

## Review

# Bioprinting of Organ-on-Chip Systems: A Literature Review from a Manufacturing Perspective

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**Abstract:** This review discusses the reported studies investigating the use of bioprinting to develop functional organ-on-chip systems from a manufacturing perspective. These organ-on-chip systems model the liver, kidney, heart, lung, gut, bone, vessel, and tumors to demonstrate the viability of bioprinted organ-on-chip systems for disease modeling and drug screening. In addition, the paper highlights the challenges involved in using bioprinting techniques for organ-on-chip system fabrications and suggests future research directions. Based on the reviewed studies, it is concluded that bioprinting can be applied for the automated and assembly-free fabrication of organ-on-chip systems. These bioprinted organ-on-chip systems can help in the modeling of several different diseases and can thereby expedite drug discovery by providing an efficient platform for drug screening in the preclinical phase of drug development processes.

**Keywords:** additive manufacturing; bioprinting; organ-on-chip



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## 1. Introduction

The development of new drugs usually takes place in phases over an extended period of time and is an expensive process [1]. A typical drug development process involves four phases before FDA review and approval [2]. In the first phase, drugs are tested in vitro, i.e., the testing occurs using cells or biological materials outside of a living animal. If initial testing is successful, the drug is then investigated in vivo, i.e., using living animal models. However, approximately half of the drugs that pass the first phase fail in later phases [3]. One cause for this high failure rate is that in vitro models are not always able to accurately represent interactions between the drug and the biological environment [4]. Another cause is that standard in vivo animal models often misrepresent the human physiology.

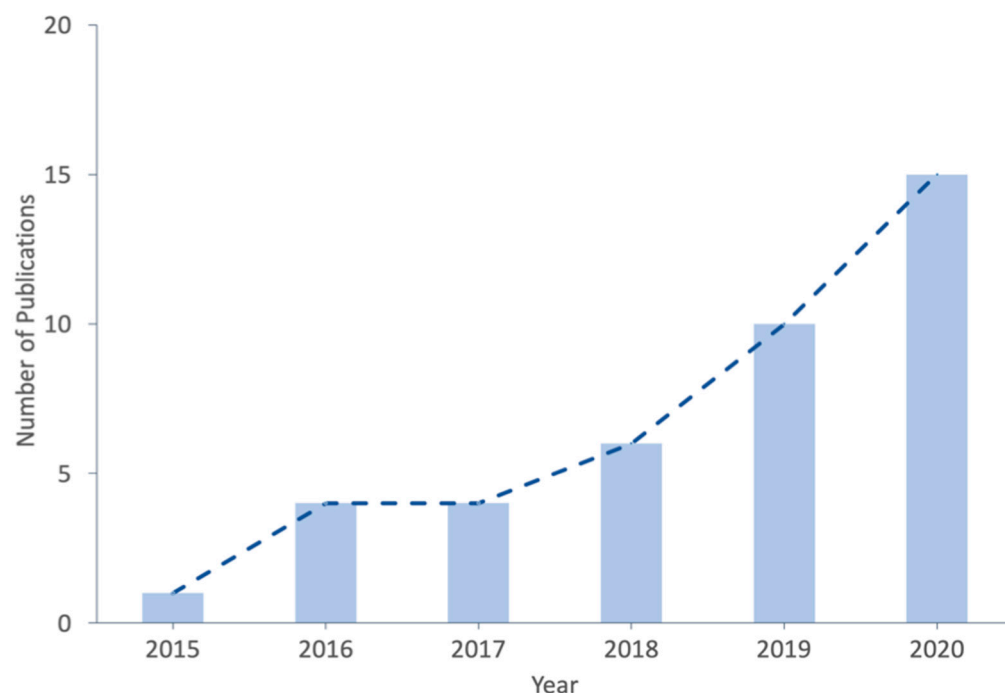
An organ-on-chip system is a microfabricated multichannel 3D microfluidic structure that emulates specific functions of human organs [2–4]. Increased specificity of organ-on-chip systems is accomplished by using dynamic fluid flow to provide nutrition and oxygenation with tissue-specific environmental cues and molecular gradients [4–8]. It is foreseen that use of such sophisticated organ-on-chip systems for modeling the activities, mechanics, and physiological responses of human tissues and organs will allow for inexpensive and faster testing of new therapeutic drugs compared with use of traditional in vitro and in vivo animal models [9].

Micro-fabrication methods, including soft-lithography and photolithography, are traditionally used to fabricate organ-on-chip systems [10,11]. In soft lithography, an elastomeric stamp with patterned relief structures on its surface is used to generate patterns and structures (also known as a mold) with feature sizes ranging from 30 nm to 100 µm. Polydimethylsiloxane (PDMS) is then poured into the mold to create a closed-circuit

channel sealed with glass slide [10]. In photolithography, a silicon wafer is covered by photoresist material, and ultraviolet (UV) light removes the photoresist material from some portions of the wafer surface. Then, some silicon is etched away from the portions of the wafer not covered with the photoresist material, to create a mold. PDMS is then poured into this mold, to create a closed-circuit channel sealed with a glass slide [11]. However, these methods are expensive and time-consuming [7]. In addition, these methods suffer from limited availability of compatible biological materials [12].

Bioprinting involves the spatial patterning of living cells and other biologics by stacking them using a computer-aided layer-by-layer deposition approach to fabricate living tissue-like constructs [13]. It has the ability to create channels that have features with complex design and is a one-step fabrication process. In addition, it has the potential to be fully automated, maintain accuracy, and be replicated with relative ease [14]. In recent years, bioprinting has been used to produce organ-on-chip systems [15,16].

Figure 1 shows the trend in the number of organ-on-chip publications involving bioprinting over the past five years. In recent years, several review papers [16–20] have been published on the state-of-art of applying bioprinting to fabricate organ-on-chip systems. However, these reviews discuss the studies primarily from physiological and biological perspectives.

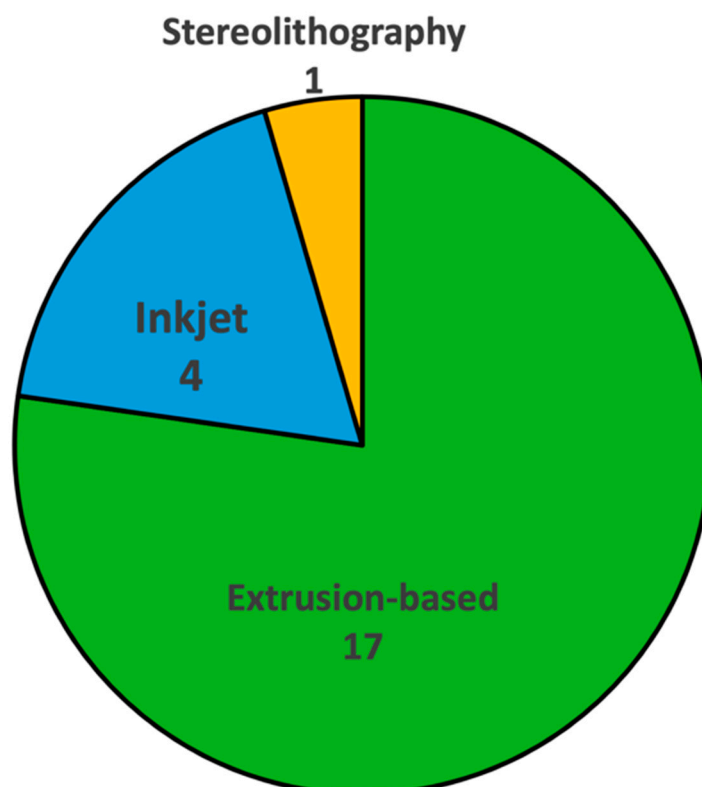


**Figure 1.** Organ-on-chip publications involving bioprinting from 2015 to 2020, based on search results in the PubMed database using search query: (organ-on-a-chip) AND (bioprinting).

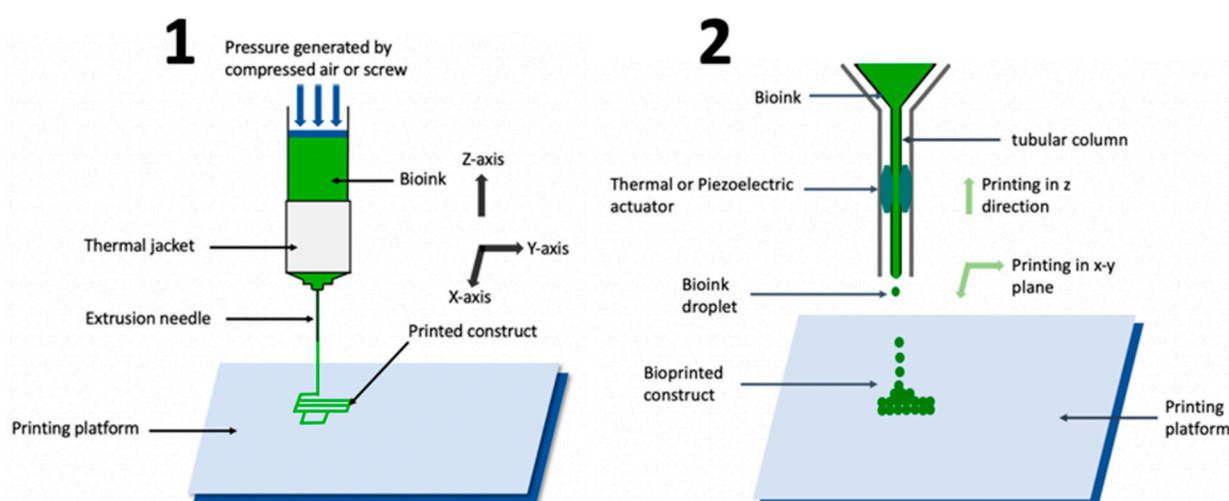
This paper is the first review paper to provide a review on bioprinting used to fabricate organ-on-chip systems from the perspective of manufacturing. Section 2 introduces the bioprinting techniques and their working principles, as well as advantages and limitations for fabricating organ-on-chip systems. Section 3 discusses recent advances in bioprinted organ-on-chip systems, categorized on the basis of tissue type, e.g., liver, kidney, and heart. Section 4 briefly presents key requirements of bioinks, the material that is printed to fabricate constructs, and use of hydrogels as bioinks. Section 5 highlights the current challenges in using bioprinting to fabricate organ-on-chip systems and presents some directions for future research. In the final section, key insights are summarized.

## 2. Bioprinting Techniques Used to Fabricate Organ-on-Chip Systems

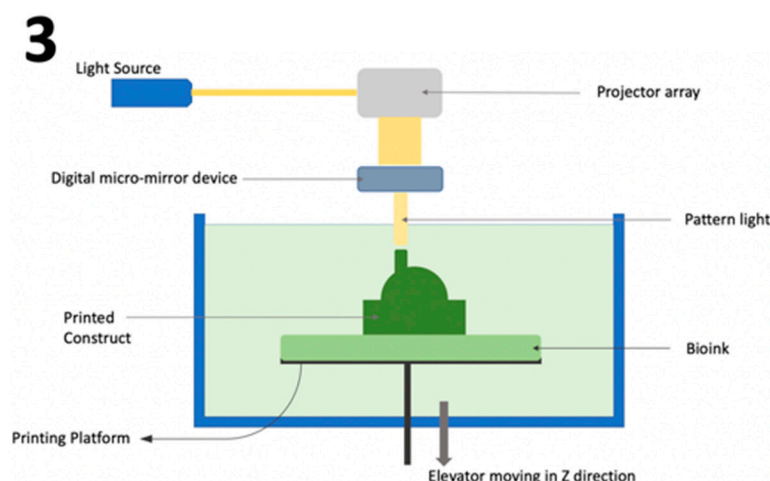
Figure 2 shows the number of reported studies utilizing different bioprinting techniques for fabricating organ-on-chip systems. Out of twenty-two reviewed studies, sixteen used extrusion-based bioprinting, four used inkjet bioprinting, and only one used stereolithography bioprinting. There were no reported studies using laser-based bioprinting. Understanding the working principles of these techniques, along with their advantages and limitations in fabricating organ-on-chip systems, can provide guidance when researchers select bioprinting techniques for organ-on-chip systems. Figure 3 shows schematic illustrations of the three bioprinting techniques that have been used in organ-on-chip fabrication.



**Figure 2.** Utilization of bioprinting techniques for fabricating organ-on-chip systems in reviewed studies.



**Figure 3.** Cont.



**Figure 3.** Schematic illustrations of (1) extrusion-based bioprinting, (2) inkjet bioprinting, and (3) stereolithography.

### 2.1. Extrusion-Based Bioprinting

The dispensing head can move along the X and Y axes in the horizontal plane, and up and down along the Z axis, as directed by the CAD (computer-aided design) model. Instructions from the CAD model are input (as a G-code file) to the robotic system, which consists of the bioprinter, and the computer hardware and software [21]. In some printers, the printing platform moves up and down along the Z axis while the head can only move along the x-y planes. The fluid-dispensing system can be driven by pressure generated from a pneumatic-, mechanical- (piston or screw-driven), or solenoid-based system [13]. In certain printers, the extrusion temperature is controlled by heating or cooling the thermal jacket that holds the syringe. Major controllable printing parameters include extrusion pressure (the pressure at which bioink is extruded), extrusion temperature (temperature at which bioink is extruded), and printing speed (the speed at which dispensing head moves in the x-y planes).

The extrusion-based process is capable of printing a wide array of biomaterials, including composite bioinks that are comparable to natural tissue [22], and therefore is suitable for fabricating organ-on-chip systems that involve several different types of tissues and extracellular matrix components [23]. In addition, the extrusion-based process usually has higher printing speed than other bioprinting processes [13]. Furthermore, the relative simplicity of the technology enables ease-of-use for researchers across disciplines [13]. However, the extrusion-based process has drawbacks such as limited resolution [24], nozzle clogging [21,25], and lower cell viability [26]. Extrusion-based bioprinting process parameters need to be optimized to balance shape fidelity and cell viability. Smaller needle diameters increase the shape fidelity of printed constructs [27] but are harmful to cells due to increased shear stress [28,29]. Increased extrusion pressure is required to print bioinks of high viscosity [21], but this also harms cells through increased shear stress [28,29]. Different cell types have different sensitivities to shear stress [30,31], so the optimization of process parameters depends on the biomaterials used during printing.

### 2.2. Inkjet Bioprinting

In inkjet bioprinting, droplets of bioink are formed through vaporization and dispensed (through an extruder controlled by an actuator) onto a platform in a layer-by-layer fashion to fabricate a 3D construct [32–34]. The actuator can have thermal [35] or piezoelectric [36,37] modality for actuation. The extruder can move in the x-y-z directions for fabrication as per the CAD design. However, in certain printers, the extruder is fixed while the printing platform moves in the x-y-z directions.

In inkjet printing, the main printing parameter that can be controlled is droplet size, which is governed by the actuator's modality. Inkjet bioprinting offers a high resolution

(~30  $\mu\text{m}$ ) which makes it suitable for fabricating organ-on-chip systems which are in sizes of hundred microns or less [38,39]. In addition, constructs printed with inkjet bioprinting offer high cell viability [38,40]. However, inkjet bioprinting is only suitable for bioinks with low viscosity (~0.1 Pa·s) [41], and the shape fidelity of vertical constructs are poorer with inkjet printing than other bioprinting techniques [38]. Because of these factors, the application of inkjet printing is limited in fabricating organ-on-chip systems that contain tissue types and biomaterials of higher viscosity.

### 2.3. Stereolithography

In stereolithography, a UV laser selectively polymerizes photosensitive resin containing cells to form a solid layer, after which the build platform shifts down in the z direction by one layer, and the process is repeated until the construct is complete [14,42]. The setup contains a projector array and digital micro-mirror device which can both be moved in the x-y plane. The photosensitive resin is composed of biocompatible, polymerizable oligomers and a biocompatible photoinitiator [42]. The position and the intensity of the UV laser focus are the parameters that can be controlled in stereolithography. By controlling the positions of the laser focus, polymerization of the resin can be precisely controlled for selective polymerization. This achieves a high resolution, which is required in fabrication of organ-on-chip systems [14]. Additionally, vertical constructs printed by stereolithography have good quality [38]. However, stereolithography is time-intensive relative to other techniques. Another limitation is need for intense UV radiation, which can have a negative effect on cell viability [20,43].

### 3. Bioink Used in Bioprinting of Organ-on-Chip Systems

In the bioprinting process, from an organ-on-chip fabrication perspective, the bioink formulation needs to satisfy certain physical and biological requirements [44]. The primary biological requirement is that the bioink needs to be biocompatible, which means that it should not be toxic to the cells and should not alter the functionality or physiology of the cells [32]. The second biological requirement is biomimicry, i.e., bioink should mimic the extracellular matrix so that cells can proliferate [45]. Physical properties that the bioink should include are shear-thinning behavior, defined as a non-linear increase in viscosity as stress is applied, and structural fidelity. Shear-thinning behavior is necessary for the bioink to be printable [46]. Bioink also needs to have suitable mechanical properties so that the printed construct is stable [47].

Unique properties of hydrogels make them ideal candidates [48] as bioink constituents. Hydrogels are three dimensional molecules composed of hydrophilic chains which are formed by cross-linking of polymer chains in an aqueous medium through various mechanisms such as physical crosslinking, chemical crosslinking, and photo crosslinking [49]. Crosslinking mechanisms have distinct advantages and disadvantages. Photo crosslinked constructs have high shape fidelity, but the UV light used during photo crosslinking can create free radicals which are harmful to cells [50]. Ionic crosslinking, a common form of physical crosslinking, forms mechanically weaker constructs than chemical crosslinking, but promotes higher cell viability [51]. Hydrogels have the ability to absorb water up to a thousand times their dry weight, which makes them suitable materials to act as extracellular matrix and support cell proliferation. The reviewed studies have used natural hydrogels like alginate, gelatin, cellulose, fibrin, and collagen, and synthetic hydrogels such as poly (ethylene glycol) (PEG), poly ( $\epsilon$ -caprolactone) (PCL), pluronic, and gelatin methacryloyl (GelMA) as shown in Table 1.

**Table 1.** List of studies using various hydrogels as bioink constituents.

Hydrogel as Bioink Constituent	Bioprinting Technique	Crosslinking Mechanism	Organ-on-Chip System	Reference
Alginate	Extrusion	Physical	Vessel, heart	[52,53]
Gelatin	Extrusion, stereolithography	Chemical	Vessel, liver, kidney	[54–57]
Cellulose	Extrusion	Chemical	Tumor	[58]
Fibrin	Extrusion, inkjet	Physical	Vessel, kidney	[55,56,59]
Collagen	Extrusion, inkjet	Chemical	Vessel, gut, lung	[57,59–61]
Poly (ethylene glycol) (PEG)	Stereolithography	Photo	Liver	[54]
Poly ( $\epsilon$ -caprolactone) (PCL)	Extrusion	Photo	Liver	[62]
Gelatin methacryloyl (GelMA)	Extrusion, inkjet	Photo	Vessel, heart, liver, tumor	[53,63–66]
Pluronic	Extrusion	Photo	Kidney	[55]

#### 4. Organ-on-Chip Systems Fabricated Using Bioprinting

This section outlines specific examples, as shown in Table 2, of the research efforts for modeling the liver, kidneys, heart, lungs, gut, bone, vessel, and tumors on microfluidic chips, and describes the fabrication processes and applications of these organ-on-chip systems.

**Table 2.** Reported studies investigating various organ-on-chip systems.

Organ-on-Chip System	Bioprinting Technique	Bioinks Used	Reference
Liver	Extrusion, inkjet, stereolithography	Gelatin, PCL, PEG, GelMA	[54,62,64,67]
Kidney	Extrusion	Fibrin, Pluronic	[55]
Heart	Extrusion	Alginate	[53,68]
Lung	Extrusion	Collagen	[61]
Gut	Extrusion	Collagen	[60]
Bone	Inkjet	PLGA	[69]
Vessel	Extrusion, inkjet	Alginate, gelatin, fibrin, collagen, GelMA	[52,56,57,59,63,66]
Tumor	Extrusion	Cellulose	[58,65,70–73]

Table 3 summarizes the major results that were found for each category of organ-on-chip system, based on collected literature.

**Table 3.** Major results for organ-on-chip-systems.

Organ-on-Chip System	Major Result	Reference
Liver	Models drug toxicity	[54,62,64,67]
Kidney	Models drug toxicity	[55]
Heart	Models drug toxicity, mimics heartbeat	[53,68]
Lung	Mimics disease response	[61]
Gut	Capable of forming tissue with multiple cell types	[60]
Bone	Disease modeling	[69]
Vessel	Mimics blood flow, disease modeling	[52,56,57,59,63,66]
Tumor	Disease modeling, drug testing	[58,65,70–73]

##### 4.1. Liver-on-Chip

Liver plays a critical role in drug metabolism and detoxification of blood. Drug-induced hepatotoxicity is one of the main reasons for drug withdrawal in later phases of clinical trials, thus highlighting the need for liver-on-chip models that can be used to evaluate hepatotoxicity during drug-screening [74]. Bhise et al. developed a liver-on-chip platform by bioprinting hepatocyte spheroid-laden hydrogel constructs directly within the culture chamber of bioreactor [60]. By using the developed liver-on-chip platform



as a model to predict acetaminophen (a drug) toxicity, with results comparable to those obtained from *in vivo* models, they demonstrated that this bioprinted liver-on-chip system has applications for drug toxicity analysis. Snyder et al., printed epithelial-laden and hepatocyte-laden Matrigel on microfluidic chip to study radiation shielding of liver cells by the prodrug amifostine [67]. This study demonstrated the application of a bioprinted liver-on-chip system to obtain an understanding of multi-cellular biological systems. The current *in vitro* models typically lose their drug metabolism functions rapidly [75]. Liver-on-chip systems capable of maintaining drug metabolism functions for a long period of time are required. Grix et al. performed a proof-of-concept study in which perfusable liver organoid was bioprinted using stereolithography technique [56]. By verifying the stable protein expression of liver organoid using immunohistology and qPCR (quantitative polymerase chain reaction), they demonstrated that bioprinted liver organoid can be cultivated on a chip to produce a liver-on-chip system. This study demonstrated that a liver-on-chip model can be developed which maintains metabolic function for long time. To demonstrating that rapid liver-on-chip modeling is possible, Lee et al. used a novel bioprinting method involving a multi-head tissue organ building system to fabricate a liver-on-chip system in a single step [62]. The bioprinted liver-on-chip system showed significantly enhanced liver function, indicating that the developed bioprinting method can be applied to fabricate organ-on-chip systems for mechanistic therapeutic studies and drug screening.

#### 4.2. Kidney-on-Chip

Drug induced kidney toxicity is responsible for nearly one-fifth of drug failures in Phase III clinical trials even if the drug passes preclinical testing [76]. Kidney-on-chip systems can act as *in vitro* kidney models that accurately predict the human drug response in preclinical testing. Homan et al. bioprinted convoluted renal proximal tubules on a microfluidic chip to develop a kidney-on-chip system [55]. In this model, the tubule structure was created by extrusion-based bioprinting using pluronic ink. A silicon gasket was first printed onto a glass slide, which was then filled via extrusion printing with gelatin-fibrinogen bioink (mimicking kidney extracellular matrix). The pluronic was then liquefied by cooling and flushed out to create an open tubule for epithelial cell seeding. The printed kidney-on-a-chip system exhibited nephrotoxicity against cyclosporine A (immunosuppressive drug).

#### 4.3. Heart-on-Chip

Although current *in vitro* models of the heart are suitable for short-term modelling of human cardiac conditions and small-scale drug screening, they are not well suited for higher-throughput drug studies and longer-term studies [11]. Heart-on-chip systems can provide a stable platform for long-term drug screening studies. Zhang et al. created a heart-on-chip model by integrating bioprinting and microfluidics technology [53]. Extrusion-based bioprinting was used to print bioink containing endothelial cells to form a microfibrillar scaffold. After endothelial cells migrated to the outside of the fibers, the scaffold was seeded with cardiomyocytes. The resultant semi-self-assembled endothelialized myocardium scaffold accurately mimicked the *in vivo* vascularized structure of the myocardium and was integrated with a microfluidic chip to be used as a platform for cardiovascular drug screening. The research team observed dose dependent responses of cardiomyocytes and endothelial cells to an anti-cancer drug, demonstrating that such a heart-on-chip model can be successfully used for drug screening. Lind et al. used multi-material extrusion-based bioprinting to fabricate a heart-on-chip system in a single step [68]. Dextran ink was used to print a film on a glass slide substrate, on which thermoplastic polyurethane (TPU) ink was used to print a cantilever base. A thermoplastic polyurethane (CB:TPU) ink strain gauge was printed on top and covered by TPU ink wire cover. PDMS ink filaments were printed on the top part of this cantilever, followed by the addition of high conductivity silver particle filled polyamide ink (ink filled with high conductivity silver particles) which was insulated with PDMS ink. The printed microfluidic device

had eight wells which acted as cell incubators and were seeded with cardiomyocytes. This heart-on-chip system was employed to study the effect of drugs (verapamil and isoproterenol) on the beating of cardiac microtissues. The beating frequency and strength were observed and recorded directly with this heart-on-chip system, demonstrating the application of this novel heart-on-chip device for toxicology and drug-screening research.

#### 4.4. Lung-on-Chip

Human lungs are exposed to environmental agents which can lead to respiratory diseases [62]. They are sites of disorders such as asthma and chronic obstructive lung diseases [62]. Lung-on-chip systems can be used as a platform for development of drugs for treatment of these diseases and disorders. Park et al. bioprinted a vascularized lung-on-chip system in a single step [64]. PCL bioink was printed to create a frame of channels and chambers on printed PDMS substrate, which was populated with endothelial cell bioink and lung fibroblast cell bioink via bioprinting, followed by printing PDMS to fix the frame. The lung-on-chip model was successfully used to recapitulate inflammatory response.

#### 4.5. Gut-on-Chip

Gut is a vital organ with a complex architecture involving multiple diverse cell and tissue types [77–79]. Gut-on-chip systems can help in learning about different gastric and intestinal cells and can contain gut microbiota to mimic a dynamic gut environment [20]. In order to better mimic one aspect of the complex architecture of gut, Kim et al. used bioprinting to fabricate a 3D intestinal villi model with capillaries [60]. In this study, bioink containing epithelial cells was printed as a layer of core region, and bioink containing adenocarcinoma cells was printed as a layer of shell region to form a mesh structure on which villus structure was printed vertically. The successful cellular proliferation of both types of cells signified the establishment of cell–cell interactions.

#### 4.6. Bone-on-Chip

Osteoporosis is a disease caused by impaired bone turnover, which increases the risk of bone fracture in men and women over the age of 50. The drugs currently used to treat osteoporosis pose problems like elevated risk of cancer, stroke, and blood clots with long-term use [80]. Due to the prevalence of osteoporosis, a drug development platform that enables the analysis of the long-term physiological response of drugs is needed. Emerging bone-on-chip systems can help in expediting the process of drug development [81]. Lee et al. used inkjet bioprinting to print micropatterns containing the antibiotic rifampicin and biphasic calcium phosphate nanoparticles dispersed in a poly(lactic-co-glycolic acid) (PLGA) matrix on a glass slide, which was integrated with microfluidic chip [69]. The microfluidic chip was seeded with osteoblast (bone) cells. The study concluded that inkjet-printed micropatterns promoted osteogenic development by osteoblasts and prevented bacterial infection.

#### 4.7. Vessel-on-Chip

Proper nutrient and oxygen supply is crucial for ensuring long-term cell viability in complex multi-tissue models, which makes developing vascular network crucial in organ-on-chip systems [82]. Lee et al. used inkjet bioprinting to create a vascular channel on a flow chamber [57]. In order to fabricate vasculature, gelatin bioink containing endothelial cells was printed on layers of collagen matrix in a straight channel form, and collagen was printed over the gelatin pattern. The endothelial cells attached to the inner surface of the channel during incubation and subsequently the gelatin in chamber was flushed after being liquefied to create a channel. It was noted that cells proliferated, and successful gene expression analysis revealed that this method can be used to create vessels in organ-on-chip systems.

In a similar study, Abudupataer et al. used extrusion-based bioprinting to fabricate a vessel-on-chip system [63]. Two layers of bioink containing endothelial cells and muscle



cells were printed on chip (fabricated with PDMA), and after the cells proliferated, a continuous flow of growth medium was perfused in the channel of the chip to mimic blood flow in the vessel. Such a vessel-on-chip model can be used to study the pathogenesis of disease and drug screening.

In another study, Kolesky et al. used extrusion-based bioprinting to print a vascular system using a sacrificial bioink, composed of Pluronic and thrombin [56]. The sacrificial bioink was printed in a crosshatched pattern with a thickness of 1 cm. Bioink containing endothelial cells, muscle cells and dermal fibroblast cells was printed around the sacrificial bioink. Removal of the fugitive ink resulted in the formation of a connected network of vessels that supported endothelialization and retained cell viability up to 95% post printing, demonstrating that such a vascular system can potentially be used to construct vessels in organ-on-chip models.

In an analogous study, Schöneberg et al. used inkjet bioprinting to print a multi-layer vasculature imitating in vivo blood vessels [59]. On a custom-made bioreactor, a layer of fibrin bioink with muscle cells and fibrinogen-collagen with crosslinker thrombin was printed on a sacrificial gelatin core containing epithelial cells, which was flushed out to create open channels. High cell viability after printing was noted along with protein expression, indicating that the system has necessary biological functionality to potentially be used in vessel-on-chip systems as a platform for pre-screening of drugs. In yet another study, Gao et al. used extrusion-based bioprinting to print a vascular structure with multilevel fluidic channels (macro-channel and micro-channel) which can potentially be integrated into organ-on-chip systems to better simulate the micro-environment of blood vessels [52].

Zhang et al. used extrusion-based bioprinting to fabricate a thrombosis-on-chip system [66]. Thrombosis is the formation of a clot in a blood vessel. In this study, a scaffold was printed with luronic, dehydrated scaffold was placed on PDMS mold filled with GelMA, and the scaffold was crosslinked followed by dissolution of sacrificial channels to produce construct with hollow channels. After the incubation of seeded endothelial cells in hollow channels, a solution of blood with added calcium chloride (to induce clotting) was injected to form a clot. The clot maturation and subsequent dissolution of clot on being treated with tPA (anti-clotting drug) demonstrated that such a thrombosis-on-chip system can be used for thrombosis drug screening.

#### 4.8. Tumor-on-Chip

Cancer is an umbrella term for a variety of diseases having the same underlying cause of unregulated division of cells, which can be a cause of morbidity and mortality. Cancer treatment is especially challenging due to different tumor characteristics (mass of tissue caused by unregulated division of cells) in different patients. Tumor-on-chip systems tackle this heterogeneity by enabling the development of patient-specific anti-cancer drugs targeted to treat the patient-specific tumor.

Yi et al. used extrusion-based bioprinting to fabricate a glioblastoma-on-chip system [70]. Glioblastoma (GBM) is an aggressive type of cancer that occurs in the brain or spinal cord. This tumor-on-chip system was fabricated by printing its chamber structures using silicon ink on a glass substrate, inside which bdECM (brain decellularized ECM) bioink containing epithelial cells was printed to construct the GBM-mimetic ring structure filled by printing bdECM glioblastoma cells, and the chamber was covered with a glass slip. The developed glioblastoma-on-chip system resisted concurrent treatment of chemoradiation and temozolomide (anti-cancer drug) in the same way as observed in cancer patients, demonstrating that such a tumor-on-chip system can be used to determine drug combinations for cancer treatment.

Hamid et al. used extrusion based bioprinting in combination with photolithography to fabricate a breast tumor-on-chip model [72]. Photolithography was used to fabricate the base of the chip using PDMS. SU-8 (epoxy-based photoresist material) was used to create internal micro-architecture of channels on the chip, followed by printing of human

breast adenocarcinoma cells in the micro-channels. Three chips with 300, 500, and 700  $\mu\text{m}$  pore sizes of channels were printed for purpose of comparative evaluation. Successful cell proliferation and metabolization of drug by cells demonstrated that such a breast tumor-on-chip can be used for investigating the efficacy of drugs.

In another study, the same research team used extrusion based bioprinting along with maskless lithography, a type of photolithography that does not use a static mask, to fabricate a co-culture tumors-on-chip system [73]. Maskless lithography was used to fabricate the base of the chip using PDMS, on which SU-8 was printed and channels were patterned using an ultraviolet light emitting head. The micro-channels were populated with liver cancer and breast cancer cells.

Mi et al. used inkjet bioprinting to fabricate a breast tumor-on-chip system [71]. Breast cancer cells and endothelial cells were printed on a PDMS chip (fabricated with soft-lithography). Post printing, the cells showed good cell viability and cell quality. A significant inhibition of tumor cell migration ability was observed when treated with paclitaxel (anti-cancer drug), demonstrating the effectiveness of such a tumor-on-chip system in aiding cancer research and for anti-cancer drug screening. Successful cell proliferation and cell integration in co-culture showed that such a co-culture tumors-on-chip system is viable for biological characterization.

Cheng et al. used extrusion-based bioprinting to print paper-based cancer tissue models which have the potential for application in the cost-effective fabrication of organ-on-chip models [58]. In this study, sacrificial petroleum jelly-liquid paraffin ink was used to print on bacterial cellulose hydrogel, the entire matrix was air-dried to form a paper-like membrane, and perfusable microchannels were obtained by removing the sacrificial ink using heat. The epithelial cells were seeded into the microchannels and cancer cells were seeded onto the surface of the paper-based device. It was observed that endothelial cells and tumor cells spread and proliferated, and cytotoxicity of cancer cells was observed on treatment with tamoxifen (anti-cancer drug), demonstrating that such paper-based models can possibly be used for producing tumor-on-chip systems for drug screening.

The available invitro anti-tumor drug screening strategies including 2D cell models are not able to mimic biological systems sufficiently since they lack true perfusion and draining microcirculation systems. Although current organ-on-chip systems are able to integrate perfusable blood vessels, only a few in vitro models are able to re-establish both blood and lymphatic vessel pair. Cao et al. fabricated a tumor-on-chip model by bioprinting a hollow blood vessel and lymphatic vessel pair which reproduced the microcirculation featuring both delivery and drainage routes to better mimic the transport kinetics of biomolecules and drugs [65]. A custom-made coaxial nozzle containing three injection channels was connected to an extrusion-based bioprinter and used to print the vessels. The bioink consisted of alginate, GelMA, photoinitiator, and PEGDA. PEGOA was extruded through the middle layer of the coaxial nozzle, while the  $\text{CaCl}_2$  solution was ejected through both the inner and outer layers to immediately crosslink the alginate and obtain tubular structures. Then GelMA, PEGDA, and PEGOA components were photo crosslinked by UV light, followed by immersion in ETA (Eicosatetraenoic acid) solution to remove the sacrificial alginate. The bioprinted vessel pair was embedded in a GelMA matrix inoculated with MCF-7 breast cancer cells to examine drug transport rate by studying the diffusion of FITC (staining agent for microscopy) through the system and to investigate the effect of Doxorubicin (a chemotherapy anti-cancer drug) perfusion on the MCF-7 cancer cells. It was observed that the permeability parameters of the bioprinted blood and lymphatic vessels in such a bioprinted tumor-on-chip system could be controlled by precisely tuning the composition of the bioink. The tumor-on-chip could meet different biological needs for delivery and drainage channels under various scenarios and offer a convenient method for in vitro drug screening.

## 5. Challenges and Future Directions

There are several challenges associated with use of bioprinting technology for organ-on-chip system fabrication. The resolution that can be achieved with available 3D bioprinters is not as good as that achievable by traditional organ-on-chip fabrication techniques like soft-lithography. Although there are a few bioprinting systems that can achieve micron-level resolution [83], their high cost makes them unattractive options compared to traditional soft-lithography and photo-lithography systems to achieve same level of resolution. Bioprinting systems have a relatively low throughput for manufacturing organ-on-chip systems in large scale production settings. There are certain stereolithography printing systems that can achieve reasonable throughput suitable for low and medium volume production [15], but they cannot compete with other techniques like injection molding [84] when it comes to large scale manufacturing of organ-on-chip systems.

The properties of bioink materials for fabricating organ-on-chip systems still need improvement. The soft-lithography has been used widely for fabricating organ-on-chip systems due to the optimal properties of PDMS material, including transparency, biocompatibility, flexibility, gas-permeability, relatively low-cost, and high shape fidelity [10]. It is challenging to find a biomaterial with all these properties. The vasculature fabricated using bioprinting has a limited resolution. With soft-lithography, the patterning of micro-channels with resolution of 5 microns is standard while sub-micron vasculature fabrication is also possible. However, the highest resolution of microchannel printed with stereolithography is 100 microns [85].

Organ-on-chip systems fabricated with bioprinting have potential for commercial applications if sophisticated bioprinting techniques are developed which are capable of bioprinting organ-on-chip systems in a scalable, accurate, and high-throughput manner. The combination of bioprinting techniques can enable creation of bioprinting process that overcomes the limitations of individual bioprinting techniques [19]. This viable strategy needs further systematic investigations. Another area that needs further investigation is the integration of biosensors in the organ-on-chip systems fabricated with bioprinting [86,87]. This integration can enhance the functionality of organ-on-chip systems to allow the monitoring of cell behavior in dynamic and controlled environments, and to monitor cell behavior and microenvironment.

Drug-screening can potentially become much more accurate if the complex organ systems of the human body, containing multiple organs, can be accurately reflected on organ-on-chip systems. There is a need to investigate multimaterial bioprinting from the perspective of printing multiple tissues in the same organ-on-chip systems that can enable printing of such organ-on-chip systems. Currently, most of the reported studies either focus on demonstrating the application of developing novel bioprinting strategies for fabricating functional organ-on-chip systems [53,57,66,74,79,80] or provide proof-of-concept applications of using organ-on-chip systems fabricated with bioprinting for drug screening [54,56,60,69,75,78]. There are very few studies that have investigated optimization strategies for bioprinting process parameters in a systematic way. For the successful application of bioprinting in fabricating organ-on-chip systems, standardization and optimization of the printing process is necessary. Therefore, there is a need to investigate the relationship between different process parameters and functions in the bioprinting process of printed organ-on-chip systems.

## 6. Conclusions

The use of bioprinting for fabricating organ-on-chip systems has the potential to expedite the drug screening process while minimizing the cost investment in drug development. While research regarding the use of bioprinting techniques to fabricate organ-on-chip systems is still in the early stages, the reviewed studies demonstrate that bioprinted organ-on-chip systems are able to functionally mimic in vivo environments and provide nearly accurate drug responses comparable to animal and in vitro models. In addition, the reviewed studies show that using bioprinting for fabricating organ-on-chip systems possesses

advantages over traditional techniques of fabricating organ-on-chip systems, including soft-lithography and photolithography, in terms of automation, cost, time-consumption, and design modifications. However, from the manufacturing perspective, for bioprinting to become the norm in fabricating organ-on-chip systems for the purpose of drug screening, investigations need to be conducted regarding the improvement of resolution, vasculature fabrication, process optimization, and process standardization. Nevertheless, with further advancements in bioprinting processes, it is expected that bioprinted organ-on-a-chip systems will be used broadly in disease modeling and drug development.

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