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REVIEW

The construction of in vitro tumor models based on 3D bioprinting

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Abstract

Cancer is characterized by a high fatality rate, complex molecular mechanism, and costly therapies. The microenvironment of a tumor consists of multiple biochemical cues and the interaction between tumor cells, stromal cells, and extracellular matrix plays a key role in tumor initiation, development, angiogenesis, invasion and metastasis. To better understand the biological features of tumor and reveal the critical factors of therapeutic treatments against cancer, it is of great significance to build in vitro tumor models that could recapitulate the stages of tumor progression and mimic tumor behaviors in vivo for efficient and patient-specific drug screening and biological studies. Since conventional tissue engineering methods of constructing tumor models always fail to simulate the later stages of tumor development due to the lack of ability to build complex structures and angiogenesis potential, three-dimensional (3D) bioprinting techniques have gradually found its applications in tumor microenvironment modeling with accurate composition and well-organized spatial distribution of tumor-related cells and extracellular components in the past decades. The capabilities of building tumor models with a large range of scale, complex structures, multiple biomaterials and vascular network with high resolution and throughput make 3D bioprinting become a versatile platform in bio-manufacturing as well as in medical research. In this review, we will focus on 3D bioprinting strategies, design of bioinks, current 3D bioprinted tumor models in vitro classified with their structures and propose future perspectives.

Keywords Tumor model · 3D bioprinting · Bioink · Tumor-on-a-chip · Drug screening

Introduction

Cancer is one of the top three fatal diseases that kill most people of the year around the world. According to the status report on the global burden of cancer worldwide, in 2018, the number of new cancer cases was estimated to be 18.1 million, and the number of cancer deaths was estimated to be 9.6 million [1]. The hallmarks of cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism and evading immune destruction enabled by genome instability and inflammation [2]. Additionally, the tumor microenvironment contains multiple cell types such as cancer cells and cancer stem cells, cancer-associated fibroblasts, endothelial cells, pericytes, immune-inflammatory cells and progenitor cells of the tumor stroma, among which there are complex interactions within the dynamic extracellular matrix during the tumor progression, and the ECM compositions are continuously erected and remodeled collectively by those various cells [2–7]. The complexity hallmarks and behaviors of cancer cells and tumor microenvironment makes it difficult to investigate the molecular biological mechanisms of cancer and the anticancer targeting therapies always demand lots of time and money. Traditional animal models and experiments are ethically controversial and unsatisfying in recapitulating drug responses of patients. Also, conventional tissue engi-

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neering methods of modeling tumors in vitro face a number of problems including oversimplified structures and limited vascularization potential which lead to size-limited models and a lack of controllable spatial distribution of tumor-related cells and ECM compositions [8]. With the development and application of 3D printing technologies in biomedical field, 3D bioprinting has been extensively used and applied to construct artificial tissues with intricate structures and various components, especially heterogeneous multicellular/material tissues [9]. Here, we will give a brief introduction of 3D bioprinting strategies and the form of bioinks, and then, we will target their applications in tumor models in vitro classified by structure. At last, we will summarize and give the prospect of the future work.

3D bioprinting strategies and bioinks

Three-dimensional (3D) printing, also known as additive manufacturing, has been used for printing multiple cells and supporting biochemical compositions encapsulated in bio-related materials to fabricate complex 3D functional tissues/organs/bio-models/bio-chips [10, 11]. In general, the strategies of 3D bioprinting are commonly divided into inkjet printing, microextrusion printing, laser-assisted printing and stereolithography [8, 12–20]. Inkjet printing is a noncontact printing technology which is based on biomaterial-containing droplet deposition onto the substrate using thermal or piezoelectric actuation. It is relatively feasible to print single cells and could provide high resolution with fast speed. The extrusion-based printing is the most widely used because of the processability it gives various biomaterials with low cost [14]. It is usually driven by pneumatic or mechanical force and suitable for a limited range of viscosity of bioinks. Laser-assisted printing deposits cell-containing droplets mainly by the optical tweezer effect and thermal shock effect of laser on trace materials. This type of technique provides high resolution but high cost and compromised cell viability due to the damage from the laser. In another type of bioprinting strategy adopted from stereolithography, an array of programmed mirrors first generate a digital mask which is then projected to a bioink reservoir, the designed patterns are photo-crosslinked following the vertical movement of the platform to form the defined architecture of the 3D structures in a layer-by-layer manner with relatively fast speed and high fidelity [8, 18–22]. With these innovative bioprinting strategies, in vitro tumor models could be built with an organized spatial distribution of multiple components and a large range of scale of defined structures, providing available platforms for the research on biological exploration of cancer and drug screening [23].

Bioinks are usually referred to cell-laden hydrogels in biofabrication with the ability of encapsulation of cells which could mimic several features of the ECM in vivo to provide certain types of biologically relevant chemical and physical signals in the 3D microenvironment with various shapes and biomechanical characteristics [24]. Existing bioinks can be generally divided into two categories: synthetic polymers or hydrogels such as PEG, PLA, PGA, and PLGA, and naturally derived hydrogels such as hyaluronic acid, alginate, gelatin, collagen and fibrin including their derivatives as well as decellularized ECMs (dECMs) derived bioinks [10, 25, 26]. Although these two varieties of hydrogels have different strengths and weaknesses, for instance, naturally derived hydrogels always show excellent biocompatibility along with poor structural formability and synthetic biomaterials usually exhibit the opposite, during and after the printing process the printability, formability and stability of the printed structures also relies on the crosslinking methods and the modification as well as the combination of diverse bioinks [27–30]. These various bioinks could meet multiple demands of different microenvironments of tissues and organs as well as distinct types of tumors.

3D bioprinting of in vitro tumor models

Although 3D bioprinting has not been adopted in tumor model construction in vitro until this decade, the remarkable advantages in locating various cells and microenvironmental factors as well as the efficient fabrication of sophisticated structures have made this unique technology increasingly applied to in vitro tumor construction for cancer studies and drug development in recent years. Here, we will give an overview about these 3D bioprinted in vitro tumor models classified by their structures (Table 1).

Spheroids and scaffold-free structures

Since tumor cells always exhibit closer behaviors and drug responses in 3D culture environment than in two-dimensional (2D) culture compared with in vivo realities, some efforts have been made to fabricate tumor cellular spheroids by 3D bioprinting techniques with controlled size and shape followed by the investigation of the impact of those parameters on cell behaviors and the uptake of drug-related ligands [35, 36]. Also, there are several scaffold-free tumor models mimicking some of the tumor progressions, phenotypes, and interactions for cancer studies (Fig. 1). Using 3D bioprinting, Xu et al. constructed an in vitro co-culture platform on Matrigel for human ovarian cancer (OVCAR-5) cells and fibroblasts (MRC-5). They micropatterned different cell types and diverse factors of the microenvironment with an accurately controlled spatial distribution and controllable biological features to maintain the viability and prolifera-

Table 1 The division of in vitro tumor models based on structures/shapes

Structure/shape	Tumor type	Bioink	Bioprinting strategy	Key research points	References
Spheroids/scaffold-free structures	Ovarian	Cell suspension	Inkjet	Cell patterning high-throughput drug screening	[31]
	Glioma	Collagen gelatin	Extrusion	Cell–cell interaction microenvironmental factors	[32]
	Glioma	GelMA-gelatin	Extrusion	Cell–cell interaction drug screening	[33]
	Multiple types of tumors	Tunable hydrogels (removed later)	Extrusion	Model patient-specific tumor phenotypes	[34]
	Breast	Gelatin alginate	Inkjet stereolithography	Cellular spheroids formation process and behavior control	[35, 36]
Fibers	Breast	Alginate	Extrusion	Modeling tissue-mimetic interactions in vitro	[37]
	Glioma	Alginate gelatin	Coaxial extrusion	Tumor-stroma cells interaction	[38]
	Glioma	Alginate	Coaxial extrusion	Cell–cell and cell–ECM interaction drug development and screening	[39]
Grid scaffolds	Cervical	Gelatin/alginate/fibrinogen	Extrusion	Comparison of cell behaviors between 3D and 2D culture	[40]
	Cervical	Matrigel gelatin alginate	Extrusion	Epithelial-to-mesenchymal transition (EMT)	[41]
	Breast	GelMA	Stereolithography	Cell–cell interaction	[42]
	Liver	Sodium alginate/gelatin/fibrinogen	Extrusion	Comparison between 2D and 3D drug screening	[43]
	Glioma	Gelatin/alginate/fibrinogen	Extrusion	Simulate brain tumor microenvironment	[44]
	Lung	Gelatin alginate	Extrusion	Comparison between 2D and 3D culture condition	[45]
Tumor-on-a-chip	Liver	PCL, gelatin, collagen	One-step extrusion fabrication	Various organ-on-a-chip applications	[46]
	Lung	Fibrin, GelMA, PLGA	Extrusion and stereolithography	TUMOR metastatic study drug screening	[47]
	Glioma	dECMs derived bioinks	One-step extrusion fabrication	Patient-specific drug development	[48]

tion of cells through a high-throughput cell printing system, which could be an available tool for studies of tumor mechanisms and drug screening [31]. In Lee et al.'s study, a 3D glioblastoma (GBM) model with vascular niche using sacrificial bioprinting was built to investigate the interaction between cells and the influence of factors in the matrix microenvironment on 3D cell behaviors. The platform could also be adapted to other systems [32]. And in 2019, a mini-brain consisted of glioblastoma cells and macrophages was printed by Heinrich and other coworkers to investigate the interactions and therapeutic-targeting between these two cell types [33]. Langer et al. developed an in vitro scaffold-free tumor model incorporated diverse cell types with defined structure through 3D bioprinting. The models could be patient-specific and able to simulate tumor tissues in vivo. In addition, the study provided a manipulable system for researches on biological mechanisms of distinct tumors [34]. Scaffold-free structures of tumor models are generally simple and convenient to build with the flexibility of spatial distributions of different cell types along with ECM compositions. However, the formability and stability of the cellular spheroids not only depend on the fabrication method but also

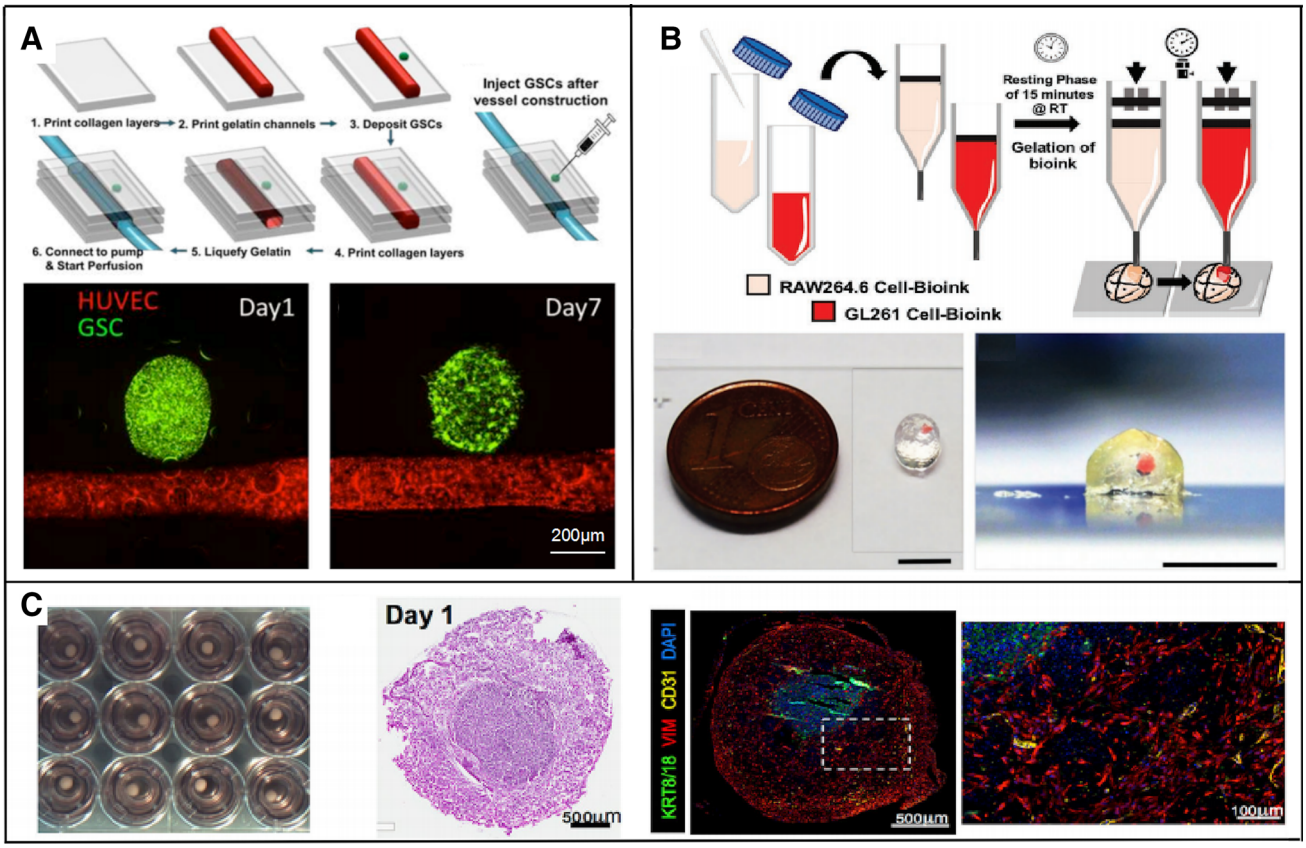

Fig. 1 Scaffold-free/spheroids structures of in vitro tumor models. **a** Glioblastoma-vascular niche with glioma stem cells spheroid (green) near a vascular channel (red). Reproduced with permission from [32]. Copyright 2015, IEEE. **b** 3D-bioprinted mini-brains consisted of glioblastoma cells and macrophages. Scale bar: 5 mm. Reproduced with permission from [33]. Copyright 2019, John Wiley and Sons. **c** H&E image and immunofluorescence of sections from bioprinted tissues for KRT8/18 (green), vimentin (VIM, red), and CD31 (yellow). DAPI (blue) is a nuclear counterstain. Scale bars: 500 μm or 100 μm as marked. Reproduced with permission from [34]. Copyright 2019, Elsevier

lie a lot on the inherent features of different types of cells. Besides, the scaffold-free structures could not always provide a complex spatial design of multiple cells and would bring about some difficulties to clearly observe the whole internal cell–cell and cell–ECM activities of the models.

Fibers

Fiber-like structures are also common for the co-culture of different cell types (Fig. 2). In 2015, Rapid 3D extrusion bioprinting method was used by Grolman et al. to develop tissue-mimetic fibers with a mouse macrophage (RAW 264.7) cell type mixed in the inner channel and human breast adenocarcinoma (MDA-MB-231) cell type in the surrounding peptide-modified alginate. Multiple architectures of spatial domains of the cell types could be controlled through the fluidic extrusion process and their impacts on the cell–cell interactions and biological characteristics could be studied in vitro [37]. In 2017, Dai et al. [38] used a coaxial extrusion 3D bioprinting system to construct self-assembled multicellular heterogeneous brain tumor fibers to study the interaction between tumor cells and stromal cells, which provided an available platform to study tumor microenvironment in vitro. And in 2018, Wang et al. adopted the same bioprinting system to locatively distribute glioma stem cell GSC23 and glioma cell line U118 separately in the shell and core of the hydrogel microfibers. A series of drug resistance experiments were conducted on different microfibers which confirmed that the microfiber could be a useful tool for drug development and screening [39]. Compared with the scaffold-free structures, the fiber-like structures of the tumor models could provide more specific designs of the spatial location of two different types of cells or components with the core–shell structure, but there are still some limitations when it comes to more than two types of cells or factors of interest and the observation of the fibers is still challenging to be more distinct and detailed.

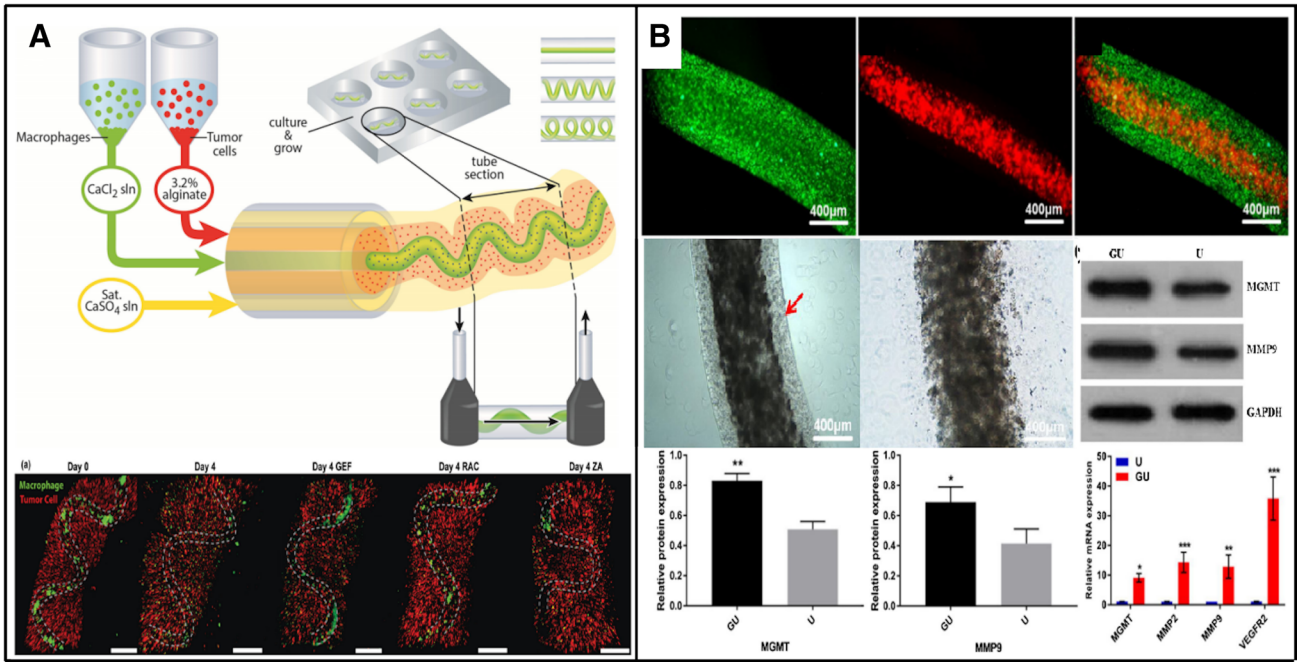

Fig. 2 Fiber-like structures of in vitro tumor models. **a** Rapid 3D extrusion of breast cancer (red)/macrophage (green) co-culture model. Scale bar: 400 μm. Reproduced with permission from [37]. Copyright 2015, John Wiley and Sons. **b** The images and expression of drug resistance-related genes in G/U and U hydrogel microfibers. Shell-GSC23 cells were labeled with PKH 67 dye (green), core-U118 cells were labeled with PKH 26 dye (red). Scale bar: 400 μm. Reproduced with permission from [39]. Copyright 2018, Elsevier

Grid scaffolds

The most widespread structure of existing tumor models is the grid matrix (Fig. 3) for its convenience for the printing process and the maintaining of cell viability due to the relatively large superficial area for the exchange of nutrients and oxygen. Also, this type of structure could provide a clear vision for the observation of cells and other experimental variables. In 2014, Zhao and other researchers developed cervical tumor models in vitro with hydrogels made of gelatin, alginate, and fibrinogen then mixed with Hela cells with high cell viability through 3D bioprinting technique. They measured and compared several tumor characteristics of the 3D models with those of 2D planar culture models. They came into a conclusion that Hela cells showed a higher rate of proliferation, matrix metalloproteinase (MMP) protein expression and chemoresistance in 3D environment and tended to form spheroids. The process could be helpful to study tumor biology and heterogeneity [40]. In 2018, similar models were applied by Pang et al. [41] to further study the epithelial-to-mesenchymal transition (EMT), and successfully proved the potential for future explorations of a therapeutic treatment targeting at cervical tumor metastasis. Also, stereolithography 3D bioprinting technique was adapted by Zhou et al. [42] to develop biomimetic bone matrices with bone stromal cells such as osteoblasts or human bone marrow mesenchymal stem cells (MSCs) loaded in nanocrystalline hydroxyapatite (nHA) contained gelatin methacrylate (GelMA) hydrogel and breast cancer (BrCa) cells were introduced into the matrices to study the interaction between tumor cells and bone stromal cells, which could provide a useful tool to reveal mechanisms of breast cancer bone metastasis. Zhou and his coworkers did some researches on a 3D liver model in vitro constructed by their own 3D bioprinting system with sodium alginate/gelatin/fibrinogen hydrogel loaded with HepG2 cells to investigate and compare the effects of several anti-cancer drugs on cells in both 2D and 3D culture conditions. And the 3D liver model could be available for drug screening [43]. Also, a 3D bioprinted glioma stem cell model was established by Dai et al. with the maintaining of the inherent characteristics of the cells and high cell viability to simulate the microenvironment of brain tumor. Glioma stem cells were found out to show differentiation potential and exhibit more resistance to chemotherapeutics (TMZ) with the comparison to 2D culture conditions [44]. And in 2018, a 3D bioprinted grid scaffold lung cancer model using gelatin–sodium alginate hydrogel loaded with lung cancer cell A549/95-D was constructed by Wang et al. with low-temperature molding principle of biological manufacturing. It was demonstrated that the model could maintain stable mechanical properties and the distributed cells showed biological functions and advanced invasion capability compared

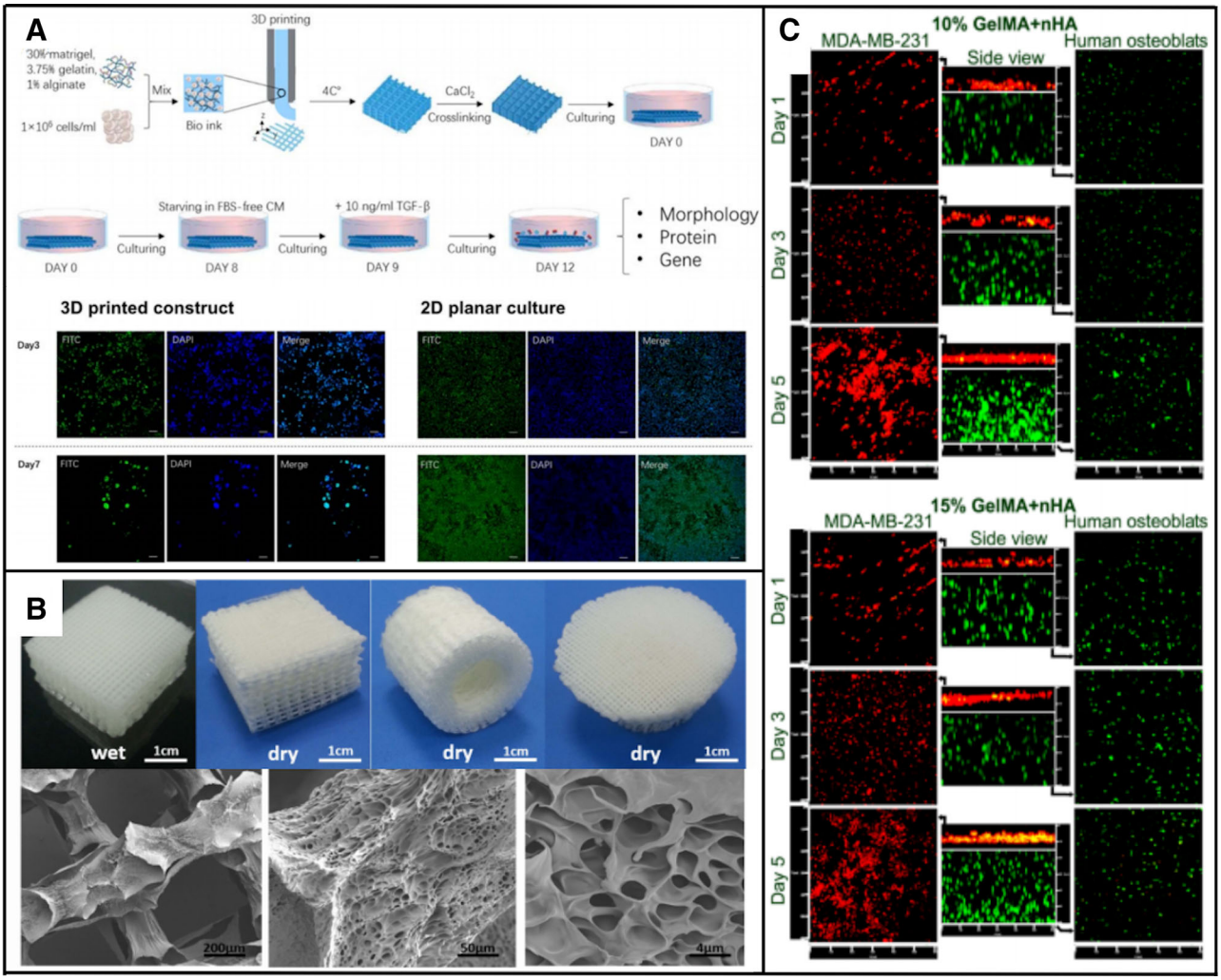

Fig. 3 Grid structures of in vitro tumor models. **a** Fabrication of the 3D HeLa/hydrogel constructs with SOX2 and DAPI staining of 3D and 2D culture. Scale bar: 100 μm. Reproduced with permission from [41]. Copyright 2018, IOP Publishing. **b** Gelatin/alginate/fibrinogen (GAF) hydrogel scaffolds and SEM images. Reproduced with permission from [44]. Copyright 2016, IOP Publishing. **c** Confocal micrographs of 3D bioprinted matrix co-cultured with osteoblasts stained by Cell Tracker Green CMFDA dye (green) and BrCa cells stained by Orange CMTMR dye (red) after 1, 3, and 5 days. Reproduced with permission from [42]. Copyright 2016, American Chemical Society

with cells in 2D culture, thus testified the 3D in vitro lung cancer model was more biomimetic and helpful for biomedical research [45]. Furthermore, the grid structure of tumor model could also provide a valuable platform for the investigation of cellular phenotypes in different bioinks, direct bioprinting of cellular spheroids to replicate the tumor microenvironment and the study of drug delivery as well as the treatment of cancer [49–51]. For all the advantages of the grid structure of tumor models, there are more demands of the formulation of the bioinks and the optimization of printing process due to the higher requirements of the precision and integrity of the grid structure. Also, how to prevent the collapse or deformation of the structures during the printing process as well as the following incubation period is still remained to be further resolved. Currently, the grid tumor models are oversimplified, and more components such as more types of cells and growth factors should be added to make these tumor models much close to the complex real tumor microenvironment. Still, people need to figure out to what extent of the simplified tumor model is acceptable regarding the structure, cell type and other components. It really depends on the applications of the tumor models, for disease models, more components should be added to faithfully reproduce the cellular behavior to mimic their in vivo counterparts, while for the drug screening model, the effectiveness and cost need to be well balanced.

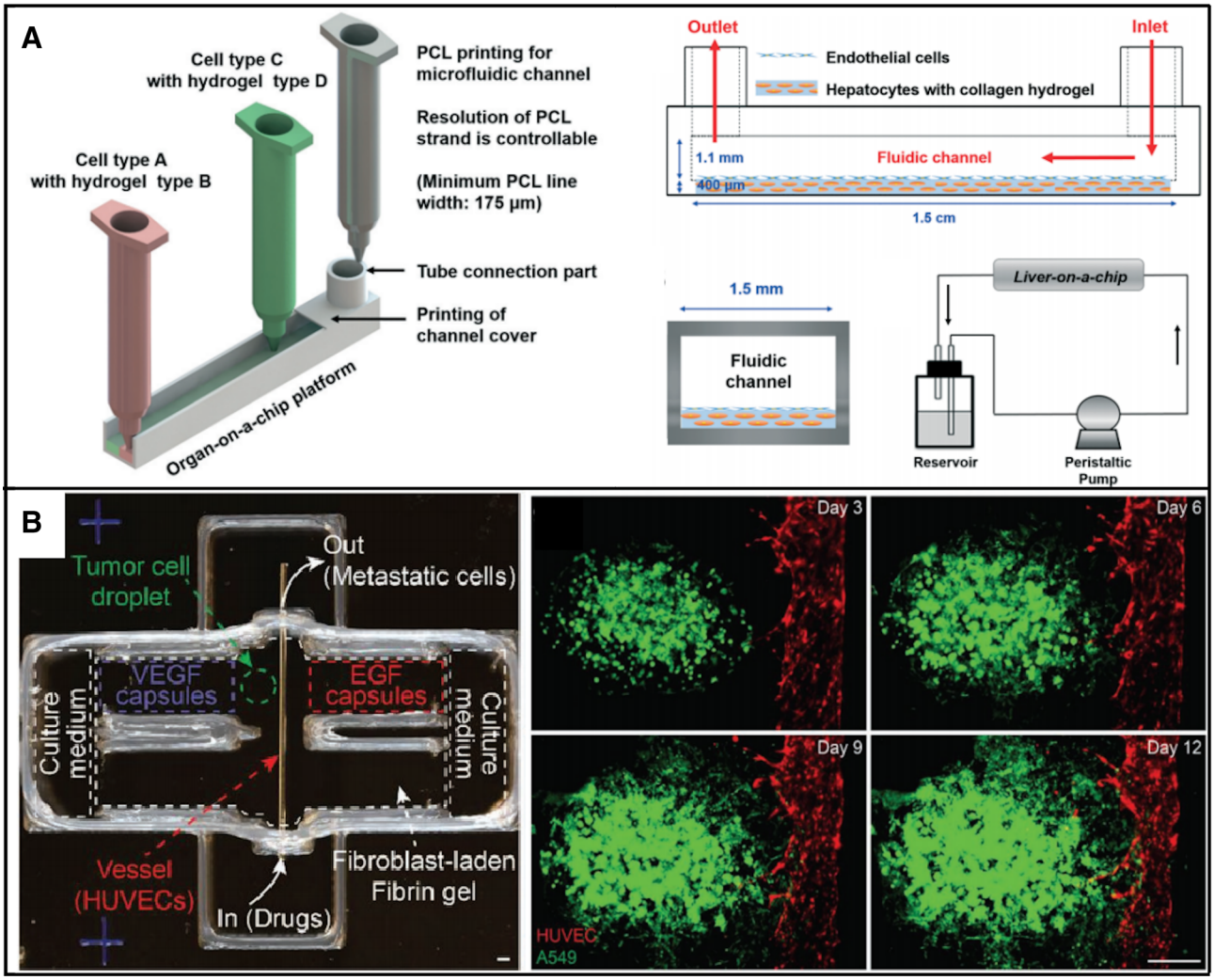

Fig. 4 In vitro bioprinted tumor-on-chips. **a** Schematic illustration of one-step bioprinting and liver-on-a-chip. Reproduced with permission from [46]. Copyright 2016, Royal Society of Chemistry. **b** Photo and fluorescence images of 3D printed metastatic model of A549s approaching and entering the vasculature through the fibroblast-laden fibrin gel (green channel: GFP-expressing A549s; red channel: RFP-expressing HUVECs). Scale bar: 500 μm. Reproduced with permission from [47]. Copyright 2019, John Wiley and Sons

Tumor-on-a-chip

In recent years, tumor-on-a-chip (Fig. 4) has come into view as the rising of 3D bioprinted tumor models. In 2016, Lee constructed an organ-on-a-chip by a one-step fabrication process with the ability of accurate measurement of metabolism and drug sensitivity for multiple organs including studies on liver function and drug development [46]. And in 2019, Meng and other scientists constructed 3D bioprinted in vitro metastatic models with a precisely controlled spatial placement of cells and biomaterials. The cellular behaviors were modulated dynamically with the application of programmable release capsules and the tumor models were vascularized to simulate the process of cancer dissemination. The model would be a convictive platform for drug screening and the study of mechanisms of tumor metastasis [47]. Also, Yi et al. [48] fabricated a patient-specific glioblastoma-on-a-chip to determine superior tumor-killing related drug combined utilization with centric hypoxia of the chip and to recapitulate clinically observed concurrent chemoradiation-temozolomide treatment resistance. The tumor-on-chips could greatly enhance the complexity of the in vitro tumor models with more approximate compositions and distributions of the multiple cell/ECM variables to recapitulate the various physiological activities and responses to drugs or environmental stimulus in vivo. With the development and improvement of biomaterials and bioprinting strategies, the tumor-on-chips are anticipated to be more extensively used and provide a valuable platform for drug screening as well as patient-specific cancer studies.

Future prospects

For all the irreplaceable strengths 3D bioprinting exhibits, there are still several problems remained to be solved. For instance, the cell viability, native behaviors, phenotypes, and functions are challenging to maintain during and after the printing process due to the influence of multiple printing and environmental parameters including the stress on cells [8, 52–56]. The accurate response to external environmental stimulus and the recapitulation of native behaviors of printed tissues/organs are crucial to the validity of the models. And the optimal formulations of bioinks alter when the types of cancer and the experimental requirements change, resulting in different demands of bioprinting techniques and printing conditions. So the combination of various printing strategies and materials is required for the replication of multiple cell types and ECM compositions of personalized tumor models, where some achievements have been made these years [20, 28, 57–60]. In addition, the evaluation standard system for the validity and reliability of the simplified in vitro printed tumor models is obliged to be explicit. Although there are still several obstacles to be overcome, we remain confident that with the continuous development and improvement of 3D bioprinting, it is possible for us to build in vitro tumor models with very close behaviors as in actual human bodies for patient-specific therapeutic studies and drug development.

Conclusions

Although 3D bioprinting has newly been applied to the in vitro tumor model establishment, the distinguished advantages it brings about have made this versatile technology commonly used in the biomedical field. In this review, we gave a brief introduction of the strategies of 3D bioprinting techniques and the formulation of the bioinks, we then detailed the utilization of 3D bioprinting in tumor model construction in vitro roughly divided into four categories by different designs of the scaffold. Although there are only about 20 works of literature on tumor bioprinting up to date, this innovative and unique bio-manufacturing method has gradually found its way in biomedical studies as well as in medico engineering cooperation with tremendous potential and gained increasing attention from researchers around the world. The achievements it makes in cancer study and drug screening are increasing at a surprising speed. 3D bioprinting is with no doubt a powerful and booming technology emerging in the application of cancer research. And it is promising to realize accurate in vivo environment reproduction for advanced anti-cancer drug development as well as the efficient, patient-specific therapeutic treatments in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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

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