Microfluidic bioprinting for organ-on-a-chip models

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Bioprinting is a revolutionary technology to assemble scaffolds for growing tissues. Microfluidic organs-on-a-chip is a useful platform with widespread applications mainly in drug screening and pathological studies. Organ-on-a-chip models are created to recapitulate the structural, microenvironmental and physiological functions of human organs. Recently, bioprinting has been applied to fabricate organ-on-a-chip models owing to its ability to print multiple materials and cell types simultaneously with good spatial resolution and reproducibility. This enables the creation of a biomimetic microenvironment with heterogeneous 3D structures. Functional vascularized tissue structure can be printed directly enabling fluid flow for transport of nutrition, gaseous exchange and removal of waste. We examine the integration of microfluidic and bioprinting technologies for organ-on-a-chip applications and discuss the future trends and challenges.

Introduction

Clinical need and manufacturing significance of microfluidic bioprinting

With the increasing cost of clinical research, drug companies are less willing to spend tens to hundreds of millions of dollars on clinical trials, which leads to fewer publicly accessible novel treatments [1,2]. Traditionally, drug testing was done with 2D approaches because of simplicity and scalability [3]. However, studies have shown that cells behave differently in 2D and 3D models, especially in terms of protein expression [4,5], drug response [5], cell morphology [6] and cell migration [7]. Owing to the enhanced cell–cell and cell–matrix interactions in 3D models, these models better reflect the physiological microenvironment. There are different ways to create 3D drug testing models [8]. Bioprinting (see Glossary of terminology used in this review) offers the possibility to generate complex 3D structures in a reproducible and controllable manner [9]. Bioprinted 3D tissue or organ models can also be scaled-up for HTS [10]. Previously, there have been few extensive reviews in which the authors have highlighted the importance of additive manufacturing (AM) in microfluidic organ-on-a-chip models [11–13]. In this review, we focus on bioprinting tissue constructs directly on the chip as well as one-step fabrication of tissue chips.

Bioprinting

Bioprinting is a revolutionary technology that deposits biomaterials, biochemicals and cells via an automated dispensing system to assemble tissue scaffolds [9,11,14,15]. Bioprinting has four different modes: extrusion bioprinting, inkjet bioprinting, stereolithography (SLA) bioprinting and laser-assisted bioprinting [9,11,15]. The main aim of bioprinting is to orchestrate a fully integrated and functional biological microenvironment, and it is essentially done via scaffold printing and subsequent cell seeding or direct cell printing [16].

Bioink is the soft biomaterial that is printed together with living cells [17,18]. A number of biomaterials have been used as bioinks for printing 3D tissue constructs [19]; naturally derived hydrogels [17,20,21] are the most common. They comprise gelatin, alginate, agar, agarose, hyaluronic acid (HA), fibrin or fibrinogen, collagen, chitosan and silk. The formulation of biomimetic bioinks is important for the successful *in vitro* fabrication of bioprinted tissue constructs [18]. Owing to their mild crosslinking conditions and

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https://doi.org/10.1016/j.drudis.2019.03.025

good biocompatibility, alginate [22] and gelatin [23] are among the most commonly used bioink materials. New hydrogel formulations, such as functionalized HA [24], that allow self-polymerization have also been developed.

Organs-on-a-chip

Development in microfabrication and microfluidic technology led to the emergence of 'organ-on-a-chip' models [25,26]. They reflect the structural, microenvironmental and physiological functions of human organs on microfluidic chips [3,26–29]. The combination of bioprinting with organ-on-a-chip technology allows creation of complex and biomimetic *in vitro* models for simulation, mechanistic and pharmacological modulation [30]. In organ-on-a-chip models, cytokines, growth factors, nutrients, extracellular matrix (ECM) components and junction proteins can all be engineered [25,31–34]. Many clinically relevant pathological phenotypes and drug responses that involve structural and functional interactions between tissue types can be studied. Fluid flow in organ-on-a-chip models reflects physiological flows such as blood flow, and tissue functions like peristalsis, breathing and heartbeat [26,32].

Most organ-on-a-chip platforms are created for mechanistic studies and proof-of-concept drug testing studies. These platforms often require the use of sophisticated microfabrication processes. Bioprinting technology could automate these processes and potentially address the throughput and reproducibility issues faced by traditional organ-on-a-chip systems [35]. Organ-on-a-chip models often consist of 3D microchannel and complex structures to replicate the architecture of real tissue and organs [30]. However, it is hard to control the property and microstructure of soft scaffolds. This could also be addressed by bioprinting, which allows fine-tuning of the mechanical property, porosity, microstructure and polymerization mechanisms of the hydrogel scaffolds [9,15]. We summarized the features and advantages of different *in vitro* cell culture models in Table 1 and compared microfabrication and bioprinting methods for developing organ-on-a-chip models in Table 2.

Advantages of 3D bioprinting on a microfluidic platform

Ability to create a biomimetic heterogeneous microenvironment

In nature, cells reside and grow in the ECM, which consists of a very complex organization of proteins and growth factors that generate biophysical, biochemical and mechanical signals between cells [11]. It has been a major challenge to achieve ECM-like heterogeneous composition of biomaterials in microfluidic devices [36]. Hence, direct printing of biomaterial formulations consistently on a microfluidic chip would be a game-changer for creating a biomimetic microenvironment. Using customized bioink compositions, it is possible to create heterogeneous tissue constructs containing multiple cell types and substrates using multiple exchangeable printheads with different material (Fig. 1a).

Ability for direct cell printing and/or patterning in microfluidic devices

Cells are usually seeded inside a microfluidic chamber either manually or with the help of a syringe pump [37,38]. This traditional method of cell seeding is usually slow and can involve a

TABLE 1

Comparing different *in vitro* cell culture models[a]

Methods	2D culture flask/dish	Hanging drop	Microwell-based 3D culture	Magnetic-force-based patterning	Microfluidics	Bioprinting	Microfluidic bioprinting
Mechanism	Seeding cells on culture flask/dish	Form cell spheroids by gravity	Form cell spheroids in microwells with nonadhesive surface	Magnetically labeled cells compacted in spheroids under magnetic force	Microfluidic-channel-mediated cell trapping and patterning	Deposit cells and bioink using bioprinter	Deposit cells and bioink on microfluidic chip with bioprinter
Uniformity	+++	++	+++	+++	++	+++	+++
Reproducibility	+++	++	+++	+++	++	+++	+++
Scalability	++	+	+	++	+	+++	+++
Low risk of contamination	+++	++	++	++	+	++	++
Ability to create microarchitechture	+	+	++	++	+++	+++	+++
Ability to create complex culture model	+	++	++	++	++	+++	+++
Ease of handling	+++	++	+++	++	+	++	++

[a] +++, high; ++, medium; +, low.

TABLE 2

Comparison of microfabrication and bioprinting methods for developing organ-on-a-chip models

	Microfabrication	Bioprinting
Methods	Photolithography Soft lithography Injection molding Micromachining	Extrusion-based bioprinting Inkjet bioprinting Stereolithography-based bioprinting Laser-assisted bioprinting
Resolution	10–1000 μm	>500 μm
Advantages	High resolution and accurate control of small features Rapid fabrication with reusable molds Wide selection of substrate material	Rapid production and easy prototyping capability Control of complex 3D tissue geometry Precise and reproducible substrate and cell scaffold
Disadvantages	High cost for mold fabrication Limited control of cell encapsulating scaffold Difficult to handle and reproduce	Printing process can cause cellular damage Limited selection of material Unable to produce small features

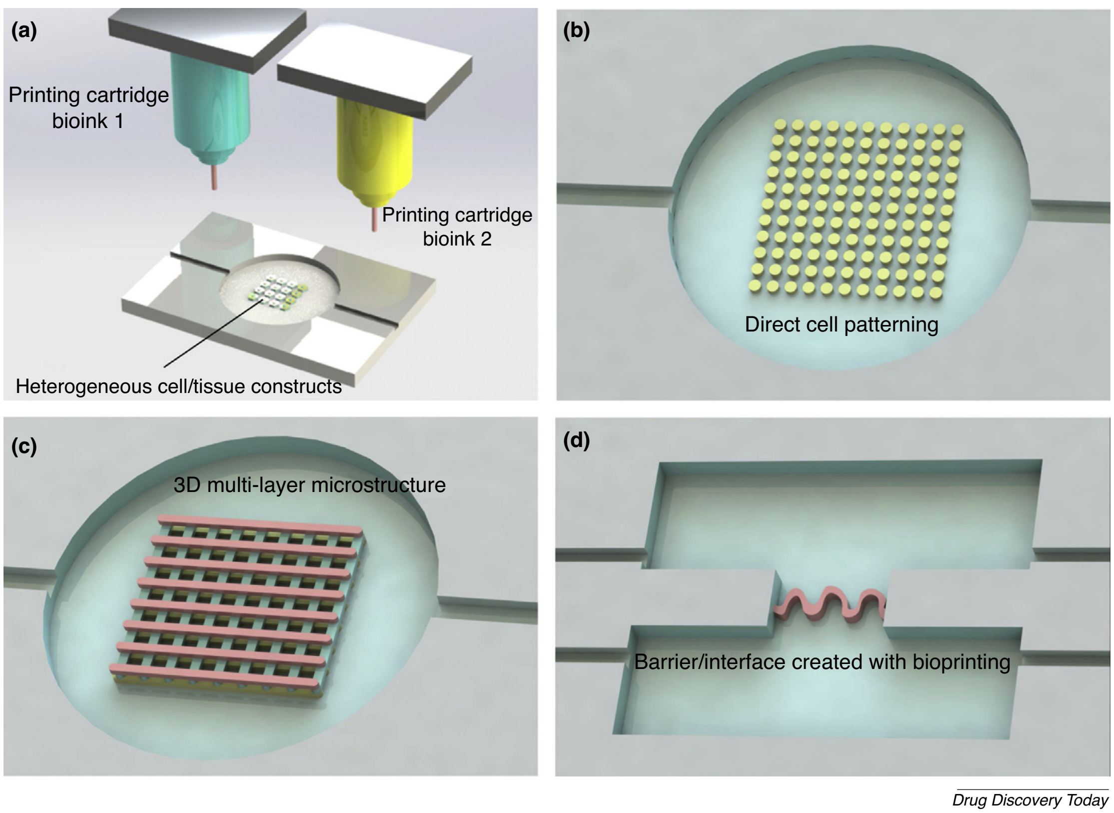

FIGURE 1

Advantages of different bioprinting on a chip. **(a)** Ability to create a biomimetic heterogeneous cellular and extracellular microenvironment. **(b)** Ability for reproducible direct cell printing and patterning in microfluidics devices. **(c)** Possibility to create complex 3D microstructure. **(d)** Ability to model barrier and interface in the vasculature.

tedious and complex set up of pumps, tubing and fluidic connections. Hence, direct multiple cell printing and/or patterning with the help of a bioprinter (mostly in inkjet [39,40] or extrusion [41]) would ensure a high-throughput and high-precision production of reproducible tissue constructs without much human intervention (Fig. 1b).

Possibility to create complex 3D microstructures

Traditional *in vitro* culture models are unable to fully reflect the organ microenvironment owing to the difference in terms of cell morphology, protein expression, cell–cell and cell–matrix interactions, and drug response [3]. By contrast, the flexibility of bioprinting modes [9] allows deposition of biomaterial–cell spheroid–tissue strands [14] in any free-form-inspired complicated 3D structures on the chip (Fig. 1c), creating cell culture models tailored for studying cell–cell and cell–matrix interactions.

Ability to model the vasculature and biological barriers

Vascularization is important to maintain tissue activities; successful vascularization has been a major challenge for producing functional tissues for *in vitro* models [42]. Vasculatures are important biological barriers that separate different tissue compartments [25]. With advanced microfluidic-assisted bioprinting techniques,

TABLE 3

Studies on microfluidic bioprinting

Organ model	3D printing technology	Bioink/hydrogel material	Microfluidic chip/ substrate material	Application	Refs
Liver	Microextrusion	GelMA and gelatin	PDMS and PMMA	Long-term culture of 3D human HepG2/C3A spheroids for drug toxicity assessment	[40]
Liver	Microextrusion	Gelatin and collagen I	PCL	One-step fabrication of liver-on-a-chip for metabolism and drug sensitivity studies	[46]
Liver	Inkjet	Alginate	PDMS and glass	Multiple cell patterning (HepG2 and U251 cells) for drug metabolism and diffusion studies	[47]
Tumor	Microextrusion	Alginate	PDMS and glass	Tumor model for *in vitro* pharmacokinetic studies	[51]
Tumor	Microextrusion	Cell suspended in media	PDMS	Cell-laden microfluidic chip for investigation of cancer cells (MDA-MB-231 and HepG2) in a co-cultured microfluidic environment	[52]
General cell culture	Inkjet	GelMA	Stereolithography resin	Simple-to-use method for long-term culture of hydrogel-encapsulated cell constructs	[53]
Bone, cartilage and muscle	Microextrusion	Gelatin, fibrinogen, HA and glycerol	PCL	Produce human-scale tissue constructs with structural integrity	[56]
Heart, blood vessel	Microextrusion	Alginate, GelMA and irgacure 2959	PMMA and PDMS	Endothelialized human myocardium-on-a-chip for regenerative medicine, drug screening and potentially disease modeling	[39]
Heart, blood vessel	Microextrusion	Alginate and GelMA	PDMS	Heterogeneous bioprinted HUVEC and cardiomyocyte scaffold on a microfluidic platform for regenerative medicine and drug discovery	[36]
Kidney	Microextrusion	Fibrinogen and gelatin	PMMA	Advanced human kidney tissue models for epithelial barrier disruption study	[64]
Placenta	2 Photon polymerization (2 P P)	Gelatin	PEGdma, PDMS and glass	Investigation of transcellular transport processes through the placental barrier	[66]
Lung	Microextrusion	Tracheal mucosa-derived dECM (tmdECM)	Polycarprolactone (PCL)	Asthmatic airway inflammation and allergen-induced asthma exacerbation model	[67]
Urothelium, blood vessel	Microextrusion	GelMA, alginate and tripentaerythritol	Bioprinted construct	Reconstruction of complex hollow tissues or organs models to create tubular tissue architectures for tissue regeneration and modeling	[86]

multiscale hydrogel-based flow networks can be generated with forms and functions close to the real vessels [43] (Fig. 1d). Bioprinting vascular networks could facilitate fluid flow for transport of nutrition, exchange of gas and removal of waste.

Organ-on-a-chip applications enhanced with bioprinting

Advanced organ-on-a-chip platforms, enhanced with bioprinting, can serve as ideal tools for mechanistic biological studies and drug testing. In this section, we discuss recent advances of 3D printing application to organ-on-a-chip models. Table 3 provides a summary of the models reviewed in this section.

Liver

Liver is one of the most important organs when assessing drug toxicity [37,44,45]. Microfluidic platforms could be interfaced with bioprinting technologies to create cell-laden hydrogel constructs on-chip. Bhise *et al*. developed a bioreactor for perfusion culture that allows direct access to cells for long-term culture [40]. The bioreactor was interfaced with a direct-write bioprinter to print hydrogel constructs embedded with HepG2/C3A hepatic spheroid (Fig. 2a). Cell viability and metabolic functions were maintained for 4 weeks in perfusion culture. This platform was used as a model to predict acetaminophen (APAP) toxicity, and the results were comparable with *in vivo* data.

In another liver model developed by Lee and Cho [46] (Fig. 2b), the microfluidic chip and the tissue model were prepared with he 3D printer, without the need to include a secondary cell-seeding process. Multiple cell types and biomaterials were applied and printed at the desired location. Liver cells cultured on this 3D-bioprinted organ-on-a-chip platform show significantly enhanced liver function. With the one-step fabrication of the chip and the tissue model, it is possible to prepare body-on-a-chip platforms with multiple culture chambers for mechanistic therapeutic studies involving multi-organ interactions.

Compared with extrusion printing, inkjet is a more precise method for microscale cell patterning, Zhang *et al*. reported the first inkjet printing integrated on a microfluidic chip [47]. In this approach, HepG2 and U251 cells are encapsulated in sodium alginate and printed into arrays on glass slides (Fig. 2c). The cell arrays are covered by a PDMS layer with microfluidic channels. Crosslinking agents are injected through the microchannels to immobilize the alginate into a gel form. The prodrug tegafur was metabolized by HepG2 into an active anticancer drug 5-fluorouracil (5-FU). The 5-FU forms a concentration gradient in the microchip which inhibited the growth of the U251 glioma cells. The adaptation of inkjet bioprinting on a microfluidic chip shows great potential to realize high-throughput and automated manufacturing of organ-on-a-chip without laborious and complicated conventional microfabrication processes.

Tumor

Tumors have highly complex and heterogeneous tissue structures that contain multiple cell types. Hundreds of unique types of

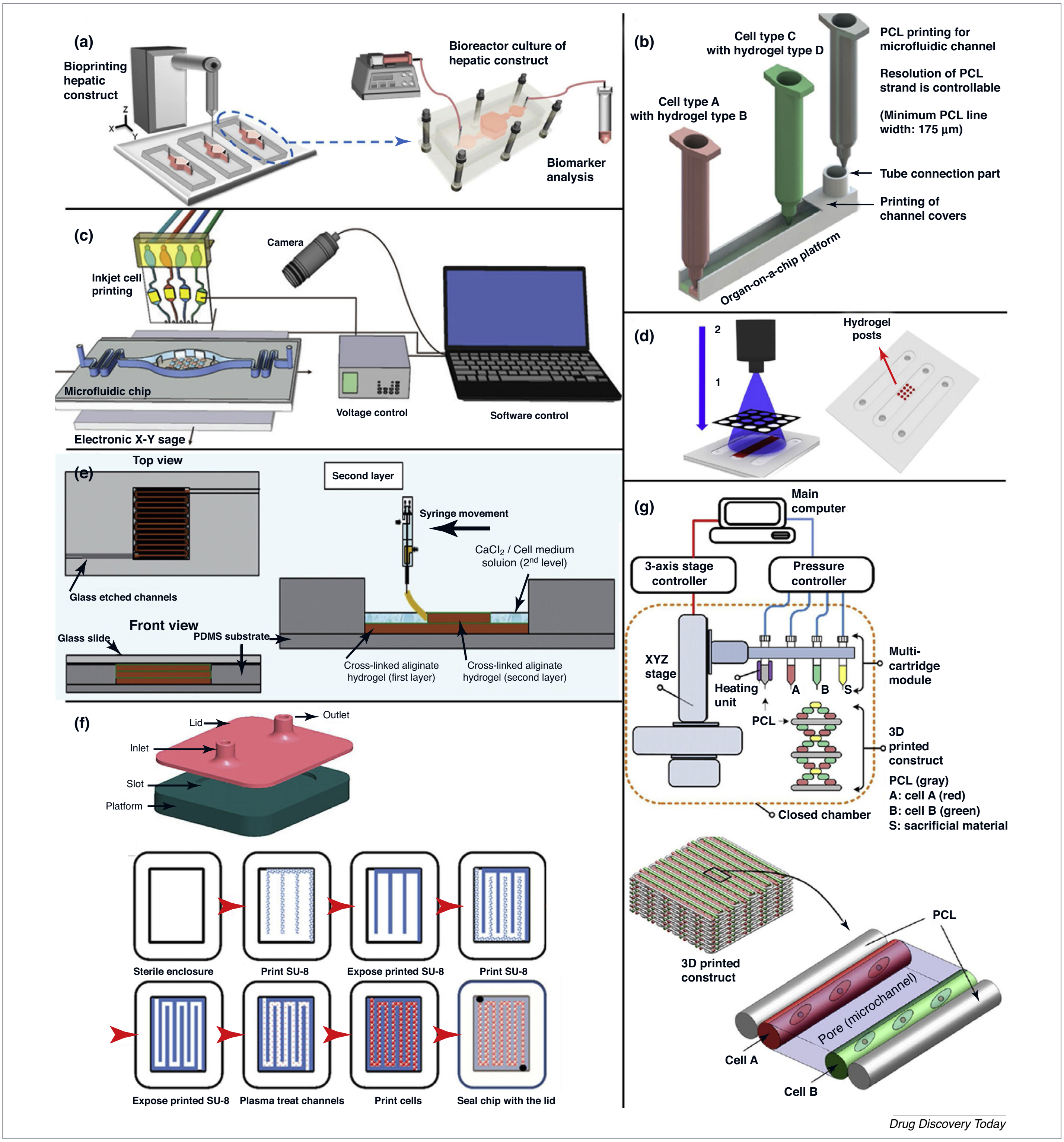

FIGURE 2

Current models of bioprinting on microfluidic platforms. **(a)** Bioprinted hepatic spheroid model. **(b)** One-step fabricated heterogeneous liver-on-a-chip model. **(c)** Inkjet bioprinting for heterogeneous cell patterning in a microfluidic chip. **(d)** Inkjet bioprinting of cell-laden construct. **(e)** tumor-on-a-chip model created by direct cell writing. **(f)** Maskless fabrication of microfluidic tumor-on-a-chip. **(g)** Bioprinted vascularized connective tissue. **(h)** Bioprinted 3D microfibrous scaffolds for heart-on-a-chip. **(i)** Bioprinted heart-on-a-chip with heterogeneous low viscosity bioink. **(j)** Bioprinted 3D convoluted renal proximal tubule for kidney-on-a-chip. **(k)** Bioprinted placenta-on-a-chip with two-photon polymerization. **(l)** Bioprinted vascularized airway-on-a-chip (VA-OC) with decellularized extracellular matrix bioink. **(m)** Bioprinted multilayered cannular tissue. Reproduced and adapted with permission from the cited references in Table 3.

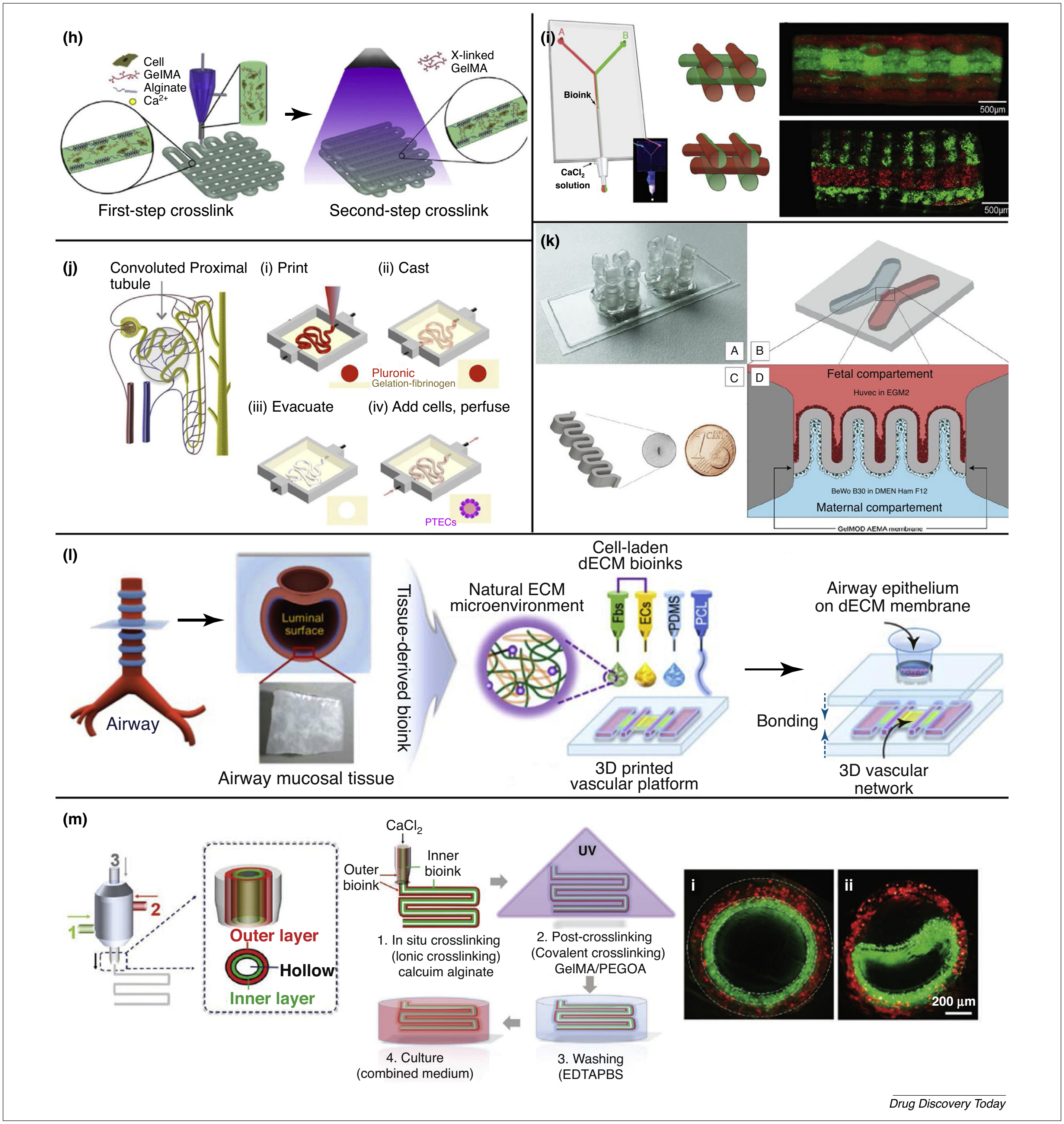

FIGURE 2. (*Continued*).

cancer have been identified [48], this makes it difficult to develop realistic tumor models for each specific cancer type. Bioprinting can create heterogeneous tissue models in a controllable and scalable manner and can potentially accelerate cancer research. Integration of a microfluidic platform with bioprinting adds another level of complexity to tumor models. Mechanical cues introduced by fluid flow are important to mimic the physiological tumor microenvironment. It has been shown that the shear stress generated by interstitial fluidic flow around the tumor tissues leads to cell cycle arrest in cancer cells [49]. Cancer cells also tend to migrate along the direction of fluid flow in 3D cell culture scaffolds [50]. Microfluidic platforms have the flexibility to create a variety of layouts and flow patterns to allow generation of chemical gradients. This enables the study of cellular responses to varying concentrations of chemicals and biomolecules that play an important part in cancer metastasis.

The easiest way to fabricate 3D tumor tissue on the microfluidic platform is direct cell writing. This method was first reported by Chang *et al*. in 2008 [51]. This is a two-step process where an automated direct cell writing (DCW) process creates the 3D tissue first, which is then housed in a PDMS device fabricated with soft lithography (Fig. 2e). In another study [52], cells are printed directly into the open channels of a PDMS device with extrusion deposition, the device is subsequently sealed. It is also possible to fabricate the chip and deposit the cells at the same time using an integrated bioprinting system (Fig. 2f) [53]. There are four print-heads in the system and each print-head performs a specific function: the first print-head deposits the photopolymer; the second one is a UV head that does the photocrosslinking; the third one applies passivation plasma treatment; and the final one seeds the cells. This system was used to create a co-culture cancer model in a microfluidic environment.

Bone, cartilage, muscle and connective tissue

Owing to its ability to deposit material layer-by-layer precisely, 3D bioprinting shows great promise for creating complex tissue-engineered constructs with clinically relevant shape, size and functional property [54]. With the direct extrusion method, it is possible to create 3D constructs with a cell-laden hydrogel. However, creating large free-form structures for bone, cartilage and muscle tissue engineering is challenging owing to the lack of mechanical strength and printability [55]. To overcome this, Kang *et al*. designed a 3D printer that deposits hydrogels containing cells together with synthetic polymers with good mechanical properties [56]. The bioprinter was composed of multidispensing modules for delivering cells and various types of polymers (Fig. 2g). The 3D tissue construct also incorporates microchannels created by the sacrificial molding of Pluronic® F-127 hydrogel. The mechanical strength was provided by polycaprolactone (PCL). This is a good demonstration of bioprinting multiple cell types at precisely controlled locations and forms complex 3D structures. After the Pluronic® F-127 component is sacrificed, the hollow microchannels can facilitate fluid flow through the construct.

To realize the full capacity of 3D cell models and microfluidic technologies and promote clinical use, bioassays must be user-friendly. In this effort, Knowlton *et al*. fabricated microfluidic chips using a desktop SLA printer and industrial inkjet-style 3D prototyping. Cells encapsulation in the 3D hydrogel are immobilized within the microfluidic channels with microposts (Fig. 2d) [57]. The platform enables fabrication of cell-laden hydrogel constructs with controllable geometries. They demonstrated a simple-to-use approach for microfluidic chip fabrication and its application as a platform for 3D culture.

Heart

Organoids are miniaturized versions of an organ produced *in vitro* that show biomimetic structure and function. They are usually derived from stem cells and can differentiate and self-organize in 3D culture [58]. Owing to the complex structure of the human myocardium and the need to integrate blood vessels in cardiac tissue, it is challenging to create cardiovascular organoids. Zhang *et al*. recently proposed an integrated 3D bioprinting strategy to fabricate endothelium myocardium (Fig. 2h) [39]. The endothelial cells bioprinted with the hydrogel scaffold can migrate toward the microfiber peripheries to form the myocardium structure. Cardiomyocytes are subsequently seeded into the scaffold; and spontaneous and synchronous contraction can be observed. The organoid is then embedded into a microfluidic device for perfusion culture. The cells in the endothelialized-myocardium-on-a-chip showed dose-dependent responses under doxorubicin treatment, the toxicity results corresponded well with rat myocardial organoids.

In another cardiac model developed by Colosi *et al*. [36], a microfluidic system was used to control the deposition of low-viscosity bioink to construct a complex heterogeneous structure using a single extruder system (Fig. 2i). The viscosity of the bioink can be easily tuned to produce different mechanical properties using the microfluidic system. The bioprinted scaffold supported the development of human umbilical-vein endothelial cells (HUVECs) along the printed structures to form functional vasculatures with synchronous beating.

Kidney

Compared with *in vivo* kidney cells, *in-vitro*-cultured kidney cells often lose their key phenotypic functions and polarities rapidly. This hinders the creation of predictive nephrotoxicity models [59]. Researchers have been trying to recreate the kidney 3D microenvironment, using hollow fiber devices [60], hydrogel scaffolds [61] and iPSC-derived organoids [62]. However, these models are limited to 1 mm in size and lack inlet and outlet for perfusate analysis. Kidney-on-a-chip devices enable collection of perfusate under a controlled microenvironment. However, it is difficult to create 3D convolution and open luminal architecture with most kidney-on-a-chip devices, which rely on a porous membrane [63]. Homan *et al*. combined bioprinting with microfluidic technologies and developed the first 3D convoluted renal-proximal-tubule-on-a-chip model with an addressable open lumen [64]. In this model, the 3D convoluted rental tubule structure is created by printing fugitive Pluronic® F127 in. onto a gelatin–fibrinogen ECM, additional ECM is then cast around the printed feature (Fig. 2j). The fugitive ink is then liquefied by cooling to 4 °C and flushed out to create an open tubule for cell seeding and perfusion. The proximal tubule epithelial cells (PTECs) seeded in the chip showed a polarized phenotype and produced kidney-specific cytokines. The toxicity of cyclosporine A (CysA) was assessed in the device. Breaks in cell–cell junctions and actin reorganization were observed at a low concentration (10 μM), whereas areas of cell death were observed at a high concentration (50 μM). The effect of CysA was quantified using a FITC-dextran leakage test at the epithelial barrier.

Placenta

The placenta is a transient organ responsible for the transport of endogenous and exogenous substances. It also acts as a barrier that separates fetal and maternal blood. The multifaceted structure of the placenta is difficult to model with current *in vitro* approaches [65]. Mandt *et al*. created a microfluidic device with a gelatin-based placental barrier to mimic the composition and properties of the placenta [66]. The microstructure of the placental barrier is fabricated with a high-resolution 3D printing method called two-photon polymerization (2 P P) (Fig. 2k). HUVECs and human choriocarcinoma cells isolated from placental tissue are seeded on opposite sides of the barrier to mimic the fetal compartment and maternal syncytium, respectively. This model creates new possibilities to investigate the effect of a mother's diabetes mellitus condition on the fetal blood supply.

Lung

The lung is the primary site for environmental agent exposure. Owing to air pollution, an increasing number of people are suffering from respiratory disease, leading to a growing demand for *in vitro* lung models. The first bioprinted *in vitro* lung model was reported by Horvath *et al*. [67]. This air–blood tissue barrier model consists of an endothelial cell layer and an epithelial cell layer, separated by a basement membrane layer made of Matrigel®. The three layers are bioprinted with a layer-by-layer approach on porous membrane substrate. Compared with manual cell seeding, the bioprinting approach allows automated and reproducible production of thinner and more-homogeneous cell layers. A bioprinted microfluidic lung-on-a-chip with decellularized ECM (dECM) bioink from tracheal mucosa was developed by Park *et al*. [68] (Fig. 2l). They reported that the dECM bioink has an increased angiogenic effect, as demonstrated by increased tube formation and higher expression of vascular markers. To show the capability of this lung-on-a-chip to mimic asthmatic epithelium and dust-mite-induced exacerbation *in vitro*, pathophysiologically relevant inflammatory responses were induced and studied.

Creating 3D vasculature structures with bioprinting

A variety of approaches have been developed to incorporate blood vessel structures in engineered cell and tissue constructs [25,69]. For instance: self-assembled endothelial cells to generate microvascular networks [42]; delivery of angiogenic factors in porous alginate scaffolds to induce sustained neovascularization [70]; use of vascular stem cells and pericyte to facilitate endothelial cell proliferation and assembly [42]; tuning scaffold properties to enable precise control of vascularization [71]; and cell-sheet technology for convenient layering of vascular cells [72]. These techniques allow the generation of a blood vessel *in vitro* but are not able to control the spatial distribution of the vasculature; random generation of new blood vessels can still occur within a printed tissue construct and lead to reduced reproducibility. Bioprinting is a potential solution for this, owing to its ability to deposit complex tissue structure at high resolution and reproducibility automatically [54,73,74]. Sacrificial hydrogel bioprinting [75–78], embedded bioprinting [79,80] and hollow-vessel bioprinting [81–85] have demonstrated the possibility of creating *in vitro* vasculature or vascularized tissues.

The heart-on-a-chip model as discussed in the previous section (Fig. 2h) is a good example for creating vasculature *in vitro*. In this approach, the bioink containing alginate and gelatin methacryloyl (GelMA) was extruded through the core of a concentric print-head. On the outer sheath flow of the print-head, a calcium chloride ($CaCl_2$) solution was perfused through to crosslink the bioink [36,39]. Microfiber is formed in this manner, whereas subsequent photocrosslinking of GelMA allows permanent curing of the multilayer microfibrous tissue scaffold. In the subsequent biological characterization, endothelial cells encapsulated within the bioprinted microfibers were found to proliferate and migrate toward the microfiber peripheries and form lumen-like structures [36,39]. These bioprinted vasculatures could also be seeded with other companion cells to further recapitulate the heterogeneity of the vascularized tissues.

In another coaxial approach to print multilayered vascularized tissues, Pi *et al*. [86] developed a multichannel coaxial extrusion system (MCCES) consisting of three concentrically assembled needles (Fig. 2m). Bioink composed of a GelMA, alginate and tripentaerythritol core is extruded directly from the device to form multicircumferential and perfusable hollow tubes. Using MCCES and customized bioink, bioprinting of heterogeneous tissue constructs is demonstrated. A urothelial tissue construct containing urothelial cells and human bladder smooth muscle cells is constructed. The bioprinted constructs support continuous perfusion of fluids through the hollow core to promote cell growth and proliferation. In this approach, no microfluidic platform is used for the perfusion; the liquid is perfused through the bioprinted hollow tubes directly. This technology is a promising step toward creating a multitude of tubular tissue architecture for regenerative medicine.

Concluding remarks and future directions

Bioprinting allows automated fabrication of reproducible tissue constructs with precise control over spatial parameters. Organ-on-a-chip platforms offer the abilities to mimic physiological, mechanical and chemical cues *in vitro*. Integration of the two technologies presents a promising direction for high-throughput drug validation and testing as an alternative to animal and human models. Bioprinted cell models on microfluidic chips that mimic the microenvironment, spatial distribution and vasculature represent a promising strategy for future 4D bioprinting, where smart materials are printed. These materials can be used as actuators that change shapes under external mechanical or thermal stimuli [87]. These stimuli mimic the specific functions of organs that are important for the *in vitro* tissue development process. In cardiomyocytes, for example, a mechanical stimulus promotes cell alignment and maturation significantly [88]. Apart from the mechanical stimuli, biochemical stimuli such as growth factors can be printed together or in sequence with scaffold with high spatial resolution.

Although a microfluidic bioprinting approach is starting to emerge as a way to build complex tissue models, the field is still in its infancy. Several issues remain to be solved. First, as the most widely used bioprinting approach, extrusion-based printing gains popularity owing to its low cost and the mild printing conditions [89]. However, owing to limited resolution and surface roughness, it is not applicable for a microfluidic platform with complex heterogeneous 3D structures. Laser-based SLA printing can achieve high resolution but the biomaterial selection is limited and cells cannot be printed together with the scaffold without harming the cells [35]. With the growing popularity of the microfluidic bioprinting field, we observe the trend to print the cell and tissue model together with the microfluidic chip in a single fabrication step [46]. The single-step fabrication process facilitates a more efficient and automated workflow. In this direction, hybrid bioprinters can be designed to print the chip and the tissue. These platforms can incorporate SLA for chip fabrication as well as extrusion for microtissue at the same time to overcome the limitation of each printing approach. Integration of on-chip sensors to monitor cell behavior and microenvironment is another area to enhance bioprinted organ-on-a-chip technology. This is crucial for clinical applications where drug testing studies are carried out for an extended period. To bridge this gap, Zhang *et al*. developed an integrated multisensor platform recently with fluidics-routing breadboard as well as physical, biochemical and optical sensing capabilities [90]. This is a great example for improving existing organ-on-a-chip models, to achieve automated control and monitor biophysical and biochemical cues in situ. Currently, these approaches are limited by the selection of material. Several commonly used resin materials in the high-precision printing process are cytotoxic and have limited optical transparency. Moreover, unlike the widely used PDMS-based microfluidic chips, most 3D-printed biochips cannot be autoclaved for sterilization [91]. Nevertheless, with more developments in the bioprinting processes and bioinks, we expect that highly efficient, automated and integrated bioprinted organ-on-a-chip platforms will be widely used to create functional tissues.

Acknowledgments

We thank Dr Wang Tianyi and Dr Wu Ying Ying for insightful comments on the manuscript. We acknowledge Prof. Walter Hunziker for dedicated support and guidance. This work was supported by Agency for Science Technology and Research (A*STAR) Joint Council Office (JCO) Grant Number 15302FG152.

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GLOSSARY

2D cell culture Cell culture models on 2D surface
3D cell culture Cells that can grow or interact with their surroundings in all three dimensions in an artificially created environment
Additive manufacturing (AM) Fabricating objects from computer 3D model by joining materials layer upon layer
Bioassay Classification of the biological activity of a substance by testing its effect on an organism and standardizing the results
Bioink Base material used for 3D printing of complex tissue models
Bioprinting Layering of cells and tissue in 3D using bioprinter
Coaxial printing Printing complex structures containing two or more materials along the extrusion direction of the bioprinter nozzle
Direct cell writing (DCW) Deposit cells directly on a substrate using bioprinting
Extracellular matrix (ECM) Collection of molecules secreted by supporting cells that provide structural and biochemical support to surrounding cells
Extrusion bioprinting Dispensing cells and matrix materials through bioink cartridge to fabricate organized tissue constructs
Hydrogel Macromolecular polymer gel constructed by crosslinking a network of polymer chains
In vitro Carrying out a procedure outside of the organism in a controlled environment
In vivo Carrying out a procedure within an organism
Inkjet bioprinting Bioprint cells by creating and releasing droplets of fluid containing cells
Lab-on-a-chip A device that integrates single or multiple laboratory function on a single integrated circuit such that automation and HTS can be achieved
Laser-assisted bioprinting The application of laser pulses for the printing of living cells and biomaterials
Microfluidics The study of systems that aid in processing small quantities of fluid by using minute channels with micro-scaled dimensions of usually tens to hundreds of micrometers
Organ-on-a-chip A multichannel 3D microfluidic platform that mimics the activity, mechanism and physiological response of organs and organ systems
Photocrosslinking The photo-induced formation of covalent bonds between two macromolecules or between two different parts of a single macromolecule
Photopolymer A polymer that changes its properties upon exposure to light, typically in the ultraviolet or visible region of the electromagnetic spectrum
Regenerative medicine A branch of tissue engineering and molecular biology that studies the process of replacing, engineering or regenerating human cells, tissues or organs for recovery from illness
SLA bioprinting The use of photocurable bioinks for the formation of constructs by exposure to lasers or projections
Tissue engineering The use of a composition of different cell types, engineering and materials methods, and appropriate biochemical and physicochemical factors, to enhance or replace biological tissues
Tissue microenvironment The immediate small-scale environment in which the tissue exists