tissues and neural networks [110].

2.1.5. Polyethylene glycol-based bioinks (PEG)

PEG is a widely used biomaterial in the construction of biomimetic scaffolds *in vitro* [111]. PEG exhibits excellent mechanical properties, is non-cytotoxic within a specific molecular weight range, and is non-immunogenic as a biomaterial [112]. However, unlike natural polymers, PEG cannot form hydrogel structures with temperature variations or ionic cross-linking properties. In addition, PEG cannot promote cell adhesion and interaction. Therefore, PEG must be coupled with functional groups (such as methacrylates) or other functional polymer materials to achieve these cellular activities [113].

Polyethylene glycol diacrylate (PEGDA) is a polymer that undergoes copolymerization with acrylate. Compared with PEG, PEGDA possesses cross-linking properties, making it a biologically active material for preparing bioinks. Using photocrosslinking methods, PEGDA is commonly used to fabricate finely structured or controllable tumor models, such as lung tumors, glioblastomas, and multiple myelomas. Pluronic® F127 (PF127), composed of PEG and polypropylene glycol (PPG), is often used in extrusion-based bioprinting to serve as a sacrificial layer for observing the biological behavior of tumor cells by dissolving PF127 [114].

We have summarized and expanded upon the information regarding the advantages, disadvantages, and potential applications of the six bioinks in Table 2 for a more intuitive comparison.

2.2. Bioprinting techniques

2.2.1. Extrusion-based bioprinting

Extrusion-based bioprinting is the most commonly used method whcih consists of three parts: a lifting platform, a nozzle, and an outlet structure [20]. With computer-assisted control, the mixed bioink is extruded from the nozzle under continuous squeezing pressure along the x-axis [115]. Simultaneously, the lifting platform moves along the y- and z-axes, depositing the material in a 2D pattern, sequentially stacking to form a 3D scaffold [116]. This method has a wide range of applications. It demonstrates good compatibility with biomaterials of different viscosities and cells of various concentrations and types [117], which enables the construction of tumor organoids with sufficient mechanical strength for building tumor microenvironments in hard tissues such as bone. Furthermore, it allows multiple nozzles to deposit different bioinks, which is suitable for constructing co-culture models to study the interactions between tumor cells and other cells. However, low resolution of hydrogel model is the key limitation of extrusion-based bioprinting [118]. The second limitation is the material nature of the bioink, which requires precise control of the nozzle temperature to prevent liquefaction and nozzle blockage, which can lead to material deformation and collapse [119,120] (Fig. 2A).

Coaxial bioprinting is an extension of the extrusion-based printing method, where the coaxial circular structure of the nozzle has the advantage of simultaneously controlling the internal and external hierarchical structures, thereby enabling the printing of hollow tubular structures, particularly in the field of vascularization [123,124]. The core-shell structure allows the co-extrusion of two different bioinks, addressing the problem of insufficient mechanical strength of a single bioink and making the printing of tubular structures more convenient. The combination of extrusion-based bioprinting with sacrificial material can also be employed to construct functional vascular networks in tumor models, creating specifically shaped tumor models such as breast ductal carcinomas [50], vascularized tumor models, and microtumor microarrays [51]. This is crucial for revealing the close relationship between blood vessels and tumors including the interaction of circulating tumor cells with stromal and infiltrating immune cells, the exchange of secreted factors between different cells, the response to external stimuli, and the adaptive behavior of the tumor to the metastatic microenvironment [112,125].

2.2.2. Inkjet-based bioprinting

Inkjet bioprinting is the first bioprinting technology and is a non-contact droplet-based bioprinter [126]. The inkjet printer consists of a liquid binder cartridge, a nozzle that moves along the x- and y-axes, and a platform along the z-axis. By electrically heating the nozzle head or inducing acoustic waves using piezoelectric crystals inside the print head [127], liquid droplets were ejected onto the substrate, adhering adjacent hydrogel inks together, forming a single layer of 2D patterns, subsequently lowering the layer and printing 3D structures layer by layer (Fig. 2B). By controlling the droplet size, deposition speed, and nozzle orientation, the bioink consisting of cells, scaffold material, and growth factors can be precisely deposited at high resolution (approximately 50 μm) and high printing speed (up to 10,000 drops per second) [128] (Fig. 2C). However, inkjet-printed models typically require long drying periods at high temperatures, which can lead to decreased utilization of bioinks. Similar to extrusion-based bioprinting, higher cell densities can lead to a high-viscosity of the bioink and nozzle clogging [129]; thus, only allowing the printing of low-viscosity bioinks. Additional crosslinking is required to ensure the stability of the printed structure [130].

Through inkjet printing, different types of cells and biomaterials can be printed at predetermined locations [18,122], simulating the complex structures and microenvironments of real tumor tissues such as osteosarcoma and ovarian tumors. This technology can also be customized according to the specific conditions of the patient, helping to simulate different types of tumor tissues better and providing more realistic and reliable *in vitro* models for drug development and treatment research.

2.2.3. Stereolithography-based bioprinting

Stereolithography can also be used to manufacture 3D printed models. It is based on the photopolymerization of photosensitive polymers [122]. Furthermore, the method uses light of specific wavelengths and intensities to scan from a point to a line focused on the surface of a liquid hydrogel in a container, resulting in a single layer of 2D cured patterns in the container. Subsequently, as the platform descends or rises to the designed single-layer thickness, the next layer continues to solidify using the abovementioned process to completely recover the previously generated 2D pattern with fresh bioink on the prefabricated structure until the 3D structure is completed [131]. Photolithography is not constrained by the viscosity of the bioink, which means that multiple bioinks with different viscosities can be used for printing, thereby enabling a more diverse range of applications for various biomaterials. Laser-assisted printing precents direct contact between dispensers and bioinks, enabling non-contact printing [132]. This method does not subject cells to mechanical stress, which is beneficial for maintaining cell viability, and provides the highest resolution among the three printing methods [133] (Fig. 3).

Bioinks based on GelMA [135] and PEGDA [136] are commonly used in photolithography technology to create scaffolds with precise structures and controllable mechanical strength [137]. These scaffolds are subsequently used to simulate tumor tissues and provide valuable tools for oncology research. Scaffolds with precise structures and controllable mechanical strengths have been used in oncology research.

2.3. Emerging 3D bioprinting technology

Laser-Induced Forward Transfer (LIFT) utilizes high-energy laser pulses to irradiate biological precursor materials, inducing instantaneous vaporization and gas formation, thereby transferring the biological precursor material from one substrate to another with rapid and precise micrometer or nanometer-level deposition. This technique is suitable for constructing intricate biological tissue structures and microscale biological chips. In the field of biomedicine, Laser-Induced Forward Transfer technology can be employed to build biomimetic

Table 2
Summary of the advantages, disadvantages, and applications of bioinks used in 3D bioprinting.

	Benefit	Constraint	Applications
Alginates Hydrogel	1. **Excellent cell compatibility:It can mimic the natural extracellular matrix, promoting the directional growth of cells and tissue regeneration.** 2. **Tunable physical properties:The concentration, crosslinking density, and other process parameters can be adjusted to meet the requirements of different biomedical applications.** 3. **Good biodegradability:It can be gradually degraded by enzymes in the body, enabling controlled tissue reconstruction.** 4. **Injectability:The good flowability allows precise positioning and shaping through injection, avoiding damage to the cells.** 5. **Compositing enhancement:It can be compounded with other biomaterials to further enhance the bioactivity and mechanical properties.**	1. **Limited mechanical properties: Alginate lacks sufficient mechanical strength and stability in certain aspects, making it prone to deformation or fracture when bearing weight or subjected to external forces.** 2. **Low precision and resolution: Issues with resolution and printing precision limit the fineness and accuracy of the printed structures.** 3. **Unstable degradation performance: The unstable degradability of alginate leads to either too rapid or too slow degradation of the printed structures *in vivo*, affecting the functionality and stability of the material.** 4. **Controllability and reproducibility: Variations in the properties of bioinks from different batches or formulations affect the stability and reproducibility of print quality.**	1. **Cell culture models: Printing cell culture models with complex structures and microenvironments to study cellular behavior, drug screening, and disease modeling.** 2. **Drug delivery systems: Utilized as drug carriers or release systems for targeted and sustained drug delivery, enabling personalized therapeutic applications.** 3. **Scaffolds and tissue engineering: Precise printing of scaffolds with specific structures and shapes to facilitate the advancement of tissue engineering.** 4. **Practical medical devices: The plasticity and deformability of alginate hydrogels can be used to fabricate personalized medical devices or assistive tools.** 5. **Biorestorative materials: Bioactive tissue repair materials for the treatment of tissue damage, injury, or disease, driving the development of tissue repair and regenerative medicine.**
Gelatin Hydrogel	1. **Excellent biocompatibility and bioactivity: It provides biological signals that are favorable for cell attachment, growth, and differentiation, enhancing the bioactivity of bioinks.** 2. **Good plasticity and printability: With superior mechanical properties and plasticity, it facilitates the support and shaping during the 3D bioprinting process. The viscosity and flowability of the bioink can be designed by adjusting the concentration and crosslinking degree.** 3. **Controllable degradability: The degradation rate of gelatin can be regulated through enzymatic or chemical crosslinking, enabling precise control over the tissue repair process.** 4. **Outstanding carrier function: Gelatin can serve as a carrier for cells and growth factors, achieving slow and controlled release of cells or bioactive molecules.**	1. **Relatively weak mechanical properties: Gelatin itself has relatively weak mechanical properties, with limited elasticity and strength. It may lack sufficient stability and durability for certain applications.** 2. **Low strength and susceptibility to deformation: The mechanical strength of gelatin hydrogels is relatively low, making them prone to deformation or fracture, which is unfavorable for maintaining the shape stability of printed constructs.** 3. **Insufficient cell adhesion: As a natural biomaterial, gelatin requires further modification or the addition of auxiliary materials to improve cell adhesion.** 4. **Rapid *in vivo* degradation: The degradation time of gelatin *in vivo* is sometimes too short, necessitating modification or the addition of crosslinking agents to adjust the degradation characteristics.** 5. **Significant batch-to-batch variability: As a natural product, the performance of gelatin can be influenced by factors such as source and manufacturing process, leading to considerable batch-to-batch differences.**	1. **Cell culture and tissue engineering: Gelatin hydrogels can be used to print three-dimensional artificial tissues, such as skin, cartilage, and muscle.** 2. **Biosensors and medical devices: Gelatin hydrogels can be used to fabricate complex biosensor structures for the detection of physiological parameters, such as blood glucose and pH.** 3. **Tissue and organ models: By using gelatin hydrogels as a matrix, combined with cells and other biomaterials, organ-like models can be constructed for disease mechanism research and drug screening.** 4. **Personalized medical devices: Leveraging the plasticity and biocompatibility of gelatin hydrogels, customized medical devices, such as prosthetics and artificial joints, can be fabricated.**
Collagen Hydrogel	1. **Good biocompatibility: Collagen is a naturally occurring component of human body structure, exhibiting excellent biocompatibility and low immunogenicity, which is beneficial for cell adhesion, proliferation, and differentiation.** 2. **Tunable mechanical properties: By adjusting the collagen concentration and crosslinking degree, the mechanical strength, elastic modulus, and other properties of the hydrogel can be modulated.** 3. **Superior mechanical performance: Compounding with other biomaterials can enhance the mechanical strength and toughness of the material, meeting the requirements for both strength and ductility.** 4. **Capability of complex structure fabrication: Complex three-dimensional structures, such as microvasculature and biosensors, can be achieved through 3D bioprinting. This provides a broad development space for the construction of bionic tissues.**	1. **Rapid gelation and uneven crosslinking: The rapid gelation and uneven crosslinking of collagen hydrogels during the 3D bioprinting process may affect the printing accuracy and structural stability.** 2. **Insufficient plasticity: Collagen hydrogels have relatively low plasticity, which poses challenges in constructing complex structures or tissues with fine features** 3. **Tissue-specific limitations: The source and type of collagen can influence its specific tissue adhesion and bioactive response to cells.** 4. **Risk of cross-contamination: Collagen extracted from animal sources may carry a risk of cross-contamination, requiring careful sterilization of the material.** 5. **Higher cost: Compared to synthetic polymers, the extraction and purification of natural collagen are often more complex and expensive, limiting its large-scale utilization.**	1. **Mimicking physiological environments: Collagen can construct more natural tissue-like structures during the 3D printing process, promoting cell adhesion, proliferation, and differentiation.** 2. **Supporting cell growth: Collagen hydrogels contain cell-adhesive peptide sequences, such as the RGD motif, which can effectively promote cell attachment and growth.** 3. **Carrier for bioactive agents: Collagen hydrogels have strong adsorption and affinity, enabling precise drug release or modulation of cell signaling pathways.** 4. **Bioprinting support materials: Collagen can assist in the printing of complex suspended or hollow structures, improving printing accuracy and enabling the achievement of desired 3D tissue constructs.**
Hyaluronic Acid Hydrogel	1. **Excellent water-retaining property: Hyaluronic acid has outstanding water-retaining capability, which helps maintain**	1. **Difficulty in controlling degradation rate: The degradation rate of hyaluronic acid hydrogels is challenging to control precisely,**	1. **Tissue engineering scaffolds: The excellent biocompatibility and biodegradability of hyaluronic acid make it suitable for the**

(*continued on next page*)

Table 2 (*continued*)

	Benefit	Constraint	Applications
	the water balance of cells and tissues, promoting cell survival and functional expression. 2. Promotion of cell proliferation and migration: Hyaluronic acid can provide a scaffold structure and signaling molecules, which helps regulate cell behavior and accelerate the integration and repair process of tissue engineering. 3. Good mechanical properties and processability: Hyaluronic acid hydrogels have tunable mechanical properties that can meet the mechanical requirements of different tissue engineering applications. 4. Carrier function: The excellent adsorption and retention capability of hyaluronic acid enables the controlled release of bioactive substances, enhancing the efficacy of tissue repair and regeneration.	which can lead to issues with the support scaffold material degrading too quickly or too slowly. 2. Limited customizability: The properties and characteristics of hyaluronic acid hydrogels are relatively fixed, making it difficult to achieve extensive customization through simple parameter adjustments. 3. Challenges in modeling and printing: Due to the rheological characteristics of the hydrogels, there may be technical challenges in the 3D modeling and printing process, such as maintaining printing accuracy and stabilizing the printed structure. 4. Weak cell adhesion: Hyaluronic acid hydrogels have limited support for cell adhesion, which can affect cell attachment, proliferation, and infiltration.	fabrication of tissue engineering scaffolds for soft tissues, such as cartilage and skin. 2. Bioink enhancement: Hyaluronic acid hydrogels can be combined with other biomaterials, such as collagen and cellulose, to develop bioinks with superior bioactivity and mechanical properties. 3. Bioinspired organs and regenerative medicine applications: Hyaluronic acid can be used to print bioinspired organ models with specific structures and functions, such as liver and kidney, for drug screening, pathological research, and personalized healthcare.
PEG Hydrogel	1. Tunability: The physicochemical properties of PEG hydrogels can be customized by adjusting parameters such as crosslinking degree, morphology, and degradation rate. 2. High transparency: PEG hydrogels have a relatively high transparency, making them suitable for printing transparent or semi-transparent biological tissue structures, such as cornea and blood vessels. 3. Compositing enhancement: PEG hydrogels can be chemically modified to introduce specific functional groups, such as cell adhesion sites and enzyme recognition sites, further expanding their bioactivity. 4. Excellent printability and plasticity: PEG hydrogels exhibit good processability and printability, facilitating their application in 3D bioprinting equipment for the fabrication of complex biological tissue structures.	1. Potential impact on bioactive stability: PEG hydrogels may interact with and negatively affect the stability of incorporated cells or bioactive agents, leading to their deactivation. 2. Lack of standardization: The standardized preparation protocols for PEG-based bioinks are not yet fully established, which can impact the consistency and comparability of printing outcomes. 3. Limited cell-directing capabilities: PEG hydrogels have limitations in their ability to induce directed cell growth, which can affect the reconstruction of some structurally complex tissues. 4. Absence of natural extracellular matrix components: PEG hydrogels lack the natural extracellular matrix components, limiting their ability to fully mimic the native microenvironment and thereby affecting the functional performance of cells.	1. Cell culture and model development: PEG hydrogels can be utilized to create cell culture models, such as tumor models and organ-on-a-chip systems, which facilitate the study of disease mechanisms, drug screening, and personalized medicine. 2. Drug delivery systems: Using PEG hydrogels as a bioink, scaffolds with microstructures can be constructed to achieve targeted and controlled drug delivery. 3. Biosensing and diagnostics: PEG hydrogels can be integrated with biosensors to fabricate 3D-printed biosensing devices for the detection of biomarkers, metabolites, and other relevant analytes.

tissue structures, biosensors, artificial bones, and provide essential tools for tissue engineering, drug development, disease diagnosis, and more [138,139].

The technology of volumetric bioprinting through tomographic scanning is an innovative approach that combines medical imaging techniques with 3D bioprinting. It leverages tomographic scanning (CT) or magnetic resonance imaging (MRI) to acquire three-dimensional biological structural information of specific parts of a patient's body, and then uses bioprinting to deposit or stack biological materials according to this model, enabling precise replication and reproduction of complex biological tissues. This technology can provide more personalized and customized solutions for tissue regeneration, transplantation, and disease treatment in the medical field. It holds potential for significant breakthroughs and innovations in medical research and treatment [140–142].

Electrospray bioprinting technology is an innovative method that utilizes the principle of electrospray to perform biological printing. The nozzle, loaded with bio-ink or cell suspension, is activated by a high electric field voltage, creating tiny sprayed droplets. These droplets then deposit onto a substrate in a controlled manner, forming the desired biological tissue structure. Electrospray bioprinting can be used to construct complex biological tissue engineering structures, such as neural tissues, vascular networks, and more. Additionally, it can be applied in areas like cell microarray preparation, biosensor manufacturing, and beyond. However, constraints such as the viscosity of biological materials and surface tension need further research and optimization before clinical application [143,144].

Plasma-enhanced bioprinting is a method that combines plasma technology with bioprinting technology. In this technique, plasma is used to modify the chemical properties of biological materials such as extracellular matrix and hyaluronic acid, altering factors like crosslinking degree and surface charge to enhance adhesion, biocompatibility, and mechanical performance. By activating the surface of printing substrates or support materials, enhancing their wettability and affinity, it promotes cell and biomaterial adhesion, thereby improving the success rate and forming quality of bioprinting. This method strengthens and optimizes the bioprinting process, showcasing innovation and cutting-edge advancements [145].

Magnetic-assisted printing technology is an advanced printing method that utilizes magnetic materials and an external magnetic field to assist in positioning and printing. It typically involves introducing magnetic particles or magnetic liquid into the printing material or support structures, and controlling the positioning and shape of these magnetic components by applying an external magnetic field. The magnetic stimulation can help overcome factors such as gravity and surface tension, induce the oriented alignment of cells, activate cell signaling pathways, enhance cell proliferation and differentiation, and achieve a flexible, efficient, and controllable printing process. Magnetic-assisted 3D bioprinting technology has brought new breakthroughs to tissue regeneration and organ repair in the biomedical field, offering new possibilities and development opportunities [146,147].

Acoustic bioprinting technology utilizes sound waves as a driving force to achieve precise positioning and organization of bio-materials. By using special piezoelectric elements or acoustic lenses to convert electrical signals into high-frequency sound waves and focusing them on specific areas, a high-energy density sound wave beam is formed. This

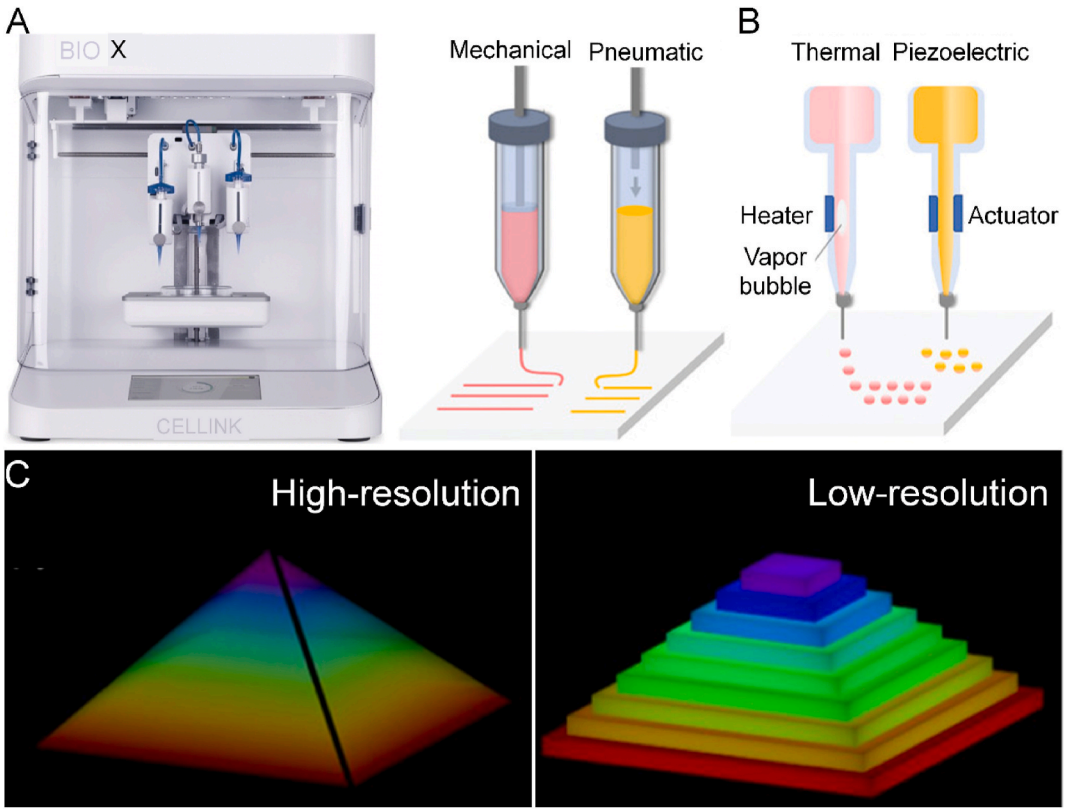

Fig. 2. Schematic diagram illustrating the principle of tumor model formation based on extrusion-based and inkjet-based bioprinting methods with deposition of bioink. (A) Cellink 3D bioprinter. (B) Extrusion-based bioprinting method, and inkjet-based bioprinting method. Reproduced with permission [121]. Copyright 2021, Wiley-VCH. (**C**) By increasing the number of layers and decreasing the thickness, the resolution of the printed structure is improved, resulting in a tumor organoid model with a smooth surface appearance [122]. Reproduced with permission. Copyright 2018, Company Biologists.

beam can generate thrust and tension on bio-materials, avoiding mechanical damage to cells, while achieving precise positioning and arrangement of cells, bio-inks, or other biological components with a resolution down to the micrometer or even submicron level. The accuracy, efficiency, and controllability of acoustic bioprinting technology contribute significantly to innovation in the fields of tissue engineering and regenerative medicine in the biomedical field [148–150].

3. Tumor organoids model for bioprinting

3.1. Breast tumor organoid bioprinting

Breast tumors are among the most common tumors in women and a leading cause of female mortality [151]. Breast tumors are characterized by the uncontrolled proliferation of breast epithelial cells in response to various carcinogenic factors. Symptoms such as breast lumps, nipple discharge, and axillary lymphadenopathy occur in the early stages of the disease. In the late stages, distant metastasis of tumor cells may lead to multi-organ damage, directly threatening the patient's life. Breast tissue has a unique structure comprising mammary glands (lobules and ducts) and adipose tissue [152]. The tumor microenvironment plays a crucial role in tumor progression. Traditional 2D *in vitro* cell cultures lack spatial heterogeneity and exhibit overly simple structures [153]. Establishing physiologically relevant *in vitro* tumor models through bioprinting, including interactions between tumor cells and the extracellular matrix of the breast microenvironment, as well as simulating hollow ductal channels of the mammary gland, is essential for a better understanding of the biological behavior of tumor cells in a natural breast tumor microenvironment [154].

Invasive proliferation and migration are key features of tumors *in vivo*. Tumor cell metastasis is significantly influenced by the biophysical properties of the tumor microenvironment. The stiffness of *in vitro* biomimetic organoid models is one of key issues that influences the behavior of tumor cells. GELMA hydrogel is a photosensitive hydrogel that has proven to be a candidate material for basic biological research [155] and is used to construct biologically relevant tissue structures. In an organoid tumor model constructed using photolithography Nitish et al. [45] observed that MDAMB231 cells moved slowly and maintained stable migration in the central region with high hardness (748 ± 90 Pa) based on the photosensitive properties of GELMA. In contrast, the opposite behavior is observed in the surrounding region with low hardness (313 ± 89 Pa). Similarly, the alginate-gelatin composite bioink with low hardness (A1G5 and A1G7) facilitated the formation of tumor spheroids and the migration of tumor cells in a 3D environment. Conversely, materials with high hardness (A3Gy and A5Gy) inhibited the formation of tumor spheroids [49]. Therefore, the stiffness of ECM can significantly influence biological behaviors such as tumor cell migration, invasion, and metastatic potential, providing new strategies and targets for tumor treatment and prevention, which warrants further in-depth research and exploration. By adding Matrigel, iterative culturing of MDAMB231 tumor spheroids was achieved in the AxGyMz composite bioink [53]. Matrigel is the basement membrane (BM) extract most commonly used for 3D organoid cultures. Matrigel is the most commonly used basement membrane (BM) extract for three-dimensional organoid culture, but it is extracted from mouse tumors and cannot fully replicate the specific microenvironment of human tumors. Additionally, the composition and mechanical properties of Matrigel vary between batches, affecting the reproducibility of experiments. In a composite bioink of 5 % GelMA +0.5 % collagen, MDAMB231 exhibited similar invasive behavior to that of Matrigel, suggesting that this stiffness-adjustable, cost-effective, and novel hydrogel has potential as an alternative to Matrigel [51]. Researching

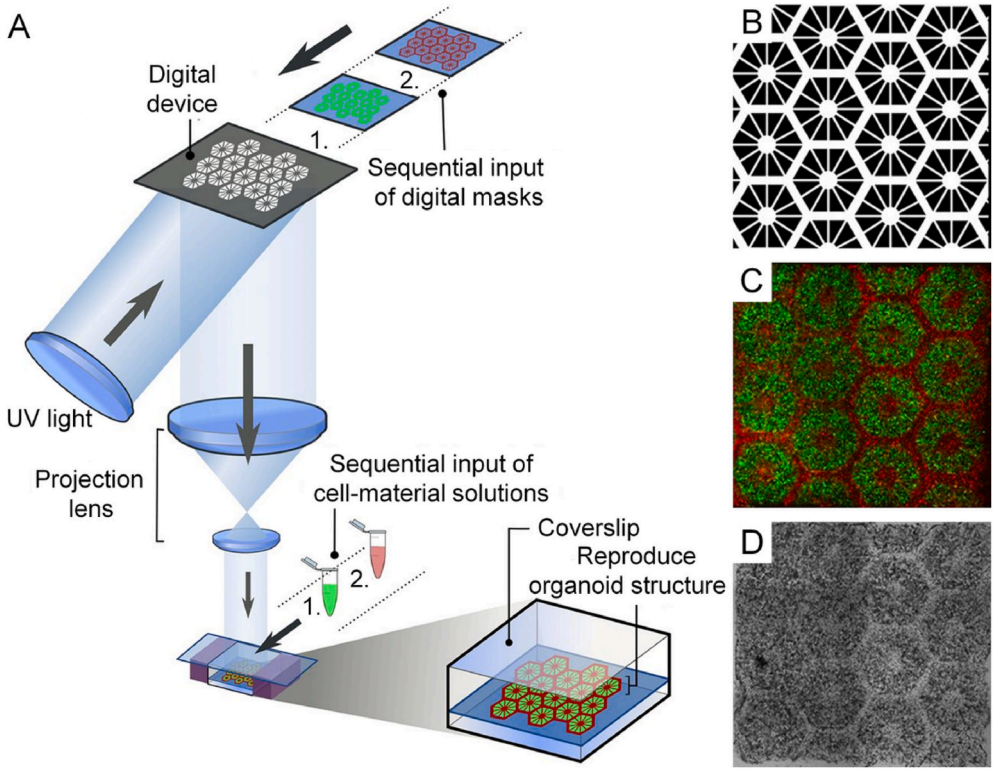

Fig. 3. Schematic diagram illustrating the principle of constructing organoid models using light-based bioprinting method. (A) Schematic representation of a liver organoid model constructed by light-based bioprinting. (B) Grayscale digital mask corresponding to the vascular structures of the liver lobules. (C, D) Images taken under fluorescence and bright field channels (5 ×) of fluorescently labeled hiPSC-HPC in GelMA [134]. Reproduced with permission. Copyright 2016, National Academy of Sciences.

new hydrogel materials as alternatives to Matrigel is of great significance. It helps improve and optimize the performance of materials, broaden the functional range of materials, achieve consistency between batches, and further drive innovation and advancement in tissue engineering and disease modeling technologies. However, MDAMB231 cells exhibited the opposite biological behavior in the novel PEG-4MAL bioink, which has highly tunable mechanical and biological functional properties. Compared to softer hydrogel systems (0.7 kPa + RGD), significant migratory behavior was observed in MDAMB231 cells within the harder hydrogel (1.1 kPa + RGD), likely because of the modification with cell adhesion peptide (RGD) [55]. Similarly, interaction with RGD promotes the migration of MCF-7 cells [50]. Therefore, future research should focus on precisely controlling the stiffness factor of the tumor microenvironment by adding different extracellular matrix components in hydrogel systems, including temporal and spatial control. Multi-level, multi-component hydrogel structures can help researchers better understand how tumor cells respond to different mechanical microenvironments, potentially providing new perspectives and methods for revealing the mechanisms of tumor initiation and development. This could contribute to the development of more effective strategies for tumor treatment and prevention. In recent years, bioprinting using dynamically cross-linked hydrogel networks has attracted significant attention because they can better mimic the mechanical properties of the ECM and respond to biological stimuli. A dual cross-linked dynamic hydrogel network based on the boronic acid motifs of laminarin (LAM-PBA) and alginate exhibited excellent cell compatibility (cell viability exceeded 90 %). By controlling the cross-linking process of both types, the processability, mechanical behavior, and stability of the bioink can be further improved [52]. This study ingeniously combines dynamic covalent crosslinking and ion crosslinking to form a double network structure, providing a new tool and perspective for the field of tissue engineering. However, detailed mechanical property data of this double network structure bioink, such as tensile strength and modulus, were not provided in the study. These data are crucial for evaluating the performance of the bioink in the 3D printing process and for further optimizing the material formulation.

Interactions among tumor, immune, and mesenchymal cells within the tumor microenvironment significantly affect tumor growth and behavior. Understanding these interactions is crucial for a deeper comprehension of breast tumor cellular characteristics and behavioral changes. In breast tumors, adipocytes are important agents that play roles in promoting tumor progression within the tumor microenvironment. They can induce inflammatory responses, influence the metabolic reprogramming of tumor cells, and provide the necessary nutrients and growth signals for tumor growth and dissemination. Hence, understanding the regulatory role of adipocytes in the breast tumor microenvironment helps deepen our understanding of the pathogenic mechanisms of breast tumors [156]. Hannes et al. [54] constructed a co-culture model of adipose tissue with breast tumor cells. After nine days of co-culturing, they observed that tumor cells induced a decrease in the lipid content of the adipose tissue and remodeling of the extracellular matrix (with a significant increase in the expression of collagens I, VI and fibronectin). This integrated 3D breast cancer-adipose tissue model illustrated the pro-tumorigenic effects of the adipose in breast cancer. In the future, the introduction of other key cell types such as immune cells, vascular endothelial cells, etc., can further elucidate the specific regulatory roles of factors secreted by adipose cells in tumor development. The bone is one of the most common sites of metastasis in advanced breast tumors [157]. To better understand bone metastasis in breast tumors, a bionic bone-specific microenvironment was created by incorporating hydroxyapatite-containing nanoparticles into a PEG/-PEGDA hydrogel, the presence of MSC increased the number of MDAMB231 cell spheroids compared to culturing tumor cells alone [47]. Similarly, in a co-culture of osteoblasts with MDAMB231 cells,

osteoblasts promoted tumor cell proliferation and tumor sphere formation, whereas MDAMB231 cells inhibited osteoblast proliferation [46]. Overall, the addition of helper cells enhanced the bionic nature of the tumor model, which is more valuable in studying the interactions of the tumor microenvironment. In the future, it may be considered to incorporate other cell types, such as inflammatory cells, to comprehensively simulate the impact of multiple factors on bone metastasis. Alternatively, integrating the 3D printing model with clinical case data for comparison of research outcomes with actual patient conditions can provide better validation of experimental results.

Resistance to anti-tumor drugs is another important characteristic of malignant tumors [64]. Using bioprinting *in vitro* organoid tumor models can prevent the false positive behavior of tumor cells exposed to anti-tumor drugs in 2D cultures. Therefore, bioprinting *in vitro* organoid tumor models can serve as a better preclinical platform for drug screening and personalized drug development. Song et al. [57] successfully maintained the growth of drug-resistant MCF-7 breast tumor spheroids in a gelatin-sodium alginate hydrogel, preserving the CD44 high/CD24 low/ALDH1 high phenotype (Fig. 4). At the same time, the EC50 values for apoptosis and necrosis concurrently induced by PTX in the resistant spheroids were 124 nM and 131 nM, respectively. In contrast, the EC50 values for PTX-induced apoptotic and necrotic cell death in larger spheroids were 59 nM and 54 nM, respectively. In the same year, the team further achieved a novel *in situ* assessment of the efficacy of PDT on tumor spheroids, with significantly higher IC50 values for the photosensitizers sTPP and Ce6 in 3D spheroids than in 2D cultures (7-fold difference) at the same radiant power. Interestingly, heterogeneous responses of individual tumor cells within a single tumor sphere to photodynamic therapy were observed suing 3D imaging. Furthermore, individual drug-resistant cells within the spheres were suggested to be responsible for the emergence of drug resistance in the tumor spheres. However, the main shortcoming of these experiments is the lack of in-depth research on the mechanisms of PDT. The analysis was conducted merely from the perspective of overall cell death. In the next step, other cell death detection methods (such as apoptosis markers and cell cycle analysis) could be combined to further explore the mechanisms of cell death induced by PDT. Similarly, in other experiments inducing tumor apoptosis with PDT, 3D tumor spheroids overexpressed the ABCG2 transporter protein, which expelled an excess of the photosensitizer PPIX from the tumor cells, thereby reducing the therapeutic effect of PDT compared to 2D models [158]. Unfortunately, this experiment did not compare the genetic changes in *in vivo* tumor

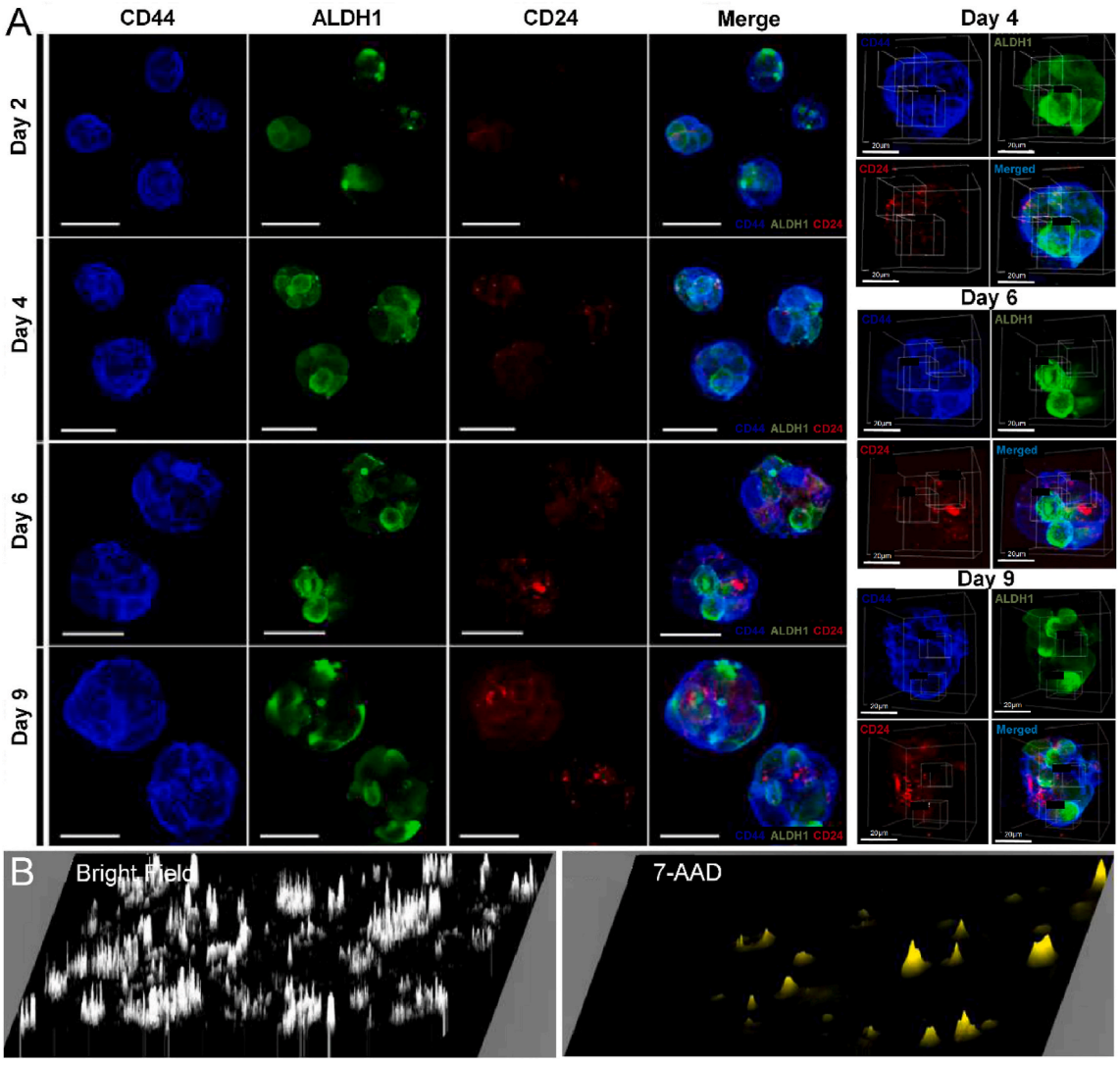

Fig. 4. Schematic representation of the construction of breast cancer organoid models for drug screening using bioprinting. (A) Confocal microscopy images showing the expression of CD44, ALDH1, and CD24 in drug-resistant spheroids. (B) Methodology for quantitative cell viability measurement in embedded MCF-7 spheroids. 3D spheroid images based on their respective intensity profiles under white light and fluorescent modes for 7-AAD [57]. Reproduced with permission. Copyright 2022, Elsevier.

models with 3D spheroids, making it unable to further demonstrate the persuasiveness of the 3D tumor model. Similarly, upon establishing a 3D culture model with a spontaneously generated hypoxia and drug resistance central core, the generation of the central core in tumors may be one of the most critical factors limiting the effectiveness of PDT in clinical practice [159]. 3D models provide a visual representation of the interaction between tumor spheroids and drugs *in vitro*. This demonstrates the ability of 3D models to effectively mimic the tumor microenvironment *in vivo*, which is crucial for understanding the response to PDT treatment and the process of hypoxic core formation [56]. In the future, patient-derived tumor cells or organ samples can be extracted for model construction with high clinical relevance to reflect the response of tumors in patients to PDT. Additionally, by combining higher resolution imaging techniques (such as multiphoton microscopy) to monitor microenvironmental oxygen levels and metabolic changes, in-depth research on the mechanism of action of PDT can be conducted.

3.2. Glioblastoma organoid bioprinting

Glioblastomas, or gliomas, are the most common primary malignant tumors of the central nervous system in adults [160]. Owing to its high malignancy rate, rapid progression, and diffuse infiltration, glioblastomas typically cannot be completely removed through surgery and tend to recur after surgery [161]. In addition, glioblastomas exist within a complex tumor microenvironment (TME) containing various cell types, including glioblastoma cells, glioblastoma stem cells (GSCs), mesenchymal stem cells (MSCs), and immune cells [162]. In addition, there are differences in cell characteristics and gene expression patterns in different regions. Traditional 2D cultures cannot simulate cell interactions and tumor microenvironment interactions. Therefore, studies of glioblastomas are limited. Bioprinting has facilitated the construction of clinically relevant brain tissue organoid models, by accurately placing tumor cells to replicate the natural tumor microenvironment. It is becoming a promising tool for creating simulated GBM structures and cell compositions and studying tumor biology.

In recent years, various laboratories have advanced the bioprinting of glioblastoma models by developing new bioprinting methods, leading to in-depth research on *in vitro* glioblastoma models. Shu et al. [58] first constructed a U87MG glioblastoma model on a sodium alginate hydrogel using extrusion bioprinting. After 11 days of printing, the cell viability remained at 88 ± 4.3 %. By utilizing photolithography and adjusting the shape of the PEGDA microcavities, they further controlled the shape, size, and thickness of U87MG glioma spheroids [59]. Similarly, in a bioink composed of fibronectin, alginate, and laminin, U87MG formed tumor spheroids, with high CD133 and DCX expression, indicating the maintenance of glioblastoma stem cell-like characteristics [62]. Erin et al. [66] utilized an immersion bioprinting method to construct a high-throughput *in vitro* glioblastoma organoid model using patient-derived tumor cells in a 96-well plate. This technology retains the heterogeneity of patient-derived tumors but requires further research to improve and expand the consistency of patient tumor organoids (PTOs). Further optimize 3D printing techniques to enhance the stability and complexity of tumor organoids, better simulating the biological characteristics of primary tumors. This high-throughput modeling method can be combined with artificial intelligence technology to achieve a more intelligent process for tumor organoid construction and drug screening. Interestingly, Matteo et al. [67] utilized FRESH 3D bioprinting to construct a glioblastoma organoid model of human

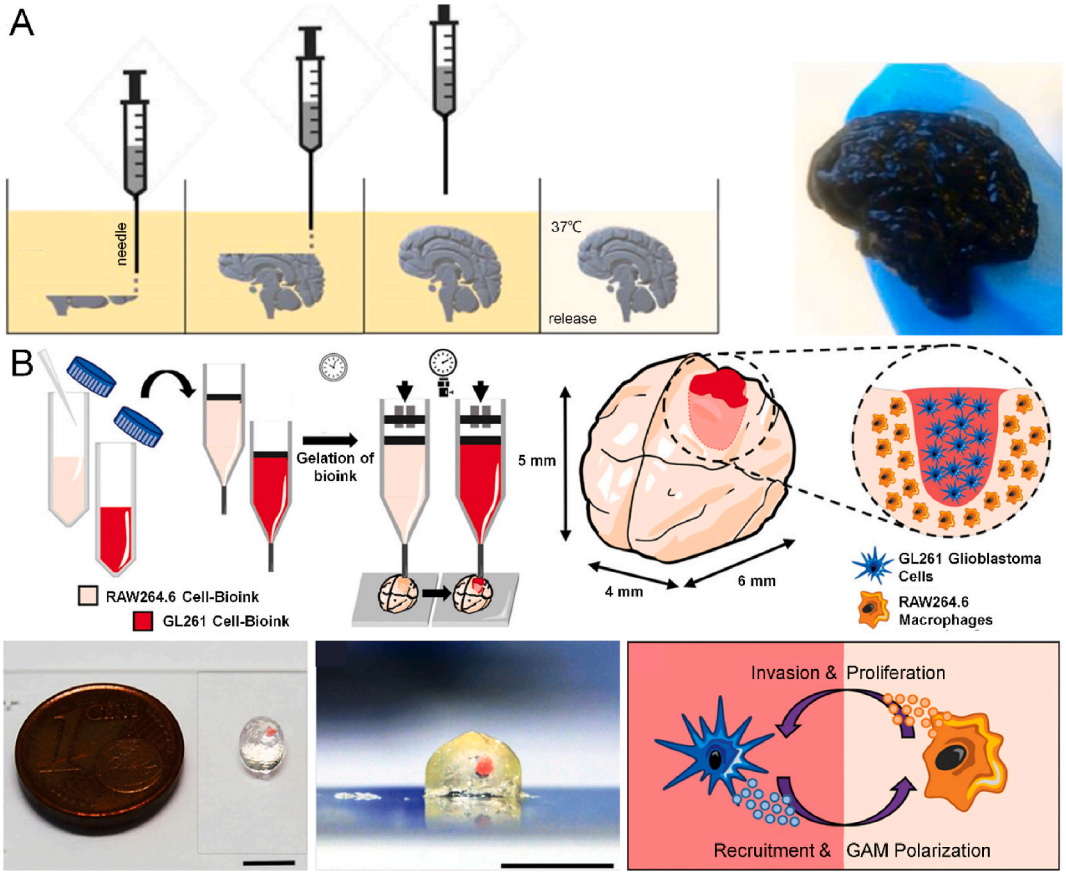

Fig. 5. Schematic representation of the construction of a glioma organoid model using bioprinting. (A) Workflow of FRESH bioprinting technology and brain-like scaffolds obtained by bioprinting with cellulose-based bioinks [67]. Reproduced with permission. Copyright 2020, MDPI. (B) Schematic representation of the bioprinting process and bioprinting mini-brains, as well as a schematic representation of the crosstalk between glioblastoma cells and macrophages [64]. Reproduced with permission. Copyright 2019, Wiley-VCH.

neuroblastoma (SH-SY5Y cell line) using conductive bioink based on cellulose nanofibers (CNF), alginate, and single-walled carbon nanotubes (SWCNTs). This model promotes the mature differentiation of SH-SY5Y cells into mature neurons and facilitates the formation of neural networks (Fig. 5A). Furthermore, this innovative experiment provides a tool for better understanding the pathophysiological mechanisms of brain tumor-related neurological disorders. However, this study did not delve into the mechanisms by which the conductive nanocellulose scaffold induces neuroblastoma cell differentiation. Future works are suggested to focus on attempting to implant the scaffold into animal models for *in vivo* validation to observe its impact on neural function restoration, thereby assessing its feasibility for clinical applications. Combined with high-throughput technologies such as transcriptomics and proteomics, a systematic analysis of the molecular mechanisms by which the scaffold promotes neuroblastoma cell differentiation can provide a deeper theoretical foundation for related drug development.

Glioblastoma is an intracranial tumor with a poor prognosis, characterized by an extensive abnormal vascular network. Furthermore, glioblastomas often use the microvasculature to guide migration. Understanding the cellular interactions between vascular and GBM cells may lead to new therapeutic strategies. By co-culturing primary adipose-derived stem cells with human umbilical vein endothelial cells (HUVECs) in a composite hydrogel of 5 % GelMA and 2.5 mg ml−1 fibronectin at a ratio of 1:0.75, a successful simulation of a vascularized tumor microenvironment was achieved. Patient-derived STA-NB1 neuroblastomas attract microvessels to approach and migrate within them [69]. To further explore the angiogenic potential of glioma cells, an *in vitro* model using neuroglioma U118 cells and glioma stem cells GSC23 cells was established. Both 3D-U118 and 3D-GSC23 cells demonstrated the ability to form blood vessels. 3D-GSC23 cells exhibit strong capabilities to form cell spheroids, secret VEGFA, and form tubular structures *in vitro* [65]. In summary, the experimental results above suggest that the glioma microenvironment model exerts a promoting effect on the vascularization process of glioma cells. Glioma cells can stimulate the proliferation of endothelial cells and the formation of luminal structures, thereby promoting the growth and dissemination of gliomas. Xu et al. conducted a series of studies on glioma stem cells. Firstly, they successfully enriched glioma stem cells using a gelatin-alginate-fibrinogen (GAF) hydrogel scaffold, and the enriched glioma stem cells retained the inherent characteristics of tumor stem cells [63]. They also showed the potential to differentiate into glial, neuronal, and vascular endothelial cells [60]. To observe the crosstalk between tumor microenvironment cells, glioma stem cells (GSC) and mesenchymal stem cells (MSC) were co-cultured using coaxial bioprinting. The interactions between GSC and MSC and their roles in tumor progression were observed. The results showed that GSC and MSC fused, and the fused cells co-expressed biological markers of both GSCs and MSCs, and exhibited stronger proliferation, clonogenicity, and invasive capabilities than GSCs and MSCs. Furthermore, the fused cells showed stronger tumorigenicity in nude mice, exhibiting pathological features of malignant tumors [61,68]. This cell fusion may be an important mechanism leading to the poor prognosis of gliomas. The newly formed hybrid cell lines resulting from cell fusion exhibit more aggressive and hypoxia-tolerant malignant phenotypes, providing insights into further understanding tumor heterogeneity and treatment resistance. Blocking cell fusion or disrupting the key signaling pathways of fused cells could potentially become a new therapeutic strategy. Similarly, during co-culture with glioblastoma cells, glioma-associated macrophages (GAM) are recruited by glioma cells and polarized into a GAM-specific phenotype. They actively secreted growth factors to promote tumor cell proliferation [64] (Fig. 5B). Therefore, by disrupting the interactions between glioma cells and GAMs, or altering the polarization state of GAMs, an important strategy for future glioblastoma treatment may be developed.

3.3. Lung tumor organoid bioprinting

Lung tumors originate from the bronchial mucosa or glands in the lungs. Lung tumors are one of the most common and deadliest malignant tumors worldwide, posing a significant problem health issue and a substantial burden [163]. The treatment options for lung tumors include surgery, radiation therapy, chemotherapy, and targeted drug therapy. The development of drug resistance often contributes to the recurrence of lung tumors, as lung tumor cells have demonstrated the ability to develop resistance to chemotherapy [163,164]. In addition, the tumor microenvironment plays a crucial role in drug resistance. Inflammatory cells and factors within the tumor inflammatory microenvironment promote tumor angiogenesis, epithelial-mesenchymal transition, cell apoptosis, and the activation of inflammatory pathways, leading to the occurrence, development, metastasis, and drug resistance of lung tumors [165,166]. Moreover, most studies on tumor occurrence, progression, and the assessment of anti-tumor drugs are based on 2D tumor models, which may lead to the loss or alteration of some original features and functions. Bioprinting offers a reliable, biomimetic 3D tumor model replicating the actual *in vivo* environment, aiding in the study of tumor development and drug screening.

Gelatin-alginate hydrogels are commonly used to construct *in vitro* models of lung tumors by simulating the *in vivo* tumor microenvironment (TME) to aid in the study of tumor growth, invasion, and drug screening. Xu et al. [70] maintained continuous proliferation of A549 cells in gelatin-alginate hydrogels for up to 28 days. Arindam et al. [71] observed upregulation of vimentin, α-SMA, and loss of E-cadherin during co-culturing of non-small cell lung tumor (NSCLC) patient-derived xenograft (PDX) cells and lung CAFs, confirming the feasibility of using gelatin-alginate hydrogel for studying cell-cell crosstalk. Furthermore, drug sensitivity testing of eight traditional anti-tumor Chinese medicines showed that, compared to 2D models, 3D models exhibited higher drug resistance [73]. This validates the practicality of using a gelatin-sodium alginate hydrogel as a 3D bioprinting lung organoid tumor model for drug screening.

In recent years, the emergence of many novel composite bio-inks has deepened our understanding of lung tumor organoid models. By printing the Hphil-CNF hydrogel on the surface of the Hphob-CNF hydrogel, hollow 3D channels were formed, allowing the real-time observation of cell morphology, cellular responses to drug stimuli, and chemical flow within the channels [72]. It provides an innovative and promising experimental platform for cell culture and biomedical research. Similarly, in GelMA-PEGDA hydrogels, the high upregulation of lung CSC-specific marker genes indicates that this model promotes the expression of lung CSC-specific markers in non-small cell lung tumor (NSCLC) cells [75]. It revealed the biological behavior of lung cancer stem cells under different conditions. Likewise, patient-derived NSCLC cells form 3D spheroids in the polysaccharide-based ink H4-RGD, showing stronger resistance than 2D monolayer cells to NSCLCPDX cells [74]. This suggests a role for the tumor microenvironment created by Ink H4-RGD in determining the variability of chemotherapeutic responses in three-dimensional spheroids.

3.4. Cervical tumor organoid bioprinting

Cervical tumors are the most common malignant tumors of the female reproductive tract, and human papillomavirus (HPV) is a primary risk factor for the development of this disease [167]. Early cervical tumors often have no obvious symptoms or signs, and in advanced stages, they can present with systemic symptoms such as anemia and cachexia. Furthermore, early-stage cervical tumors are prone to lymphatic metastasis, leading to a relatively poor prognosis, while late-stage metastasis results in poor prognosis and a high mortality rate [168]. However, the potential mechanisms underlying metastasis remain unclear [169]. Therefore, establishing biologically relevant organoid models to elucidate the mechanisms of cervical tumor cell migration and

invasion is crucial for providing a platform for *in vitro* mechanistic studies and personalized treatment of HPV-related cervical diseases [170].

Chen et al. [76] used photolithography-based bioprinting technology to construct a 3D *in vitro* microchip with a honeycomb-like branched blood vessel structure in a PEGDA hydrogel. The migration speed of HeLa cells increased as the width of the microvascular channels decreased, revealing a close correlation between tumor cell migration and blood vessel diameter. Although this 3D model provides a rapid and cost-effective tool for studying tumor migration, it does not elucidate the mechanisms of tumor cell migration. To further investigate the crucial stage of epithelial-mesenchymal transition (EMT) in cervical tumor cell metastasis, Sun et al. added the main inducer of EMT, TGF-β [171,172]. They observed the disintegration of 3D HeLa cell spheroids formed in collagen-sodium alginate-Matrigel, with immunohistochemistry showing activation of the Smad2/3 pathway, promotion of the transcription factor Snail, and suppression of E-cadherin, indicating achievement of the EMT process [77]. By further combining gene editing technologies (such as CRISPR-Cas9) and single-cell RNA sequencing, it is possible to study in greater detail the changes in gene expression and signaling pathways during the TGF-β-induced EMT process. Therefore, establishing an effective environmental stimulus as a regulatory sign in a 3D tumor model can help us better understand the occurrence and development of cervical tumors and subsequently regulate tumor metastasis by modulating the tumor microenvironment. Overall, this study provides new methodological tools for EMT research in cervical cancer, which is of significant importance. Further optimization and application of this model are expected to bring more discoveries regarding EMT and tumor progression mechanisms.

3.5. Ovarian tumor organoid bioprinting

Among all gynecological malignancies, ovarian tumors have the highest mortality rate [173]. Early ovarian tumors lack symptoms, and when symptoms appear, they are nonspecific, leading to a poor overall prognosis and propensity for metastasis and recurrence [174]. Traditional 2D cell culture systems have led to significant medical advancements in oncology research; however, the progression of ovarian tumors remains unclear. Organoid models constructed based on bioprinting can recreate the unique glandular structure of ovarian tissue *in vitro* and, through temporal and spatial control of the tumor microenvironment, simulate the interactions between different cell types in a high-throughput and reproducible manner. This allows for a systematic study of the various unknown regulatory feedback mechanisms between tumor and stromal cells and provides a tool for researching tumor biology [175,176].

Fibroblasts play a crucial role in the malignant progression of ovarian tumors [175]. An *in vitro* ovarian tumor model was constructed by co-culturing OVCAR-5 cells and MRC-5 cells on Matrigel™. Through the use of a 150 μm micro-nozzle, precise cell positioning and assembly were achieved, and it was observed that tumor cells spontaneously formed glandular structures resembling ovarian tumor micro-nodules *in vivo*. Where tumor cells spontaneously formed glandular structures resembling micro-nodules of ovarian tumor *in vivo* [79]. In future studies, various stromal cells could be introduced into this 3D ovarian tumor *in vitro* model to systematically study many unknown regulatory feedback mechanisms between cells, facilitating high-throughput drug screening and therapeutic interventions.

3.6. Liver tumor organoid bioprinting

Intrahepatic cholangiocarcinoma (ICC) cells are the second most common primary liver tumor cells within the liver [177]. The incidence and prevalence of ICC have been increasing every decade, and most patients with ICC present with advanced or refractory metastatic disease [178]. Moreover, treatment options for ICC are limited, with only approximately 20–30 % of patients qualifying for surgical resection, which is considered the only potentially curative treatment [179]. However, drug therapy has shown limited effectiveness. Mouse models are a crucial tool for drug screening in ICC, but they are associated with ethical controversies, time-consuming processes, high costs, and complex operations [180,181]. The bioprinted ICC tumor model exhibits higher resistance to anti-tumor drugs than 2D cultures, highlighting the potential role of patient-derived tumor models created through bioprinting in oncology research and the development of personalized treatments.

Patient-derived ICC cells are likely to have more clinical significance compared to ICC cell lines, as the cell lines have already lost their heterogeneity. Mao et al. [78] employed patient-derived primary ICC cells in a gelatin-alginate-MatrigelTM composite hydrogel system to construct an *in vitro* tumor model. Compared to 2D culture, the tumor markers CA19-9 and CEA, cancer stem cell markers CD133 and EpCAM, and liver damage-related liver function markers ALT, AST, and ALB were upregulated by 1.9, 5.7, 3.7, 9.7, 3.7, 1.9, and 2.0 times, respectively, in 3D bioprinting models. Therefore, the 3D *in vitro* culture model can more accurately mimic *in vivo* tumor phenotypes and thus can more precisely simulate treatment responses.

3.7. Osteosarcoma organoid bioprinting

Osteosarcoma is the most common primary malignant bone tumor in adolescents [182], characterized by the direct production of bone-like tissue from tumor cells. The development of drug resistance and metastasis is closely associated with poor prognosis. In addition, the osteosarcoma microenvironment is now recognized as essential for its growth and spread [183]. To identify new therapeutic targets, a better understanding of the mechanisms underlying tumor drug resistance and metastasis is urgently needed. Therefore, it is crucial to elucidate the interactions between osteosarcoma cells and the complex bone and bone marrow microenvironments [184]. Bioprinting technology can manufacture novel bone tissue engineering scaffolds with customized shapes [185], mechanical strength, and cellular composition, providing accurate *in vitro* migration and drug screening experiments [186].

Pellegrini et al. [82] constructed a 3D *in vitro* osteosarcoma model by embedding U2-OS cells and their drug-resistant strain U–2OS/CDDP 1 μg in collagen hydrogel. The cells grew uniformly within the scaffold, and the tumor cell clusters degraded the collagen matrix, creating lacunae through which they migrated, similar to acellular scaffolds. This invasion behavior is akin to that of tumors *in vivo*. This osteosarcoma model successfully maintained the biological characteristics of OS cells in their natural microenvironment, making it a promising tool for drug screening and OS cell biology research.

3.8. Melanoma organoid bioprinting

Melanoma is one of the most aggressive and progressive forms of skin tumor [187]. It primarily occurs in the skin but can also develop in various locations and tissues such as the mucous membranes and meninges. As the disease progresses, melanoma exhibits regional and distant metastases, leading to a poor prognosis, with a 5-year survival rate of less than 5 %. Although several new drugs have been developed in recent years, most patients do not show a lasting response to these treatments [188]. Therefore, new biomarkers and drug targets are required to improve the accuracy of melanoma diagnosis and treatment [189]. 3D bioprinting based on *in vitro* cell culture is a novel and creative method for creating a simulated microenvironment for the growth of malignant melanoma cells that mimics the human body environment.

A 3D scaffold composed of GelMA-PEGDA composite hydrogel was fabricated to construct an *in vitro* tumor model simulating the growth microenvironment of human malignant melanoma cells (A375) [80]. The melanoma cells on the 3D scaffold exhibited higher proliferation rates, elevated MMP-9 secretion levels, and increased invasiveness

compared to those in a 2D environment. Conducting longer-term cultivation experiments to observe the long-term behavior of tumor cells within the scaffold could further advance this technology's application in cancer research and drug development.

3.9. Multiple myeloma organoid bioprinting

Multiple myeloma (MM) is a malignant proliferative disease of plasma cells that accounts for approximately 12 % of malignant tumors of the hematopoietic system. A characteristic feature of this disease is the uncontrolled proliferation of plasma cells in the bone marrow, leading to organ or tissue damage [190]. MM is characterized by the following four main features: bone destruction, renal dysfunction, hypercalcemia, and anemia. MM occurs almost exclusively within the bone marrow microenvironment, which provides the necessary signals and stimuli to induce cell proliferation and/or prevent apoptosis, promoting the development of drug resistance [191]. Therefore, reproducing the specific bone marrow microenvironment of MM cells is crucial for understanding the molecular mechanisms driving MM progression and treatment

resistance (Fig. 6).

Using coaxial bioprinting, a composite bioink simulating the outer cortical bone composed of GelMA, alginate, PEGDA, and nHA, as well as the inner bone marrow-like microenvironment composed of GelMA and MM cells, was printed simultaneously to mimic the human bone marrow niche [81]. With this method, a 3D core-sheath model was employed for the first time to achieve co-culturing of MM and HS-5 stromal cells. Moreover, it was observed that IL-6 secreted by HS-5 promotes the proliferation and aggregation of MM cells, demonstrating this co-culture model held significant implications for guiding combination drug therapy in tumors.

3.10. Chronic lymphocytic leukemia organoid bioprinting

Chronic lymphocytic leukemia (CLL) is a complex and heterogeneous hematologic malignancy of the blood system [192], and it is the most

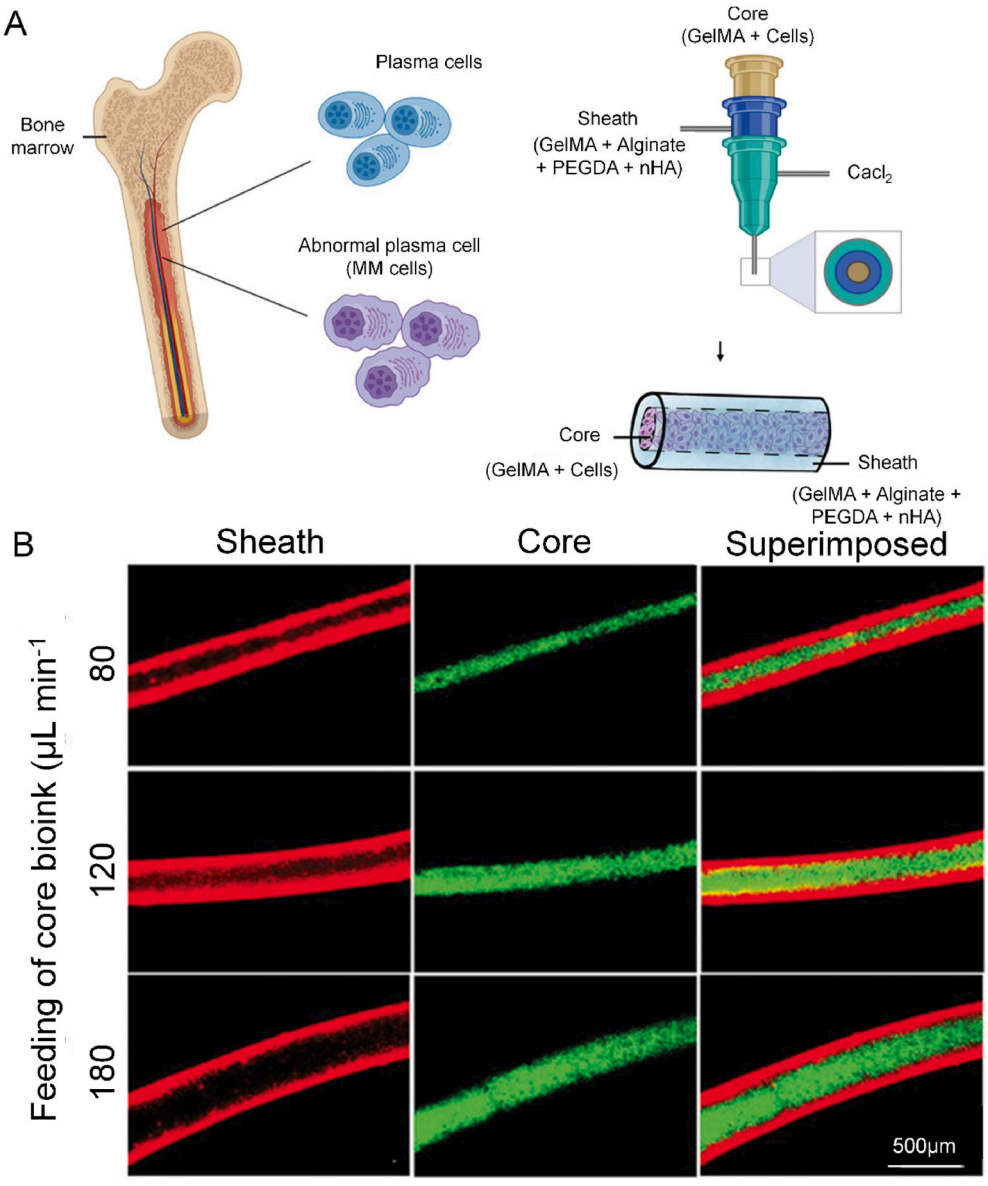

Fig. 6. Schematic representation of an osteosarcoma organoid model constructed using coaxial bioprinting. (A) Schematic of the coaxial nozzle used for bioprinting. MM cells filled with a low concentration of GelMA represent the inner core of the cartilage marrow, the sheath used to mimic the surrounding cortical bone. (B) The inner/outer diameter of bioprinting nucleus-sheath structures positively correlates to the supply rate of core bioinks [81]. Reproduced with permission. Copyright 2022, Wiley.

common adult leukemia in Western countries [193]. Studies have shown that CLL pathogenesis is closely related to the tumor-supportive microenvironment and immune system dysfunction. The disease exhibits significant clinical variability and remains incurable [194,195]. In addition, culturing primary CLL cells *in vitro* is challenging, and traditional two-dimensional *in vitro* models lack the cellular and spatial complexity present *in vivo*, leading to an incomplete understanding of the actual events occurring at these sites. Francesca et al. [83] established the first long-term 3D culture model for CLL by embedding CLL cells in a fibronectin 411 hydrogel matrix, maintaining the growth of primary CLL cells for up to 28 days while preserving the expression of the characteristic surface markers CD19 and CD5. This represents a groundbreaking advancement and provides a reference for simulating the physiological settings of other non-solid tumors (Fig. 7).

4. Conclusion

This study detailed the construction of *in vitro* organoid tumor models using various bioinks combined with different printing methods. The emergence of 3D bioprinting technology has led to significant breakthroughs in developing various *in vitro* organoid tumor models and hydrogel tissue engineering. Using bioprinting technology, significant technological innovations have been achieved in material selection and overall construction. Multiple printing techniques and bio-materials are being used to address the limitations of traditional tumor organoid modeling. *In vitro* organoid tumor models modify the heterogeneity of the microenvironment, including the presence of non-tumor cells and their functions, the signaling of soluble cell factors, and changes in extracellular matrix components.

Tumor treatments are increasingly shifting towards personalized therapies targeting individuals with unique and heterogeneous diseases. Some studies have linked *in vitro* model responses to drugs with patient outcomes. The use of *in vitro* models for high-throughput drug screening is widely applied in oncology research and holds promise as a potential tool for screening effective drugs for patients with tumors. The main limitations of functional precision medicine are the establishment of physiological culture models, the development of high-throughput systems, and difficulties in measuring tumor heterogeneity. Bioprinting overcomes these barriers, with 3D *in vitro* tumor models being promising for precision medicine, allowing for rapid *in vitro* modeling using tumor cells sourced from patients to accurately simulate patient responses to treatment. They are physiologically relevant, personalized tumor models that are highly suitable for drug development and clinical applications and facilitate individual tumor response analysis.

Although bioprinting organoid tumor models are continuously advancing and providing innovative biomedical and clinical translational research approaches, some obstacles remain to be addressed. First, current tumor models only simulate a single type of tumor and cannot simultaneously simulate the development of multiple tumors. In the future, the various advantages of bioprinting will make it suitable for developing human tumor microchip models, and microchip technology is expected to achieve connectivity and communication between

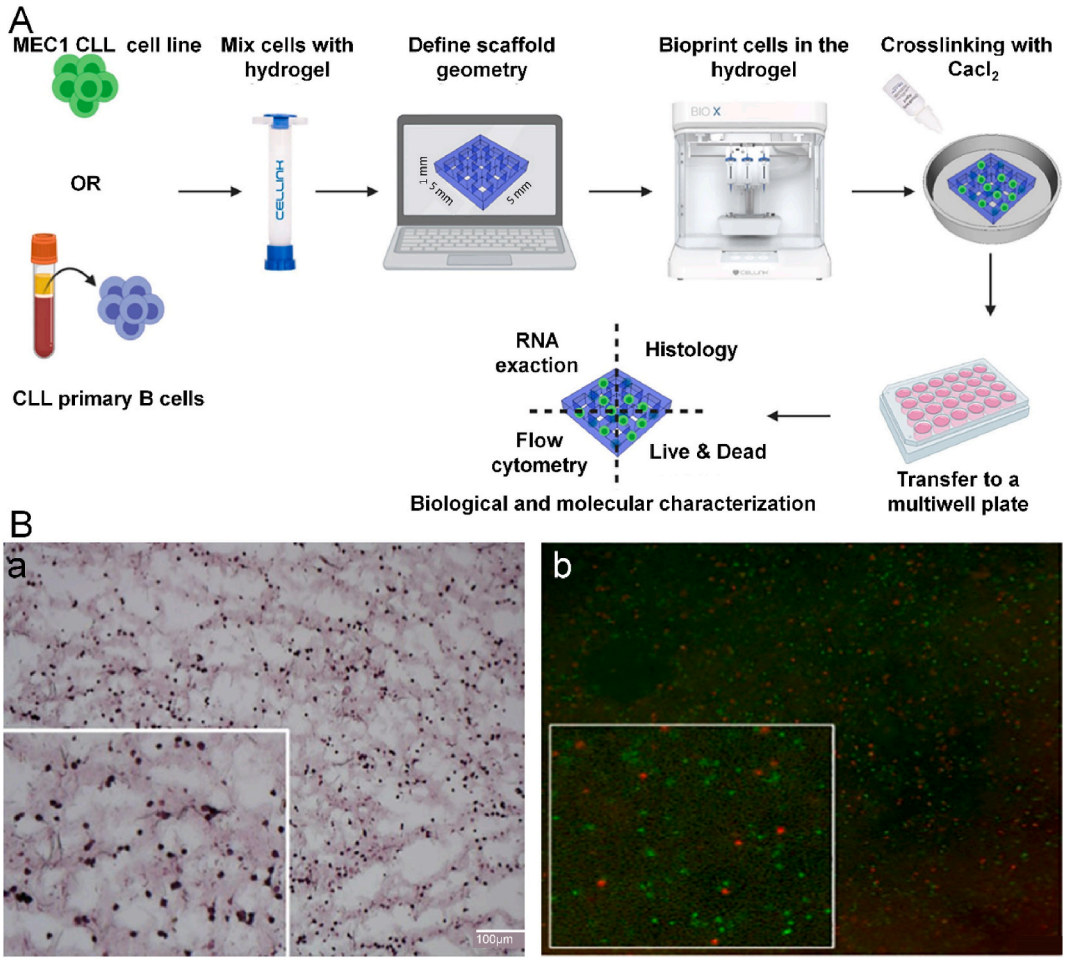

Fig. 7. Schematic representation of the construction of a chronic leukemia organoid model using bioprinting. (A) Schematic representation of the bioprinting strategy using the CLL cell line MEC1 or CLL primary B cells. (B, a) Representative H&E staining of 5 μm cryosections of 3D bioprinting CLL progenitor cells showing their distribution in the scaffold. (B, b) Representative images of bioprinting CLL progenitor cells acquired using Axio Observer Zeiss fluorescence microscopy for live/dead assay at day 28 [83]. Reproduced with permission Copyright 2021, Frontiers Media.

multiple tumor models, treating various organs/tissues as one model. Using this multi-organ microchip model, complex drug metabolism that single-organ tumor models cannot simulate can be achieved, similar to the human body environment.

Second, limited reproducibility is a significant barrier to generating organoid tumor models and maintaining their functionality. The primary factors affecting the reproducibility of tumor models include batch-to-batch variability, production scalability, cell composition, and tumor model structure. Future material research should focus on developing and designing bioinks with better biological performance or composite bioactive factors to functionalize bioinks continually, thus mimicking *in vivo* growth factors or other mechanical and chemical stimuli to functionalize and maintain the reproducibility of printed structures. By improving the standards of bioprinting materials, the reproducibility of tumor models will continue to improve, ultimately leading to significant progress in clinical trials.

Further expansion of bioprinting technology is needed to elucidate the interactions between multiple cells and build organoid tumor models that better reflect physiological states, thereby increasing their usefulness in clinical trials. Meanwhile, 4D printing, a promising technology platform with highly tunable material selection, allows biomaterials to respond to external stimuli, enabling the development of bio-folding hydrogel scaffolds with self-folding behavior and allowing pre-printed 3D configurations to change over time, advancing the manufacture of functional 3D tissues significantly. 4D printing technology is also attractive for drug delivery systems, allowing the programmable release of drugs or cells, reducing drug leakage, and improving drug delivery efficiency. So far, existing self-assembly or self-folding 4D printing systems have been limited to macroscopic deformations, restricting the precise spatial manipulation of 4D printed structures. Therefore, the exact construction of organoid tumor models requires the integration of advanced technologies from various fields.

CRediT authorship contribution statement

Xiangran Cui: Writing – original draft, Visualization, Validation, Resources, Methodology, Formal analysis. **Jianhang Jiao:** Validation, Resources, Methodology, Funding acquisition. **Lili Yang:** Writing – review & editing, Validation, Resources. **Yang Wang:** Writing – review & editing, Supervision. **Weibo Jiang:** Writing – review & editing, Supervision. **Tong Yu:** Writing – review & editing, Validation, Resources. **Mufeng Li:** Writing – review & editing, Supervision. **Han Zhang:** Writing – review & editing, Validation, Resources. **Bo Chao:** Writing – review & editing, Validation, Resources. **Zhonghan Wang:** Writing – review & editing, Supervision, Resources, Conceptualization. **Minfei Wu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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