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Bioprinters for Organs-On-Chips

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Abstract

Recent advances in bioprinting technologies have enabled rapid manufacturing of organ-on-chip models along with biomimetic tissue micro-architectures. Bioprinting techniques can be used to integrate microfluidic channels and flow connections in organ-on-chip models. We review bioprinters in two categories of nozzle-based and optical-based methods, and then discuss their fabrication parameters such as resolution, replication fidelity, fabrication time, and cost for micro-tissue models and microfluidic applications. The use of bioprinters has shown successful replicates of functional engineered tissue models integrated within desired microfluidic system, which facilitates the observation of metabolism or secretion of models and sophisticated control of a dynamic environment. This may provide the wider order of tissue engineering fabrication in mimicking physiological conditions for enhancing further applications such as drug developments and pathological studies.

Keywords

Microfluidics; bioprinting; organ-on-chip; multiscale

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Conflict of Interest
None.

1. Introduction

Recent advancements in microfabrication and tissue engineering have accelerated the growth in novel organ-on-chip models used in pharmaceutical industry and other sectors [1, 2]. The main element of such platforms is the micro-tissue model that includes biomimetic structured cellular components to represent a specific organ or tissue connected to perfused channels [3]. The level of functionality in micro-tissue models is defined by physiochemical interfaces between cellular components, extracellular microarchitectures, and circulation of nutrients [4]. Analysis of biochemical markers and metabolic activities of cellular components are studied in such devices using molecular protocols and real-time imaging tools [5]. In addition, developments of complex biomimetic micro-physiological systems can enable the use of organ-on-chip models for studying pathological conditions [2]. Recent attempts have focused on recapitulating physiologically relevant microarchitectures by utilizing spatial arranging capability of bioprinting techniques to improve the similarity to native complex tissues [6, 7].

Desired biological function dictate not only design of micro-tissue models but also microfluidic assembly and integration of (any) pumps, valves, mixers and sensing parts (such as optical, electrochemical, and mass spectrometry biosensors) onto the device [8]. The conventional prototyping process occurs through curing poly-dimethyl-siloxane (PDMS) and other thermoplastics in prefabricated molds (built by soft lithography) [9]. Computer aided design (CAD) files are commonly employed to design microfluidics patterns before molding onto a master metal or cutting the pattern by laser engraving. In some cases, oxygen plasma has been used to augment the bonding strength between the PDMS and the glass slide [10]. The resulting PDMS molds have led to the fabrication of microfluidic channels with high resolutions [11]. The complexity of the channels has varied by the desired application, starting from simple hollow channels coated with cells to porous PDMS membranes and three-dimensional (3D) cavities filled with extracellular matrix (ECM)-like constructs [12].

Translating microfluidic technology to biomedical markets has been facing practical challenges. The generation of multiple high-resolution photomasks is costly and their alignment for the sequential layers of photoresist is very challenging [13]. The modeling process that includes curing, assembly, and inlet connection involves time-consuming manual operations and it cannot be easily automated. Indeed, molding chips may take from a few hours to one or two days. The leakage from inlet/outlet interfaces is another challenge associated with molded chips. Although molded chips have the advantage of higher throughput, researchers have been investigating cheaper, more robust, and more user-friendly fabrication approaches [14]. These challenges become significant when microfluidic systems are used for biomedical research and studies on cell biology. For example, the lengthy process of chip preparation can affect cell viability and functionality of biological agents. Lack of support for cell attachment to the chip and accuracy in cell patterning are other common issues associated with molded chips, which hamper the effectiveness of organ-on-chip systems.

Organ-on-chip systems, which are microfluidic-based *in vitro* tissue models, have been developed to study pathology and improve drug development [15]. To better mimic physiological environment for higher functionality *in vitro*, 3D fabrication techniques are required. By taking advantages of well-developed microfabrication techniques, many of the complex architectures of natural tissues can be engineered, such as heart-on-a-chip, lung-on-a-chip, liver-on-a-chip, and tumor-on-a-chip [16–19]. However, fabrication processes become sophisticated and labor-intensive because of the requirement of multi-step lithographic processes, alignment, and integration. The high expensive and time-consuming processes hinders the industrializing tissue model manufacturing business into biomedical markets. Thus, more automatic, robust and cheaper methods are desired.

Three dimensional bioprinting is one of the most promising biofabrication techniques for next-generation tissue engineering [20, 21]. The combination of cell-laden bioink and three dimensional printing allows the constructions of functional tissues and organs with complex architectures from digital models [22]. The automated process potentially allows for the scale-up of tissue fabrication from lab scale to production for drug development requirement [23]. In addition, the spatial heterogeneity of tissues can be easily achieved by 3D bioprinting compared to traditional microfabrication techniques [24]. This is discussed in more details in the next section.

2. Fabrication Benefits

One-step manufacturing process from the digital sketch to the final structure, followed by post-processing treatments, such as surface modifications, advances the traditional multi-step process of fabrications of microfluidic organ-on-chip platforms [25, 26]. The use of 3D printing has led to reduced processing time and it has offered ground-breaking solutions for the commercialization of microfluidic systems. Extrusion-assisted 3D printing which dispenses material through an extruder or nozzle while a computer-controlled arm moves the nozzle creates 3D shapes. Direct extrusion 3D printing of micro-channels is challenging because of the collapse of top layers into channels; hence, such 3D printing technology should be combined by a support material. In this process the support material is removed post-printing, also called as sacrificial printing [27]. Selective removal of the sacrificial material leads to a microfluidic system in the bulk structure. In addition to extrusion-assisted 3D printing, recent attempts have focused on light reactions for solidifying the structure and making micro-channels [28]. Known as stereolithography (SLA), light exposure solidifies a liquid resin or hydrogel through a photochemical reaction [29]. While conventional SLA depends on the movement of laser beams to raster scans across the liquid, digital light processing has been proposed to pattern 2D shapes onto the liquid starting material [30]. Compared to extrusion-assisted printing, photo-patterning benefits from superior resolution (i.e., laser spot size or projector pixel size) and less chance of cell injury [31]. These methods and their applications are summarized in Table 1.

Three dimensional printing has been used to create microfluidic devices in terms of flow channels and connections, such as the membrane-based valves fabricated by SLA [41]. This work showed a *fail fast, fail often* concept in which prompt experimental feedback quickened device development. In addition, CAD models allow the ability of integrating

commercially-available parts into the device, such as electrochemical detection systems [42]. Indeed, soft lithographic masters are replaced by the design files and they can be transferred to any fabrication stations or labs. 3D printing also enables the fabrication of interface compartments to improve established microfluidic chips with multiple functions [43]. Any insights into the development of 3D printing technologies and the underlying mechanisms will become valuable tools for creating microfluidic systems at better precisions [44].

We divided existing 3D printing methods into nozzle-based and optical-based methods (see examples in Table 1). Nozzle-based approaches use a nozzle that deposits the ink onto a substrate, normally with sufficiently high pressure to enforce the ink transfer. Optical-based 3D printing may involve direct printing or transferring the ink onto a receiving substrate by ejection or crosslinking the ink by light-material interactions. Both approaches have merits and shortcomings, depending on the desired application. We discuss the limitations of each technique for creating microvascular tissue constructs, and project the trend of bioprinting techniques toward fluidic channels in organ-on-chip models (Figure 1). We then review novel methods toward 4D printing of microvascular tissue and microfluidics. This article would help researchers to select proper bioprinting methods and design strategies for micro-tissue models and microfluidic encasements.

3. Nozzle-Based Bioprinting Techniques

Nozzle-based 3D printing techniques directly place the ink through a nozzle system while the nozzle tip is in contact with the construct. An example in making the novel 3D-printed ECM based immune-array for multiple protein detection using automated reagent delivery in early cancer detection [49]. Recent advancements in micro-patterning inside of biomaterial scaffolds have allowed handling the microenvironment and delivered understandings in the biological behavior of stem cells [50]. As an example, micro-patterning of hyaluronic acid hydrogels with peptide-crosslinked spots was used to control the diffusion of mesenchymal stem cells [51], which demonstrated the benefit of stem cell remodeling. In addition, Jeon *et al.* showed that within a alginate hydrogel system with dual crosslinking, the size of the 3D micropattern encapsulating the cells exhibits some control over stem cell proliferation as well as the level of osteogenic and chondrogenic differentiation [52].

3.1 Inkjet bioprinting of vascularized systems

The first bioprinting technology introduced into lab-on-chip platforms is inkjet bioprinting [53], which directly deposits droplets of the ink onto a substrate by using a heating or piezoelectric head attached to the nozzle (Figure 1). Under pneumatic pressure or a mechanical load, the biomaterials and/or cells are dispensed in droplets at desired positions [32]. In one work, alginate-based bioink formulation was placed along a bifurcating microvasculature capable of supporting physiological flow rates [54]. They showed that cells can be patterned in the channels using inkjet bioprinting. Regarding the practical limitation of inkjet bioprinting, such as the requirement of low viscosity bioink and the inherent inability to perform continuous flow, constructing 3D architecture is very challenging while applying hydrogel bioinks. Therefore, this method has been less used for 3D bioprinting.

3.2 Extrusion-assisted bioprinting

Extrusion-assisted bioprinting is the most multipurpose process and enables the broadest range of bioink viscosities to be used for bioprinting for clinical applications [55]. Sometimes extrusion-assisted bioprinting is called pressure-assisted bioprinting (PAB) [56]. In such a case, the motion of air or plunger-based pressure is released in the form of a continuous filament through a micro-nozzle or a micro-needle on a motionless substrate. Li *et al.* believe that it benefits from homogenous distribution, room temperature processing and direct combination of cells [57]. PAB has been applied to the printing of cell and organs with confirmed retention of activity in many applications. Bioprinted cells include but not limited to human mesenchymal stem cells (hMSCs), mouse pre-osteoblasts, endothelial cells, and osteogenic progenitors [58]. Kolesky *et al.* made-up a single tissue by co-printing three different cells and multiple inks including Pluronic F-127 filaments and hMSCs-laden gelatin/fibrin filaments and neonatal dermal fibroblasts inside a modified ECM together with embedded vasculature (Figure 2A), which is then coated by human umbilical vein endothelial cells (HUVECs). Towards having a dense osteogenic tissue, culture media containing growth factors for osteogenic differentiation was perfused [33]. A mixed tissue model was fabricated and transformed into a vascular network filled by HUVECs. Brigham *et al.*, have fabricated semi-interpenetrating networks of hyaluronic acid and collagen to achieve adequate mechanical properties and to present a new class of hydrogels in 3D printing. They designed many synthetic materials, such as the biodegradable polycaprolactone, poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and PLGA, PLA-PGA co-polymer as an alternative to natural polymers [59].

Recently researchers have started using decellularized ECM as a promising bioink to reproduce the natural microenvironment of the cells to mimic the native tissue. [60]. Homan *et al.* reported 3D human renal proximal tubules *in vitro* (Figure 2B) by placing the renal proximal tubules inside an ECM. The perfusable tissue microchips was then preserved for greater than eight weeks. Their proposed technique offers a new way for programmable fabrication of human kidney tissue models [34]. Toprakhisar *et al.* have established a novel approach in bioprinting of dECM hydrogel bioink for use as micro-capillary-based bioprinting (Figure 2C). The main advantage of this method is bioprinting without any need to use support structure or any cross-linker components.

Freeform reversible embedding of suspended hydrogel (FRESH) has been applied for creating vascular constructs, where bioink is extruded into a buoyant support from granular dense medium [35]. The presence of a sacrificial support bath allows direct printing of bioinks such as alginate and Matrigel® with high fidelity [35]. FRESH has been utilized to fabricate vascular networks. In a recent effort, xanthan gum was deposited into a CaCh-infused gelatin to form sacrificial channels as rapid prototyping of microfluidic platforms [61]. Proper characterization of bioinks and the role of processing parameters such as pressure on the biological properties of printed constructs have been neglected [62].

In summary, extrusion-assisted has shown great potentials in fabricating mechanically-stable microfluidic constructs through both sacrificial and direct 3D printing. The wide range of materials and low costs associated with extrusion-assisted bioprinting have made it the first choice for many efforts on bioprinted microfluidic platforms. However, it suffers from a

resolution limitation of around 100 μm for current bioink systems. Optical-based techniques have provided a better resolution, as explained below.

4. Optical-Based Bioprinting Techniques

Traditionally, microfluidic systems were fabricated by lithography-based methods, where a photo-lithographic and photoresist filter with chemical treatments were used to obtain micro-patterning of proteins and other molecular agents. These patterns were created from a silicon master [70], or PDMS to fabricate stencils [71] or stamps [72]. After coating the master or stamp with the expected material, the printer starts creating the micro-pattern. Removable masks also have been used to protect some parts of substrate [73]. This is done by washing the substrate with cells/proteins after the mask is placed and they will only be deposited where the substrate is exposed and the excess is removed from the system. At the end, by removing the mask, a highly accurate pattern is obtained. Regarding the nature of these techniques, manual manipulation makes them nozzle-based approaches. Optical-based techniques are commonly known as light-assisted printing that uses light interaction with the subject ink to either polymerize a photo-curable ink or help the deposition of the ink from a donor plane onto a substrate. We classify the optical-based bioprinting technique to three main technologies: laser-induced forward transfer (LIFT), stereolithography (SLA), and two-photon lithography (TPL). We discuss the principals, highlights and challenges of each technique for microfluidics systems.

4.1. Laser-induced forward transfer (LIFT)

The basic configuration for LIFT includes a laser system which produces pulsed focused beams, an absorbent layer where a sheet of ink is coated, and a collecting surface on which the ink is deposited (see Figure 3A) [74]. The laser energy yields absorption of ink onto a transparent substrate and the light-matter interaction at the interface results in a strong pressure at the irradiation region. A small pixel of the biological material is ejected onto the collecting substrate. The properties of the incident laser spot controls the final shape and size of transferred ink. This method has a bioprinting resolution of around 50 μm [75] but it is costly due to the complexity of the laser source and the control of the laser.

The LIFT technique was employed to repair Pt-based heater elements with promising results on the ability of LIFT to write electrodes for the control of electro-osmotic flow in microfluidic platforms [76]. The LIFT technique has been used to pattern cellular compositions with almost any desired cell density and suspension viscosity [77]. In one study, LIFT was used to print thin layers of fibroblasts- and keratinocytes-laden collagen for skin tissue formation [36]. Wu and Ringeisen have fabricated tissue models loaded by human umbilical vein smooth muscle cells (HUVSMCs) and HUVECs using small droplets with diameters of approximately 50 μm [37]. Such work showed that the LIFT technique can be used for tissue biofabrication and it can be scalable to manufacturing macroscale constructs.

4.2. Stereolithography (SLA)

Conventional SLA is popular in manufacturing industries, while most SLA printers are configured so that the light source polymerizes a bath filled with a resin onto a moving 3D stage (Figure 3). Once one layer is polymerized, the stage descends a predefined distance for another pre-defined layer to be polymerized (Figure 3B). The stage is then elevated by a distance equivalent to the thickness of the layer, thus this method suffers from a lower resolution [80]. Despite these techniques, digital light processing (DLP) generates dynamic masks using computercontrolled array of micromirrors to polymerize a unit volume of the resin thus significantly expediting the curing process (Figure 1) [81]. During the exposure time, the DLP chip can be selected independently to deflect the light or to project it, which results in a light pattern. Each layer is solidified based on the pattern generated by the DLP chip and the light is modulated and transferred through a reduction lens onto the liquid resin. Compared to other 3D bioprinting technologies, light-assisted 3D bioprinters have the advantage of higher printing speed and better bioprinting resolution [31]. Due to the high degree of directionality in laser light and less light scattering perpendicular to laser light, the bioprinting resolution in SLA- and DLP-based bioprinting is predominantly determined by the thickness of the photocured resin. The thickness can be tuned by modifying laser characteristics such as pulse duration, wavelength and energy, repetition rate, and beam focus diameter, as well as the resin properties such as viscosity and surface tension [82]. Although the polymerization kinetics of the curing process can be very complex, the following relationship is often used to describe the thickness of the photo-cured resin [83]:

SLA bioprinting has been initially used for creating tissue constructs. In a study, the authors used a 100- μm -resolution SLA printer to solidify a biodegradable bioink made of diethyl fumarate, poly(propylene fumarate), and bisacylphosphine oxide towards healing critical-sized bone defects [84]. SLA has been recently employed to the rapid fabrication of microfluidic systems using transparent biocompatible polymers, with potential outlooks in organ-on-chip platforms [38]. Since SLA eliminates the need of expensive molds and it has the advantage of rapid prototyping, SLA-based microfluidic systems may lead to an efficient commercialization of organ-on-chip platforms.

The printing speed of light-based printing technology has been greatly enhanced by maskless DLP-based bioprinting. A manual exchange of two bioinks was used, including GelMA as the parenchymal tissue and GelMA-glycidal hyaluronic acid as the vasculature. The bioinks were mixed with human-induced pluripotent cell-derived hepatic progenitor cells and HUVECs. A recent study by Zhu *et al.* demonstrated DLP bioprinting of prevascularized tissue models with complex geometries of varying widths and heights of 50 μm using endothelial cells that were encapsulated in a mixture of glycidal methacrylate-hyaluronic acid and gelatin methacryloyl (GelMA) [39]. This study not only highlighted the versatility and the accuracy of the maskless method, but since all tissue models were bioprinted under 1 minute, it also emphasized the high speed of bioprinting associated with DLP. A recent effort on making multi-material DLP-based bioprinter has led to high-resolution fabrication of micro-tissue models, such as tumor model shown in Figure 3C [31]. They used a novel microfluidic chamber to switch different bioinks.

4.3. Two-photon lithography (TPL)

TPL does not rely on the use of complex optical systems or photomasks to print in a photosensitive bioink and it involves the use of a near-infrared ultrafast femtosecond laser. A multi-photon absorption process occurs in a medium that allows penetration of the emitted light. The freedom that laser systems offer in terms of scanning and tightly modulating the energies at the point of interest allows for submicron features. Applegate *et al.* prepared 3D multiscale patterns in protein hydrogels made of soft silk with low-energy femtosecond lasers without the use of exogenous or chemical crosslinkers [88]. Since then several improvements, such as multi-material bioprinting and patterned geometries, have been made in the direction of manufacturing of tissue models via TPL [87, 89]. Indirect bioprinting based on TPL has been applied to generate hollow models. In a recent work, multiscale channels were created within polyethylene glycol (PEG) tetrabi-cyclononyne hydrogels by photodegrading the bioink at the target pattern [40].

5. New Directions: 4D Bioprinting

Recent advancements in the fields of microfluidics and tissue engineering have opened new directions to design and construct *in vitro* models of diverse pathophysiological conditions. Although 3D bioprinting technologies have attracted a great deal of attention in the last decade and there has been a great progress in this field, several intrinsic limitations can be found in these approaches. Despite the capacity of 3D bioprinting techniques for building complex biological constructs, they still suffer from proper building hollow constructs [90]. From the perspective of tissue engineering, the development of functional tissue constructs (for instance, vascular constructs with multiscale vasculatures from small capillaries to large vessels) has still remained very challenging in the field of biofabrication [91]. Furthermore, printed 3D structures are unable to provide the growth and proliferation of the cells in thick structures. To address these limitations, four-dimensional (4D) bioprinting has been introduced by integrating *time* into 3D bioprinting process, in which changes within tissue configurational and biophysical characteristics occur in response to environmental stimuli, leading to a better mimicking of native tissues [92].

When time is considered in 3D bioprinting, the shapes or functionalities of printed constructs change over time under external stimulations [93]. In a broad classification, 4D bioprinting includes (i) based on the shape deformation of the material (either conventional materials or smart materials) (ii) based on the maturation of the engineered construct (time dependent process) [93]. The latter one can be a promising strategy to tackle challenges of 3D bioprinting and better mimic the native tissue [94]. Its noteworthy that 4D biofabrication not only includes shape transformation of a 3D bioprinted structure but also different strategies of biofabrication such as casting, spin/dip coating, photolithography, and so on. Therefore, this advanced technology offers creating a vast range of structures with the most complexities and highest resolution.

Figure 4A depicts the concept of 4D biofabrication. The way how shape transformation is utilized for 4D biofabrication is classified into three basic ways (Figure 4A) [94]. The first approach includes shape transformation of acellular constructs and loading them with cells. This method is comparable to a traditional scaffold-based approach which is less-attractive

since living cells put the most restrictions on selection of the type of materials, fabrication method as well as fabrication conditions. The second approach includes deposition of cells onto printed construct, and shape conversion of the cell-loaded construct. The third approach is fabrication of the structure with a non-vital material and encapsulation of cells at the same time, and afterwards its shape transformation. This approach is very challenging due to practical limitations regarding how to control cell deposition during printing. The latter two approaches are very favorable because shape transformation can occur while cells are already incorporated to the construct. Regarding the type of shape transformation, this physical transformation can induce by various stimulus which leads to the shape morphing of the object, either manually or using materials with shape-changing properties [94].

5.1. Current approaches

There are currently four different approaches for shape transformation of a material/ biomaterial: 1) manual transformation 2) spontaneous shape transformation 3) cell contraction 4) stimuli-responsive [94]. Among these, the stimuli-responsive process may occur with different stimulus such as moisture, temperature, *pH*, magnetic field, electric field, light and so forth [92]. Use of stimuli-responsive (bio)materials offer several advantages over the other conventional methods, such as precise control on the moment when shape transformation is needed, simultaneously folding of multiple objects made of different materials, and the ability to fold micron-sized objects. These important characteristics may allow the biofabrication with cells being seeded onto the stimuli-responsive material or encapsulated within the smart material [94].

Temperature stimulation has been used to induce shape transformation in bioprinted constructs more than any other factor. Figure 4B shows a folding star-shaped thermos-responsive polymer bilayer. As it can be seen, both theoretical (simulation) investigations and real microscopy images indicate that this thermos-responsive hydrogel swells and deforms into a capsule-like structure at reduced temperatures, and it unfolds and releases the encapsulated cells by increasing temperature, offering a promising approach for drug delivery applications [95, 96].

Another frequently-used stimulus is water, based on the level of water sorption (i.e., swelling) in various hydrogel systems. For instance, the swelling of the bioprinted scaffolds occur through water sorption when they immersed in an aqueous solution. Figure 4C shows a doublelayered printed construct consisted of patterned PEG following self-folding controlled by the swelling differences of individual layers in water environment, leading to the deformation of the constructs [93]. When cells were embedded in the PEG bilayers, water exposure will induce the self-folding behavior of the construct into cylinders of various sizes, with minimal damage to the cells.

Another approach for shape transformation of a biomaterial is cell contraction. Cells, with the ability of exerting traction force, can adhere to a substrate/biomaterial surface and induce self-folding based shape transformation to fabricate complex cellular constructs (Figure 4D). In this approach, the angle θ (i.e., the angle between the folding microplate and glass substrate and can be measured by the cell amount adhered onto microplates) plays a vital role in making desired 3D cell-laden microstructure.

5.2. Proposed solutions for vascular chips

There has been a great effort on developing novel 4D biofabrication approaches for vascular systems. For instance, a group of researchers have employed shape-morphing hydrogel systems to fabricate very small internal diameter tubes, as low as 20 μm . This is comparable to blood capillaries *in vivo* and is not yet feasible by other bioprinting approaches (Figure 4E) [90]. The self-folded hydrogel-based tubes fabricated via their proposed approach supported cell growth and proliferation, as well as cell survival, while maintaining high viability of the cells by day 7.

Although the 4D bioprinting has been recently developed, several groups tried to provide the bioprinting scientific community with a better understanding of this approach through writing comprehensive review papers on this matter [93, 94]. But in a general overview, with the aim of creating *in vitro* models that resemble tissue structures found in nature, 4D bioprinting promotes dynamic, structural, and cellular changes of a tissue over time, overcoming the static nature of 3D bioprinting [97].

6. Conclusion and Future perspective

6.1. Concluding remarks

Three major factors for the design of microfluidic organ-on-chip models are chip function, degree of integration, and application [8]. To mimic complex architectures of micro-tissues or organoids in traditional organ-on-chip models, the fabrication of multiple PDMS layers and sequential integrations are required, which are expensive, time-consuming, labor-intensive, and difficult to get automated for continuous production. The emergence of 3D bioprinting techniques have led to novel opportunities for economic and rapid prototyping of micro-tissues, and they have shown potentials for constructing organ-on-chips in automated procedures for continuous production. Adapting 3D bioprinting in microfluidics platforms allows the construction of desired micro-tissue in organ-on-chip models using inexpensive desktop 3D bioprinter platforms, while the microfluidic set-up includes expandable electrochemical monitoring, sensing elements for electrochemical transductions, and enzymes and biomarkers. This can be achieved by the integration of supporting technologies with bioprinting platforms.

6.2. Future directions

There are still some challenges for creating functional microfluidic constructs towards commercialization. When applying 3D bioprinting to produce micro-tissue models, the post-integration of microfluidic chips and micro-tissues is important. Compared to traditional organ-on-chip models, bioprinted micro-tissues inside microfluidic chips will encounter much more shear stresses given by perfusion flows due to their spatial architecture. Optimum scaffold structure for better adherence and preserving structural integrity over long-term will be another challenge. For example, three-(trimethoxysilyl)-propyl methacrylate coating has been applied for better adherence onto glass substrate for bioprinted GelMA- and PEGDA-based bioinks [31]. Creation of customized supports and connecting scaffolds are also straightforward using 3D printing techniques. Furthermore, micro-tissue fabrication has become more automatic and robust, which will ultimately lead

to single-step biofabrication of organ-on-chip for scaling-up from lab scale to mass production. Microfluidic chips can be also made in an automated manner since 3D printing has shown the capability to construct microfluidic platforms [41, 43], as well as some advances in microelectronic systems [99]. The combination of 3D printing of microfluidics fabrication and bioprinting techniques shows great promise in the direction of single-step organ-on-chip engineering. In addition, we can develop new capabilities, such as repeatable microelectrodes, which are unrealistic by traditional microfabrication methods. We envision further technologies that will enable online rapid fabrication of (electrically-) conductive materials and microchannel encasements along with integrated micro-tissue constructs. This way, the required labor works to make such assemblies will be significantly reduced during and after the printing process. In this perspective, we propose using multi-material, extrusion-assisted printers to deposit a mixture of electrically-conductive, nonconductive, and sacrificial materials as an integrated construct. This strategy would pave the path towards commercializing organ-on-chip platforms.

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Abbreviations:

(hMSCs)	human mesenchymal stem cells
(HUVECs)	human umbilical vein endothelial cells
(PAB)	pressure-assisted bioprinting
(PDMS)	poly-(dimethyl-siloxane)
(CAD)	Computer aided design
(ECM)	extracellular matrix
(SLA)	stereolithography
(PLA)	poly (lactic acid)
(PGA)	poly (glycolic acid)
(LIFT)	laser-induced forward transfer
(TPL)	two-photon lithography
(HUVSMCs)	human umbilical vein smooth muscle cells
(DLP)	digital light processing
(3D)	three-dimensional
(4D)	four-dimensional

(PEG)	polyethylene glycol
(GelMA)	gelatin methacryloyl

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Figure 1. Current bioprinting techniques used in the fabrication of organ-on-chip models. Adapted with permission from references Katja et al. [45], Malda et al. [46], Xuanyi et al. [47], and Jeong Han et al. [48]

Figure 2.

Examples of nozzle-based bioprinted models: **A**) Schematics of the fabrication process: (i) fugitive vascular ink of Pluronic F127 and thrombin as well as cell-laden gelatin are printed within a pre-fabricated encasement, (ii) ECM material is cast over the printed structure, followed by fibrinogen cleavage and fast polymerization into fibrin in the cast matrix, (iii) the fugitive ink is liquefied by cooling, leaving a vascular network, and (iv) endothelialized and perfused. **B**) Schematic of a nephron highlighting the convoluted proximal tubule, in which (i) a fugitive ink is first printed on a gelatin-fibrinogen ECM, (ii) additional ECM is cast, (iii) the fugitive ink is evacuated, and (iv) cells are seeded within the tubule. **C**) Bioprinted cell-laden ECM hydrogel chips: (i) aspiration of pH and salt neutralized ECM pre-gel from the temperature-controlled ink reservoir, (ii) incubation of aspirated ECM pre-gel, and (iii) extrusion of gelled ECM bioink directly on the substrate. **D**) Images of support structure-free bioprinted ECM hydrogel structures with (i) two and (ii) four printed layers (scale bar: 3 mm). **E**) Three dimensional development of cellular organization after (i) one and (ii) three days of culture (scale bar: 100 μ m). *Reprinted with permission from reference Kolesky et al. [33], Kimberly et al. [34], and Burak et al. [63],*

Figure 3.

Examples of light-assisted bioprinted models: **A)** DLP-based bioprinted triculture liver model: bright field images demonstrating the patterns of (i) 5% w/v GelMA and (ii) 2.5% w/v GelMA with 1% w/v GelMA-hyaluronic acid without cells, (iii) bioprinted hepatic construct with patterns of hepatic cells (green) and supporting cells (red) (scale bar: 500 μ m). **B)** SLA bioprinted PEG-based model: (i) experimental setup, (ii) a multi-layer PEG structure, (iii) NIH 3T3 fibroblasts stained with Cell-Tracker green or orange, (iv) side view and (v) Z-optical section of cell-loaded gelatin-methacrylate model. **C)** DLP-based bioprinted tumor-on-chip model: (i) schematic of the patterning mask; (ii) bioprinted microvasculature using 50% v/v PEGDA; (iii) bioprinted MCF7 cell (blue)-laden microvascular bed of 7% w/v GelMA further seeded with HUVECs (green) in the channels. *Reprinted with permission from reference Xuanyi et al. [47], Amir et al. [78], and Anthony et al. [79].*

Figure 4.

Examples of 4D bioprinted models: **A)** Schemes of 4D biofabrication, where 3D structure is fabricated and reshaped by transforming over time: (i) fabrication of non-vital constructs and loading with cells (colored in blue), (ii) fabrication of non-vital construct, and (iii) fabrication of vital construct with cells and its shape transformation. **B)** Thermo-responsive materials: (i) theoretical (simulation) images indicate that the folding (shape transformation) action depends on temperature and swelling function [96], and (ii) shape transformation of poly-(*N*-isopropylacrylamide)-based layer with cells and corresponding experimental images. **C)** Water-responsive bioprinted scaffolds: (i–iii) fabrication of the PEG bilayers, (iv) conceptual schematic illustrating the self-folding of bilayered PEG hydrogels, (v) representative fluorescent image showing Hoechst-stained fibroblasts (stained in blue) encapsulated in the inner hydrogel layer and calcein-stained fibroblasts (green) loaded in the outer hydrogel layer. **D)** Conceptual illustration of cell origami: (i) cells adhere and stretch across two microplates (green: actin; blue: nucleus), (ii) cells are cultured on micro-fabricated parylene microplates, (iii) various 3D cell-laden microstructures can be produced by changing the plate geometry. **E)** Methacrylated alginate tubes: cartoons (upper panel) and demonstrative photographs (lower panel) of self-folded tube (i) water post-fabrication, (ii) same tube from immersed in a 0.1 M CaCl_2 solution, which leads complete unfolding of the tube, and (iii) unfolded film immersed in a 0.1 M EDTA solution, leading to tube refolding.

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Table 1.

Advantages and disadvantages of 3D printing methods for microfluidic platforms

Approach	3D Printing Method	Advantages	Disadvantages	Refs
Nozzle-based	Sacrificial inkjet	Ease of handling, low cost, rapid prototyping	Low resolution, low printing speed, instability for vascular channels	[32]
	Sacrificial extrusion-assisted	Inexpensive, rapid prototyping, resolution	Low biocompatibility, low-printing speed, long postprocessing	[33], [34]
	Extrusion-assisted embedded	High 3D stability, structural complexity, resolution	Cost, long preparation, slow fabrication	[35]
Optical-based	Laser-induced forward transfer	Resolution, biocompatibility, speed, high printing speed	Cost, 3D instability, post-processing	[36], [37]
	Stereolithography	Resolution, rapid prototyping, ease of preparation	Low biocompatibility, limited material	[38]
	Digital-light projection	Resolution, very high printing speed, complexity	Cost, limited material	[39]
	Two-photon absorption	Very high resolution	High cost, low compatibility, very slow printing speed	[40]

Table 2.

Examples of micro-tissue models fabricated by nozzle-based bioprinters (cell viability values indicate the average of reported results).

Organ	Main components	Feature size	Cell Viability	Comments	Refs
Heart	Mature cardiomyocytes oriented along conductive-ECM	~ 150 μm	~ 80% (channel perfusion)	Easy to connect to microfluidic encasement	[19]
Kidney	Proximal tubule embedded in epithelial cell-filled ECM	~ 100 μm	~ 95%	Sacrificial Pluronic printing used to make channels	[34]
Skin	Keratinocyte/fibroblast-embedded ECM	~ 100 μm	~ 90%	2D printing of single elements	[36, 64]
Gut	Permeable membrane-like ECM, seeded by gut epithelial cells & gut microbes	~ 100 μm	N.A.	Very stable fluidic channels, potentially can be patterned by cell printing	[65]
Vascular System	Endothelial cells seeded in sugar (acellular) channels	~ 200 μm	~ 85%	Very robust channels under blood perfusion over time	[66]
	Endothelial-filled channels in gelatin-based ECM	~ 250 μm	~ 80%	Sacrificial Pluronic printing used to make vascular tissue model	[27]
	Alginate-based vessels in cell-laden gelatin-based ECM	~ 200 μm	~ 85%	Core/shell method used to direct print channels based on alginate	[67]
Bone	Stem-cell filled gelatin-based ECM and poly-ethylene-glycol Droplet based bioprinting	~ 50 μm	~ 80%	Extrusion based techniques: fused deposition modeling, compatible with various cells and GFs;	[68]
	For hydrogels	~ 50 μm	~ 90%	Inexpensive, flexible, and commercially available	[69]
Cartilage	Poly-ethylene-glycol ECM filled by mouse myoblasts onto micro-sized cantilevers	~ 100 μm	~ 80%	Inkjet printing used for thin structures	[68]

Table 3.

Examples of micro-tissue models fabricated by optical-based bioprinters (cell viability values indicate the average of reported results).

Organ	Main components	Feature size	Cell Viability	Comments	Refs
Liver	Hepatocyte (gelatin) filled by endothelial cell	~ 20 μm	~ 75% (short-term)	Multi-material used as there are more than one cell type	[47]
Skin	Stem cell-filled gelatin embedded by keratinocytes/fibroblasts	~ 100 μm	~ 90% (short-term viability)	LIFT gives good 2D resolution	[85], [86]
Bone	Nano-hydroxyapatite and human osteoprogenitors filled by bone osteosarcoma cells	~ 50 μm	~ 95% (local)	LIFT using quartz ribbon coated with a thin absorbing layer of titanium and bioink	[84]
Vascular system	Tubular layers of ECM filled by smooth muscle/endothelial cells	~ 20 μm	~ 85%	DLP-based printing of photo-crosslinkable gelatin	[30]
Tumor	Endothelial-seeded capillaries in cancer-cell-encapsulated ECM	~ 20 μm	~ 90%	Cancer cells encapsulated in gelatin methacryloyl	[31]
Capillaries	Seeded cells in capillaries with small diameters	~ 1 μm	~ 60% (local)	Small-sized channels made by two photon lithography	[87]