



# Microphysiological Systems: automated fabrication via extrusion bioprinting

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**Abstract:** Microphysiological systems (MPS) offer great potential for improving pre-clinical testing for pharmaceutical treatments and novel therapies. These advanced *in vitro* models are designed to recapitulate the basic functions of living tissues or organs through dynamic culture and biomimetic microarchitecture. Increasing advancements in MPS design require advanced fabrication methods, such as extrusion bioprinting. Extrusion bioprinting presents the ability to automate fabrication of these systems in a simplified, one-step process, while providing the ability to fabricate complex, reproducible designs that incorporate dynamic culture, 3D microtissues and integrated sensors for analysis into a single system. This work reviews the main components that constitute these systems, current state-of-the-art MPS fabricated via extrusion bioprinting, and future considerations for the development of MPS.

**Keywords:** Extrusion bioprinting; biofabrication; Microphysiological systems (MPS); 3D culture

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## Introduction

In 1965, Gordon Moore predicted computing would increase in power and decrease in cost at an exponential rate. While this law has continued to uphold in computing, advances in drug development have followed a troubling alternative trend, referred to as EROOM's law (1). Since 1950, the number of novel treatments approved per billion US dollars spent on R & D has halved approximately every 9 years (1). A large factor in EROOM's law is the high failure rate of potential therapies in clinical trials, highlighting the ineffectiveness of current *in vitro* testing methods. Many current *in vitro* models use static 2D cultures that bear little resemblance to native tissue environments. In 2015, less than 12% of drugs entering clinical trials resulted in an approved medicine, costing millions of dollars and exposing patients to harmful side effects (2). MPS models have provided

great promise for improving the rate of innovation in drug development and off-setting EROOM's law through more physiologically relevant *in vitro* models (3). These systems are designed to recapitulate the functions of living tissues or organs through relevant microarchitecture and dynamic stimulation of cultures (4).

The fabrication of these devices involves two main parts: microfabrication of the tissue cultures and design of the microfluidics, sensors and other dynamic systems for the delivery of nutrients, chemicals, biological factors and mechanical or electrophysiological signals in a controlled microenvironment. Several techniques are available for this fabrication process, including photolithography, soft lithography, dry etching, sputter coating, chemical vapor deposition and stereolithography (4). While each of these fabrication methods provides individual advantages, many involve non-biocompatible hazardous reagents and require

laborious multi-step processes (4).

Recently, extrusion biofabrication methods have been applied for the fabrication of MPS. Bioprinting technologies allow scientists to pattern cell types and create more complex tissue structures, creating more physiologically relevant models useful in translational research (5). This biocompatible process allows the potential for a single-step, automated fabrication of MPS, creating a more efficient fabrication process. Additionally, bioprinting allows for the integration of 3D tissue designs within MPS devices, as opposed to 2D cultures often used with other methods. This review article details design components and considerations for these devices, recent advances in MPS fabricated through extrusion biofabrication and future considerations for this technology.

### 3D biofabrication technologies

Advantages of 3D culture, such as cell adhesive sites in three dimensions and tunable mechanical properties, became apparent with the development of spheroid cultures in the 1970s (6). As research on 3D cultures progressed, technologies were developed for increased control over the 3D microenvironment. The development of the first 3D bioprinter in the 1990s allowed scientists to design more intricate 3D microarchitectures (7,8). Since the 1990s, biofabrication technologies have become increasingly complex. Today there are a variety of biofabrication methods available, which can be split into two general groups: jetting-based and extrusion-based bioprinting (9).

Jetting-based bioprinting involves non-contact techniques to fabricate 2D or 3D structures using picolitre bioink droplets layered onto a substrate (9). Common jetting-based methods include piezoelectric, thermal, pneumatic and laser-assisted technology (9). Piezoelectric actuators apply a piezo-crystal pulse to eject a small droplet of a bioink (7,9). Thermal methods create bioink droplets by locally increasing temperature within the bioink compartment to generate a bubble (9). Pneumatic pressure and valves generate droplets through opening and closing of microvalves (9). Focused laser energy methods generate vaporization through laser systems to produce small droplets (9). Jetting-based technologies offer advantages such as high resolution ranging from 20–100 µm, but are limited to low viscosity bioinks and have a long processing time, limiting the size of structures that can be created with these methods (7,9).

Extrusion-based bioprinting dispenses continuous

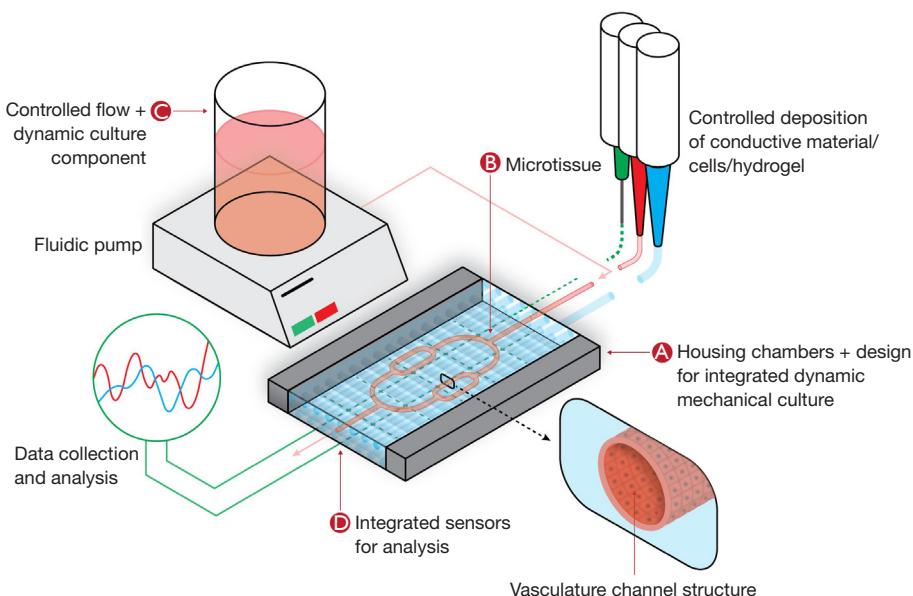
filaments of bioinks through micro-nozzles to build 2D or 3D structures (9). These methods dispense bioinks through pneumatic pressure or syringe pumps (9). The speed and volume of material dispensed can be adjusted by controlling the pressure level or displacement of the piston or pump (7,9). While these methods offer lower resolution compared to jet-based technologies, they allow for the fabrication of constructs with clinically relevant sizes in a realistic time-period and provide versatility in material compatibility. Because of these advantages, extrusion-based bioprinting is often regarded as the most promising and is the most common biofabrication method used to develop 3D microphysiological systems (MPS) (4,7,10,11). A more detailed review on various biofabrication technologies can be found in Seol *et al.* (9).

### MPS design components and considerations

The components of MPS can be broken into four key elements: a microfluidic chip or culture chamber, live microtissues, components for dynamic culture and sensors for results readout (12). A visualization of these systems is demonstrated in *Figure 1*.

#### *Microfluidic housing/culture chamber*

The microfluidic chip contains the housing, or culture chamber, for the live microtissues and often connects this chamber to a dynamic flow system for perfusion of nutrients, bioactive molecules and drug compounds to the live microtissues. Ideally, a culture chamber provides a sterile environment that can be fabricated or molded into custom geometries and can be easily analyzed noninvasively. For this portion of the device, many microfluidics have utilized silicone-based inks such as poly(dimethylsiloxane) (PDMS), which are highly compatible with a variety of fabrication methods and provide optical clarity, low cost and high reproducibility (13). However, PDMS comes with some significant disadvantages for MPS including deformation, evaporation, absorption, leaching and hydrophobic recovery (13–15). The most prominent disadvantage of this material for use in MPS is the absorption of proteins, drugs and hydrophobic molecules, complicating the pharmacokinetic and pharmacodynamic models of drugs. A variety of alternatives to PDMS have been developed, with some of the most promising including acrylonitrile butadiene styrene (ABS), poly(lactic acid) (PLA) and polycaprolactone (PCL). These materials, which overcome the disadvantages



**Figure 1** Components of MPS. The components of MPS can be broken into four key elements: (A) the housing, or culture chamber; (B) the live microtissue, the primary component of any MPS; (C) controlled flow and dynamic culture components for applied mechanical, shear and electrical forces as well as (D) integrated sensors for real-time analysis of constructs. MPS, Microphysiological systems.

**Table 1** Materials for microfluidic housing

Material	Advantages	Disadvantages	Ref.
PDMS/silicone-based inks	Compatible with variety of fabrication methods; optically transparent; non-degradable	Deformation; absorption of hydrophobic drugs; leaching; hydrophobic recovery	(10,15-19)
PCL	Biocompatible; less prone to bulk absorption of hydrophobic drugs; low melt temperature; rigid	Not optically transparent	(20,21)
ABS	Biocompatible; less prone to bulk absorption of hydrophobic drugs; rigid	Not optically transparent; high melt temperature	(15)
PLA	Biocompatible; less prone to bulk absorption of hydrophobic drugs; rigid	Not optically transparent; high melt temperature	(15)

The microfluidic chip contains the housing, or culture chamber, for the live microtissues and often connects this chamber to a dynamic flow system for perfusion of nutrients, bioactive molecules and drug compounds to the live microtissues. While PDMS is the most common material used for this component, disadvantages including absorption of hydrophobic drugs have led to the development of alternative housing materials, including photocurable resins, PCL, ABS and PLA (18). PDMS, poly(dimethylsiloxane); PCL, polycaprolactone; ABS, acrylonitrile butadiene styrene; PLA, poly(lactic acid).

of deformation, absorption, leaching and hydrophobic recovery with PDMS, can be used with transparent glass or polystyrene substrates to fabricate chambers for MPS. Additionally, because of their printability, these materials can be used in extrusion biofabrication platforms for one step biofabrication of both the culture chamber and the live microtissues. A summary of these materials, along with advantages and disadvantages, is presented in *Table 1*. Many

recent publications have attempted to move away from the use of PDMS towards these alternative materials.

### Live microtissues

The primary component of any MPS is the live microtissue. Live microtissues can vary in complexity, from simple 2D cultures along the walls or grooves of the microfluidic chip

to intricate, multicellular 3D microarchitectures. Extrusion biofabrication offers the capability to create both simple, uniform cell layers and more complex 3D architectures. A variety of tissue types have been modeled as MPS through extrusion bioprinting, some of which are detailed in Section IV below and in *Table 2*.

### 3D Microtissues

Biofabrication of MPS allows for spatial control of cell-laden materials to mimic multi-cellular microphysiological architectures. A variety of materials can be used for this purpose. Briefly, common materials used in 3D microtissues for cell deposition include naturally-derived and synthetic hydrogels. While some MPS utilize scaffold-free cell slurries, matrix bioinks allow for enhanced resolution and control over cell deposition. Additionally, use of matrix bioinks allows for control over substrate mechanical properties and biological factors. This material and cell selection are not the primary focus of this review, although more can be learned from our previous publication on this topic (8). Some examples of matrix bioinks used in MPS can be found in *Table 2*.

### Channel fabrication in live 3D microtissues

Vasculature is a major component of many tissues and is critical in modeling physiologically relevant *in vitro* models. Vasculature is particularly important for drug screening technologies, as many compounds are injected directly into the blood stream. Many MPS incorporate some type of channel to mimic vascular flow, ranging from simple straight channels to more complex branched features.

Extrusion biofabrication offers a variety of methods for incorporation of microchannels to apply flow and recapitulate vasculature structures within microtissues (4,11,22,24). Often, sacrificial bioinks are used to fabricate complex channels within a microtissue. Some common materials used for this purpose are pluronic F127, agarose, gelatin and carbohydrate glass (8). Bioinks such as pluronic, agarose and gelatin offer a thermal reversible gelation that allow the materials to be printed at room temperature then washed away either through heating or cooling. Bioinks such as carbohydrate glass offer increased mechanical stiffness compared to other softer sacrificial materials that allow for easier construction of more complex designs (8).

Temporary support baths utilizing the sacrificial material gelatin have also demonstrated great promise for the fabrication of microtissues with complex channels (8). The FRESH method, further described in a previous

publication, provides temporary support of soft, fragile bioinks during fabrication. This method has not yet been used for the fabrication of MPS, but has great potential for the automation of highly complex vasculature (8,25).

### **Dynamic culture: flow, mechanical and electrical stimulation**

MPS may also contain extra components to stimulate flow or incorporate additional mechanical or electrical stimulation of tissues during culture.

### **Stimulating flow: pumps for MPS**

To create better *in vitro* models of dynamic *in vivo* cellular systems, pumps are often used to expose cells to flowing media, as compared to static *in vitro* cultures. Pumps can also act as an automated way to change cell media, and can be either “closed” (recirculating liquid) or “open” (refillable main liquid reservoir) systems. The application should determine the type of pump used, as different types offer various advantages and disadvantages. Some characteristics to consider are flow rate, flow type (pulsatile or constant), duration of flow, mechanical effects, and electrical requirements. The most common pumps used in MPS are gravity-driven flow, peristaltic pumps, syringe pumps and electroosmotic pumps (*Table 3, Figure 2*). For a more comprehensive review on pumps, refer to reference (26).

#### **Gravity-driven flow**

Gravity-driven flow is a passive flow technique that is simple to implement, as it requires no mechanical parts or power. It is comprised of only two liquid reservoirs with a height difference between them, creating a flow of liquid from the higher reservoir to the lower one (*Figure 2*). In a closed system, the flow rate will decrease as the height difference between the reservoirs decreases. However, this effect can be minimized by orienting the reservoirs horizontally (*Figure 2*). In an open system, refilling the higher reservoir will also negate this problem and prolong the flow. While gravity-driven pumps are only able to produce a constant flow rate, the simplicity of these pumps can be beneficial in complex applications where tubing and multiple pumps would otherwise be needed.

#### **Peristaltic pumps**

Peristaltic pumps are a type of active, positive-displacement pump that results in sinusoidal, pulsatile flow (24,26). With these systems, liquid is pushed through a tube that is wrapped around rotors that are turned by a rotating shaft (*Figure 2*). Peristaltic pumps are very versatile as they are

**Table 2** MPS fabricated via extrusion bioprinting

Tissue/disease model	Cell type(s)	Materials	Housing material	Length of culture	Analysis	Culture method	Ref.
Liver	HepG2/C3A Spheroids	GelMA	PDMS/PMMA and glass slide bottom	30 days	Hepatocytes responsive oxygen concentration and shear stress; compared to static culture spheroids in GelMA; acetaminophen exposure	Bioreactor platform (custom design) with peristaltic syringe pump	(10)
Liver	HepG2, HUVECs	Gelatin and 2% Collagen type I	PCL with glass slide bottom	6 days	Quantitative analysis of albumin and viability	Perfusion with peristaltic pump	(20)
Liver	Cryopreserved hepatocytes spheroid; Zucker fatty rat hepatocytes spheroids	Scaffold-free	PDMS/PMMA and glass slide bottom	77 days, 23 days (rat)	Measured gene expression, glucose production response to insulin, and bile acid secretion	Cultured in perfusion chamber for 4 days with syringe pump, then cultured with moderate rocking or shaking for more than 2 weeks	(19)
Heart	HUVECs (bioprinted); neonatal rat cardiomyocytes hiPSC-CMs	Alginate, GelMA	PDMS/PMMA gaskets	33 days	Live/dead staining; quantified beating of cardiomyocytes observed with optical microscope	Perfusion with peristaltic pump	(17)
Heart	hiPS-CMs, NRVMs	Dextran ink, TPU ink, CB: TPU ink, Ag: Pa ink	Soft PDMS ink, Rigid PDMS ink, PLA, ABS	28 days (hiPS-CMs)	Quantified resistance changes through custom connected device; response to isoproterenol and verapamil dosing on tissues	Static culture with built in sensors for electronic readout of contractile stresses	(15)
Kidney	Human immortalized PTECs, A498 renal cancer cells, HNDF	Fugitive pluronic, gelatin-fibrin matrix	Silicone elastomer with glass base	65 days	Albumin uptake; response to cyclosporine A	Perfusion of continuous, unidirectional flow with peristaltic pump	(11)
Nervous System	Schwann cells, hippocampal neurons cell suspensions	Scaffold-free	Silicone, Polycaprolactone in combination with glass dishes	14 days	Response to infection of PRV	Static culture	(21)
Vessels (thrombosis model)	HUVECs, HNDFs	GelMA, pluronic F127	Petri dish	7 days	Formation of thrombi; response to thrombolytic agent	Perfusion with syringe pump	(22)
Cancer	Human breast adenocarcinoma cell line (MDA-MB-231)	Scaffold-free	PDMS	14 days	Cell proliferation and morphology	Perfusion with syringe pump	(18)

**Table 2 (continued)**

**Table 2 (continued)**

Tissue/disease model	Cell type(s)	Materials	Housing material	Length of culture	Analysis	Culture method	Ref.
Lung	Human alveolar epithelial type II cell line A549, EA.hy926 hybrid human cell line (human umbilical vein endothelial cells fused with A549 cells)	BD matrigel BM matrix growth factor-reduced	Millicell-CM organotypic tissue culture plate inserts	3 days	Comparison of manually seeded and bioprinted monocultures; immunofluorescent imaging, histological staining and brightfield microscopy	Static culture on Millicell-CM organotypic tissue culture plate inserts	(23)
Brain	Mouse brain endothelial cell line (bEnd.3)	Collagen hydrogel	PMMA plate with glass bottom	21 days	Characterization of cell adhesion and construct permeability, diffusivity; disruption of barrier with mannitol	Laminar flow with syringe pump (0.1 mm/s)	(16)

A variety of tissue models have been developed via extrusion bioprinting. This table details some of the MPS fabricated with this method, including the cell types, microtissue materials, housing materials, length of culture, analysis and culture methods utilized in each model. HNDF, human neonatal dermal fibroblasts; PMMA, poly(methyl methacrylate); PDMS, poly(dimethylsiloxane); PCL, polycaprolactone; ABS, acrylonitrile butadiene styrene; PLA, poly(lactic acid); MPS, Microphysiological systems; GelMA, gelatin methacrylate; HUVECs, human umbilical vein endothelial cells; NRVMs, neonatal rat ventricular myocytes; hiPS-CMs, human induced pluripotent stem cell derived cardiomyocytes; TPU, thermoplastic polyurethane; CB, carbon black nanoparticles; BD, Becton Dickinson; BM, basement membrane.

**Table 3** Pumps for MPS culture

Pump	Advantages	Disadvantages	References
Gravity-driven flow	Simple, inexpensive; no power required; prevents air bubble formation; works in open or closed systems	Cannot produce pulsatile flow; difficult to control	(26,27)
Peristaltic pumps	Produces pulsatile and reversible flow; works in open or closed systems	Power required; mechanical parts can have large footprint	(24,26,28)
Syringe pumps	Produces pulsatile or constant flow; fine control of flow	Power required; mechanical parts can have large footprint; only works in closed systems	(22,29,30)
Electroosmotic pumps	No mechanical parts; reversible flow	Power required; high voltages can cause cell lysis; only works in open systems	(27,31)

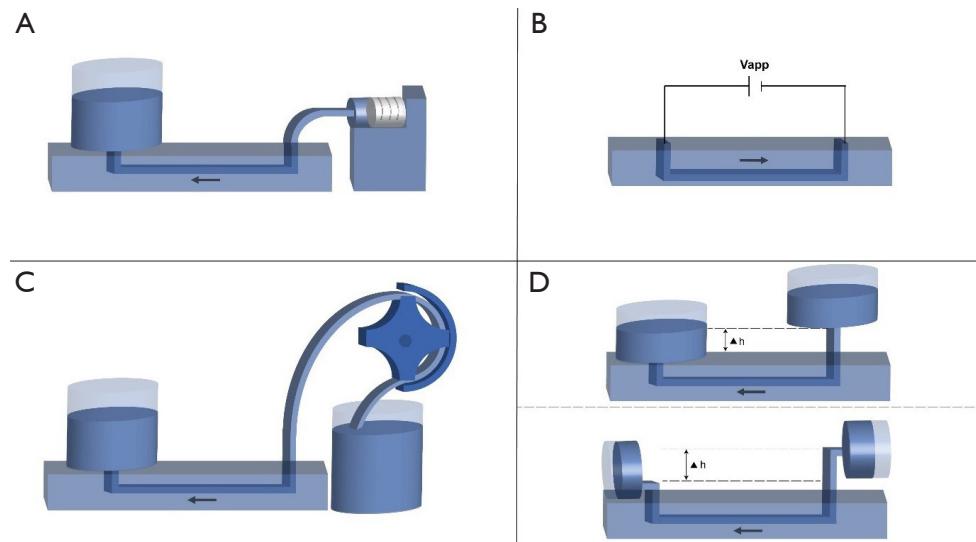
To create better *in vitro* models of dynamic *in vivo* cellular systems, pumps are often used to expose cells to flowing media, as compared to static *in vitro* cultures. Common pumps used in these devices include gravity driven flow, peristaltic pumps, syringe pumps and electroosmotic pumps. MPS, Microphysiological systems.

bi-directional, have large ranges of flow rates, and can be used as either closed or open systems. However, peristaltic pumps require power and have mechanical components that can wear with time. Additionally, these systems can have a large footprint, which brought about the push for smaller pumps such as the peristaltic micropump (28). Wagner *et al.* utilized a peristaltic micropump in a multi-organ-chip system that was integrated directly onto the MPS (32). This

pump, no more than a few millimeters in width, provided pulsatile flow at a circulation rate of 40  $\mu\text{L}/\text{min}$  to two culture compartments (32). Continued development and miniaturization of peristaltic pumps provides great promise for the advancement of MPS.

#### Syringe pumps

Another type of active, positive-displacement pump is the syringe pump (22,29,30). These pumps utilize a motor-



**Figure 2** Pumps for applied flow [adapted from (26)]. To create better *in vitro* models of dynamic *in vivo* cellular systems, pumps are often used to expose cells to flowing media. The most common pumps used in MPS are syringe pumps (A); electroosmotic pumps (B); peristaltic pumps (C); and gravity-driven flow (D). Gravity-driven flow systems can be set up with reservoirs oriented vertically (top) or horizontally (bottom). Ah, change in height; Vapp, voltage applied; arrows indicate direction of flow. MPS, Microphysiological systems.

driven piston to create either pulsatile or constant flow stemming from a syringe full of liquid (*Figure 2*). Since all liquid must originate from the syringe, it is a closed system. This can become an obstacle in longer term projects where the piston syringe would need to be refilled. However, these pumps offer significant control over flow, making them very popular and widely available.

#### Electroosmotic pumps

As opposed to many other pumps, electroosmotic pumps are not mechanical, meaning that they have no moving parts. Instead, these pumps use electricity for active flow generation in microchannels in an open system. A surface-ionizable material, such as silica, becomes negatively charged due to deprotonation (31). This charge attracts cations, which form a “positively charged solution layer” close to the channel’s surface. When an external electric field is applied, the cations move, pulling the solution with them. The opposite can be done with a positively charged surface. Electroosmotic flow (EOF) is simple but requires caution, as electric fields above 1 kV/cm can cause cell lysis (27).

#### Electrical stimulation and integrated sensors

A variety of biocompatible conductive materials have been used to provide electrical stimulation to microtissues (33,34). Electroactive biomaterials have the ability to deliver electrical, electrochemical and electromechanical

stimulation to cells and can also be used to create integrated sensors in MPS (35). Bioprinting provides the opportunity to incorporate these materials directly into microtissues and MPS. Some common conductive materials utilized in bioprinting include gold, graphene, carbon and silver-based inks as well as electrically conductive polymers such as polymer polypyrrole (PPy).

Gold nanoparticles are commonly incorporated into biomaterials for a variety of applications and offer an array of benefits, including high surface energy, high conductivity, and biocompatibility (36). Gold-based materials have been utilized for the fabrication of biosensors and have been incorporated into live microtissues for functionalization and electrical stimulation. A gold nanorod-incorporated (GNR) gelatin methacrylate (GelMA)-based ink was used to print functional cardiac tissue constructs. These GNR constructs demonstrated increased cell adhesion and organization as well as promotion of synchronized contraction of cardiac cells compared to non-GNR constructs (37).

Due to its unique structure, graphene provides a variety of advantages including high fracture strength, electrical and thermal conductivity, fast mobility of charge carriers and high biocompatibility (38). This material was incorporated into a GelMA-based ink to fabricate graphene nanoplatelets for nerve tissue regeneration (39). The conductive substrate provides cues to developing cells

to reinforce electrical connections and promotes formation of a neural network (39).

Carbon-based inks are another popular material for biofabrication due to their electrical conductivity and mechanical properties. Several carbon-based bioinks have been fabricated over the past year, but a particularly promising one from Adams *et al.* demonstrates a biocompatible carbon-nanotube ink that can fabricate 3D functionalized structures (40). This bioprinted MPS demonstrated high cell attachment and differentiated actin cytoskeletal structures when electrical impulse was applied compared to unstimulated scaffolds (40).

Recently, electroconductive materials have also been used in MPS to incorporate sensors for automated, quantifiable results readout. Extrusion biofabrication presents the opportunity to fabricate these sensors directly into the live microtissues, as described in the cardiac MPS from Lewis *et al.* shown in Section IV and *Figure 3* below (15). This system utilized a silver particle-filled, polyamide (Ag:PA) ink to fabricate electrical leads and contact pads, as well as a TPU-CB-TPU (TPU, thermoplastic polyurethane; CB, carbon black nanoparticles) ink within the structures (15). These sensors allowed for noninvasive analysis of the MPS (15).

Electroactive biomaterials have also been used for mechanical stimulation in MPS. Svennersten *et al.* utilized a PPy, which exhibits good biocompatibility, chemical stability and high conductivity, to fabricate a microactuator system on a chip that delivered mechanical stimulation to renal epithelial cells (35,41). This stimulation resulted in increased internal calcium levels, a documented response to mechanical stimulation (35).

### Mechanical stimulation

Many tissues are exposed to continuous mechanical stimulation *in vivo*. To properly mimic this environment, many MPS aim to include mechanical stimulation (42,43). In addition to use of electroactive biomaterials, this stimulation can also be achieved through application of uniaxial, cyclical, or biaxial strain.

Huh *et al.* successfully applied uniaxial strain in a lung on a chip model through generation of displacement along one axis (43,44). This model incorporated 3D microarchitecture and dynamic mechanical activity to recapitulate the function of the alveolar-capillary interface (44). In a different study, cyclical stain was used to promote differentiation of murine embryonic stem cells into cardiomyocytes, demonstrating upregulation of  $\alpha$ -MHC in mechanically stimulated

embryoid bodies (43). While sometimes more complex than uniaxial or cyclical strain, biaxial strain has high potential for high-throughput devices and can be incorporated in a variety of ways. Biaxial strain was applied with a stretching device and applied vacuum chamber on a lung-on-a-chip device (43). Alternatively, another model designed wells with a deformable bottom to apply biaxial strain to cardiac progenitor cells, demonstrating a successful activation of  $\beta$ -catenin (43).

Mechanically active MPS have the potential to reproduce dynamic physical forces that are critical for organ function and disease development. To effectively recapitulate *in vivo* environments, careful consideration should be made in designing these forces. A more comprehensive review of these forces and methods to apply them can be found in reference (45). Biofabrication platforms have the potential to simplify the fabrication of these incorporated components through the automation of chamber designs used to apply these forces.

### MPS fabricated via extrusion bioprinting

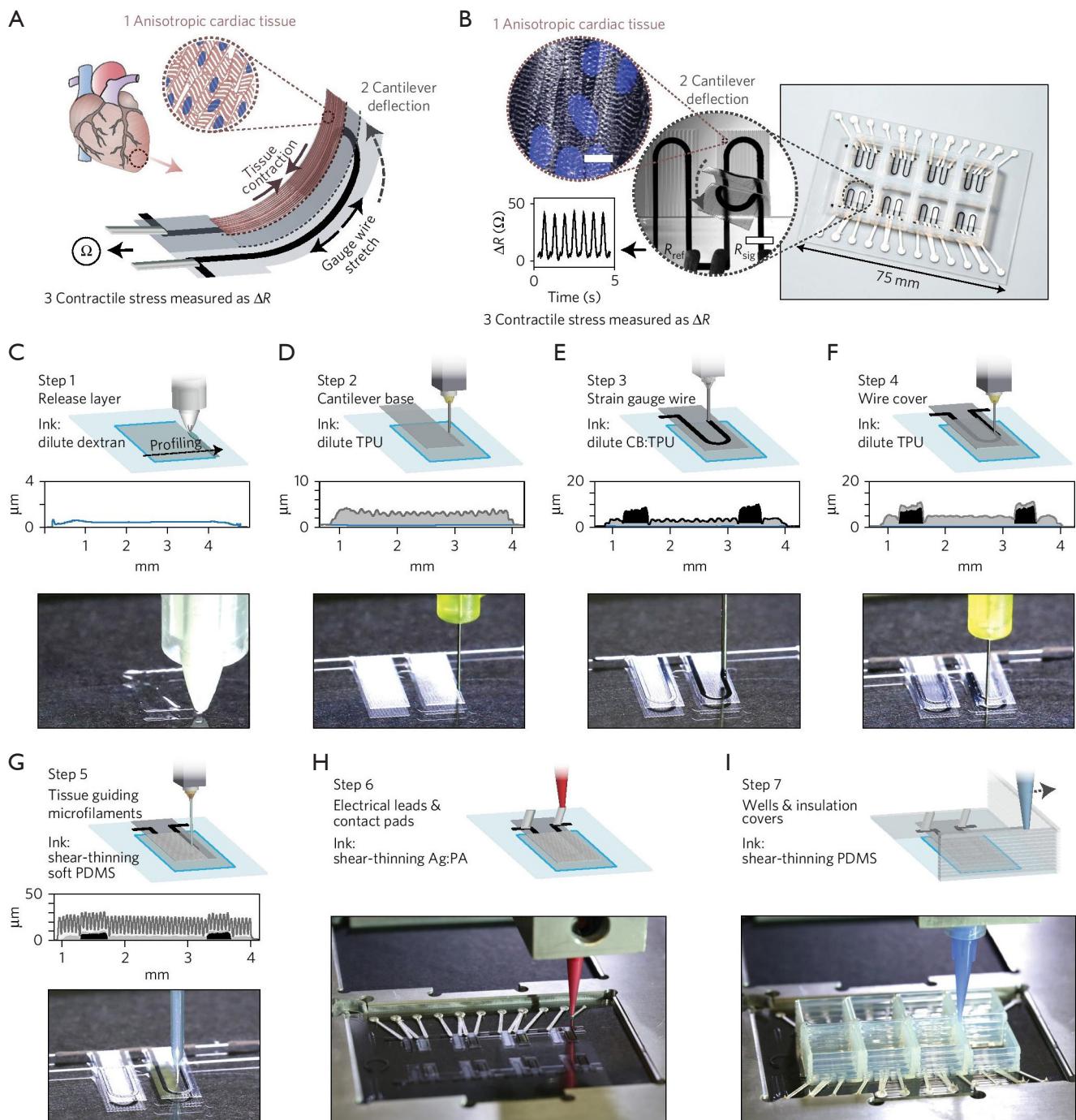
Utilizing the components described above, many MPS of varying tissues have been fabricated through extrusion biofabrication. Some of these models are summarized in *Table 2*.

### Brain-on-a-chip

The blood-brain barrier (BBB) plays an integral role in regulating transport phenomena of small- and macromolecules as well as maintaining homeostasis of the brain microenvironment (16). Dysfunction of the BBB is associated with a variety of neurological disorders, including neurodegenerative disease and ischemia, and as such, there is a high interest in developing more effective *in vitro* models of this system (16). Kim *et al.* developed a model by 3D co-printing a polymeric frame with dissolvable poly(vinyl alcohol) to create a microvasculature structure to encase cell-laden collagen. This model was used to develop a time-dependent evolution of the barrier function for up to 3 weeks (16).

### Heart-on-a-chip

Cardiovascular disease prevails as the leading cause of death worldwide (46). Additionally, a large number of treatments for other diseases present cardiotoxic side effects, leading



**Figure 3** Cardiac microphysiological device principle and microscale 3D-printing procedure [adapted from (15)]. (A) Principle sketch of the device. Contraction of an anisotropic engineered cardiac tissue [1] deflects a cantilever substrate [2], thereby stretching a soft strain gauge embedded in the cantilever. This generates a resistance change proportional to the contractile stress of the tissue [3]; (B) the fully printed final device. Insert 1: confocal microscopy image of immuno-stained laminar NRVM cardiac tissue on the cantilever surface. Blue: DAPI nuclei stain, White:  $\alpha$ -actinin, scale bar 10  $\mu\text{m}$ . Insert 2: still images of a cantilever deflecting upon tissue contraction. Insert 3: example resistance signal; (C-I) automated printing of the device on a 2 inch  $\times$  3 inch glass slide substrate in 7 sequential steps. For each step, a corresponding still image from the printing procedure is displayed.

to withdrawal of therapies from the market (46). While a biomimetic cardiac model is clearly necessary for the advancement of medicine, the complex geometry and mechanisms of the heart is especially difficult to recapitulate and requires advanced fabrication methods.

Vasculature in cardiac models is necessary for the culture of thick cardiac tissue, but its complexity is not able to be replicated through traditional fabrication methods. Zhang *et al.* explored a novel strategy for fabricating endothelialized myocardial tissues via the use of extrusion bioprinting, microfluidics and stem cells (17). When combined with a microfluidic perfusion bioreactor, the bioprinted model remained viable for up to 15 days with perfusion, compared to significant cell death by day 7 without perfusion (17). This model was also tested with hiPSC-cardiomyocytes and with further development has a potential for use in personalized drug screening (17).

While 3D models provide enhanced physiological relevance, the increased complexity often causes challenges when analyzing tissue constructs. The Lewis and Parker group worked to integrate embedded sensors into a 3D bioprinted cardiac MPS for easier analysis of contractile forces of cells (15). Using a custom extrusion biofabrication platform, the group was able to sequentially print multiple functional materials for a one-step fabrication of a comprehensive chip to test contractile force and drug response of cardiac tissues (15). The constructs were fabricated with an array of PDMS wells as the microfluidic housing with a sandwich structure of TPU-CB-TPU printed inside each well. The electrical conductivity of the embedded CB structures is affected by strain and was used to measure the contractile forces of cells cultured on the constructs (15).

### *Kidney-on-a-chip*

While 20% of failures in Phase III clinical trials are caused from renal toxicity, only 2% of drugs tested in pre-clinical phases fail from this issue (11). Therefore, it is imperative to create more physiologically relevant 3D models of the kidney tissue to improve pre-clinical testing accuracy of therapeutics and drugs (11). The proximal tubule, responsible for 65–80% of nutrient absorption and transport from the renal filtrate to the blood, is the most frequently damaged site in the kidney from prescription drugs (11). Prior to the study by Homan *et al.*, models of the proximal tubule consisted of either 3D static cultures limited to 1 mm in size or perfusion models of 2D

culture (11). Through extrusion bioprinting, this group was able to fabricate complex 3D perfusable architecture that was cultured for over 2 months. The model was tested with a nephrotoxin, cyclosporine A, and demonstrated disruption of the epithelial barrier in a dose dependent manner (11). This model significantly enhanced epithelial morphology and functional properties relative to the same cell types grown on 2D controls or without perfusion. The automated, customizable, one-step fabrication method demonstrates the power of extrusion biofabrication for modeling MPS (11).

### *Liver-on-a-chip*

Drug-induced-liver-injury (DILI) is the most common reason for regulatory actions post drug approval, yet almost 50% of drugs that cause substantial DILI produce little or no toxicity in animal or preclinical models (47,48). In vitro liver models have increased in complexity over the past few decades, from simple organoids of hepatocytes to 3D patterned cells in microfluidic chambers (49). Comparisons of various models have demonstrated the superiority of 3D cultures as well as fluid flow over 2D, static cultures (48-50). Extrusion biofabrication presents the capability to create advanced 3D architectures and culture chambers in one step, and a variety of liver-on-chip models have been fabricated through this method (51-53).

Bhise *et al.* utilized extrusion biofabrication to control deposition, placement and thickness of spheroid-laden GelMA solutions to fabricate a 3D hepatic construct (10). Through bioprinting technology, variables such as spheroid concentration, hydrogel composition and geometry of printed constructs could be easily changed and controlled (10). The biofabrication process was designed to interact with a pre-fabricated bioreactor and culture chamber, which was used to provide a controlled flow rate of media to constructs at 200  $\mu$ L/h (10). Cultured spheroids maintained viability for up to 30 days of culture and demonstrated similar response to APAP treatment as animal models, demonstrating potential for its use as a model for drug toxicity analysis (10).

Lee *et al.* not only created geometrically complex multicellular constructs, but also utilized extrusion bioprinting for a one-step fabrication process of the construct and culture chamber (20). The group tested various geometries, including a 2D printed scaffold-free model, a single-cell 3D model and a multi-cellular 3D model, all encased in a 3D printed polycaprolactone or PDMS chamber (20). Results demonstrated lower protein absorption

in PCL chamber constructs compared to platforms fabricated with PDMS, and liver function was significantly improved in 3D models compared to 2D ones (20).

### Lung-on-a-chip

One of the most common methods of entry for drugs, pathogens and other agents into the human body are the lungs. Modeling this process via MPS is important for studying disease pathways and to analyze the transport of aerosol based drugs (49). Most systems aim to model the epithelial-endothelial layers that create a semi-permeable barrier that separate air and fluid channels (49).

The automation of fabricating simple cell layers through biofabrication was demonstrated by Horváth *et al.* by printing cell-laden Matrigel layers of human alveolar epithelial type II cell line A549 and hybrid human cell line EA hy926 onto transwell culture plate inserts (23). Compared to constructs fabricated via pipetting, the cultures fabricated via extrusion bioprinting demonstrated improved uniformity and tightness of mono- and co-cultures (23). This automated fabrication process, combined with pneumatic channels and cyclic wall stretching used in Huh *et al.*, described in Section III above, could create a truly superior model for analysis (44). Additionally, the chambers utilized in Huh *et al.* could be potentially bioprinted as well, creating a simplified, one-step fabrication process (44).

### Nervous-system-on-a-chip

Enhanced models of the nervous system are necessary to better understand the complex neurological phenomena and to develop effective treatment for neurological disorders. Johnson *et al.* developed a bioprinted nervous system model through fabricated PCL microchannels with compartmentalized grease and silicone chambers and cell suspensions of rat embryonic hippocampal neurons, rat embryonic sensory neurons, rat embryonic Schwann cells and porcine kidney epithelial cells (21). These components migrated throughout the channels to form an interconnected nervous system that was used to investigate the preferential transmission of pseudorabies virus (PRV) from the cell body of PNS, CNS or the terminal site (21).

### Vessels-on-chips

*In vitro* models focused on vascular-related diseases are

integral for developing improved treatments of these diseases. Thrombosis in particular is a leading cause for morbidity and mortality from cardiovascular diseases (22). Through the commercially available Allevi (previously BioBots) platform, Zhang *et al.* fabricated a fibroblast laden GelMA hydrogel with endothelialized channels and induced thrombi through the infusion of blood into the model (22). Migration of cells in response to the clot and the pathology of fibrosis were analyzed with this model. To form the vascular channels, Zhang *et al.* utilized pluronic F127, a sacrificial material that can be washed away after fabrication (22). This model is illustrated in *Figure 4* (22).

### Cancer-on-chips

In addition to healthy tissue models, there is also a large need for disease models for *in vitro* testing. Tumor MPS have the potential to offer more physiologically relevant models through tumor heterogeneity, vasculature incorporation and biomimetic spheroid structures (18). Bioprinting has demonstrated improvement over previous fabrication methods for cell deposition into microfluidic devices, and offers great potential for advanced cancer *in vitro* models (18).

### Multi-tissue platforms: towards “Body-on-a-chip” devices

The advancement of single MPS platforms has led to research on multi-tissue devices towards full “body-on-a-chip” or “human-on-a-chip” devices. These devices aim to create more systemic in-vitro models of metabolic pathways within the human body for more relevant pharmacokinetic and pharmacodynamic analysis.

One notable multi-tissue MPS fabricated through extrusion bioprinting is a three-tissue organ-on-a-chip model from Skardal *et al.* (54). To mimic inter-organ responses and create a multi-tissue biomimetic model, Skardal *et al.* developed a three-tissue MPS consisting of liver, heart and lung microtissues connected in a closed, circulatory perfusion system. The system demonstrated secondary toxicity in cardiac tissues likely caused from the release of inflammatory cytokines from lung microtissue after exposure to a known toxic compound bleomycin (54). Skardal *et al.* utilized biofabrication to construct liver structures and the PCL microchambers (54).

While few multi-tissue systems have been developed through biofabrication, there is high potential for advancement of multi-tissue systems with this method,

which lends easily to the scaled complexity of these systems (55,56). A more comprehensive review of other multi-tissue MPS can be found in reference (57).

### Discussion: current limitations and future considerations

Advances in biofabrication technologies, specifically in extrusion biofabrication, have led to the progression of increasingly biomimetic MPS. Extrusion biofabrication offers many advantages over other methods, including biocompatibility, the versatility to pattern a variety of materials, and the automated one-step fabrication of multi-material, complex microstructures. These advantages have led to the development of superior tissue models previously impossible to fabricate through other methods. These models incorporate 3D cell culture, advanced sensors for analysis and dynamic culture of systems through controlled flow and electrical and mechanical forces.

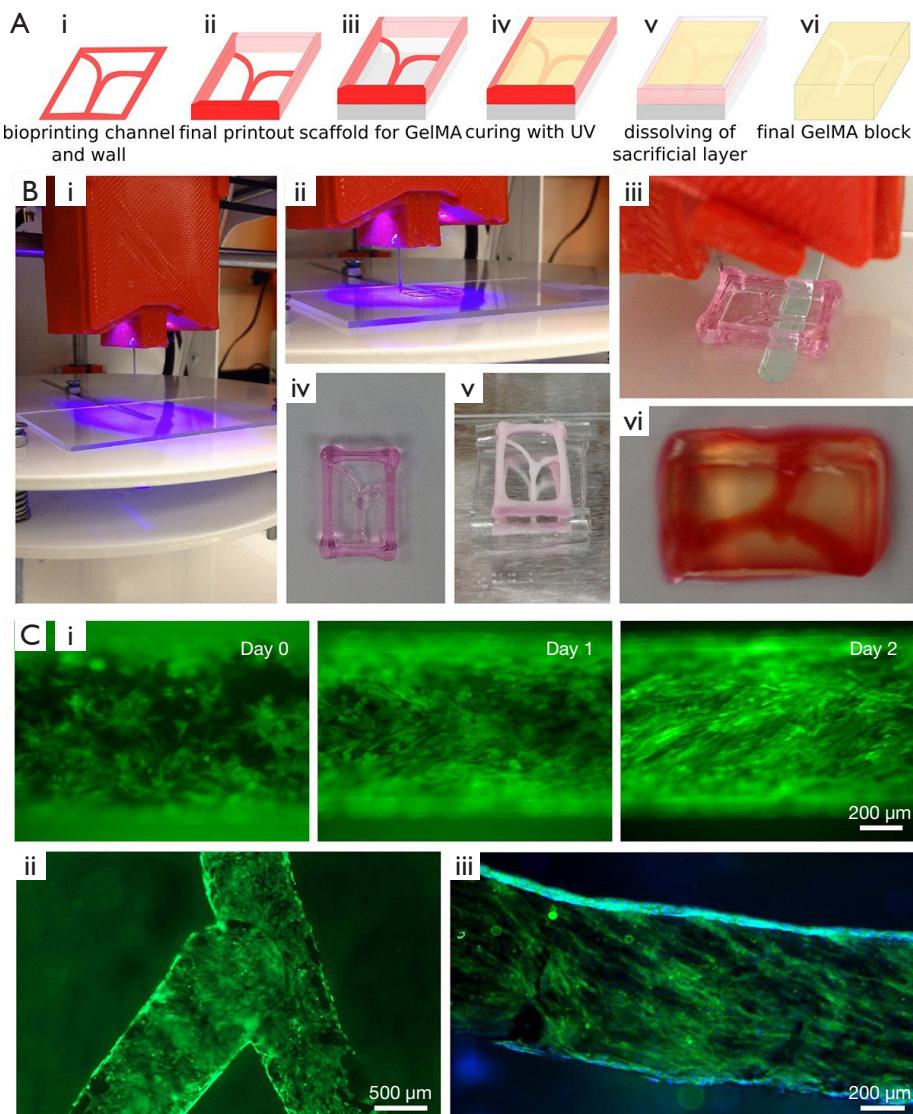
Yet many fabricated MPS still utilize outdated, laborious methods for fabrication of these models. This lack of adoption is likely due to the initial barrier of access to the advanced technology of extrusion biofabrication. Many models above were fabricated on expensive, complex, custom-built platforms, which most labs do not have the expertise or funding to develop. These custom-built devices, while extremely powerful, limit the ability for reproducibility or collaboration. Likewise, initial commercially available extrusion biofabrication platforms were extremely expensive and difficult to use. This landscape is changing, however, with accessible, low-cost biofabrication platforms that incorporate the advanced technologies of these other platforms. The potential of these standardized, low-cost platforms is evident through advancements such as the thrombosis model depicted in *Figure 4* and developed by Zhang *et al.* (22). With a standardized system like the one used in this work, advancements in the field can be easily reproduced and scaled. This capability will be especially important for the translation and mass production of these systems. A standardized platform also presents promise for translation of MPS into clinical practice for personalized medicine. Personalized therapies are becoming more and more relevant, and it will be necessary to develop methods to test compatibility of such therapies with individual patients (58,59). Consideration not only in MPS design but also manufacturing method, scalability and fabrication time must be considered. Standardized extrusion biofabrication platforms present great promise for this development

and translation. In addition to standardization of these systems, continued improvement in extrusion biofabrication technology, as well as matrix bioinks, is necessary to provide enhanced resolution and manufacturing capabilities for the continued advancement of MPS.

Many of the current MPS fabricated with extrusion biofabrication incorporate some but not all advantages of this method. Systems focus on uniform patterning of 2D cells, or controlled architecture of vasculature channels, but do not always incorporate sensors for analysis, multiple cell types, 3D culture environments or design for application of additional mechanical or electrical stimuli. As more research institutions gain access to this technology and the field continues to mature, we predict the development of even more biomimetic, complex MPS that incorporate all of these features. Likewise, more multi-tissue platforms will likely be developed for the prediction of multiorgan toxicities and analysis. As these advancements rapidly develop, it would be useful to create a standardized method of analysis for success or failure of each of these systems.

These methods of analysis will need to be tailored for each tissue and tissue model developed, and should be based on functions measured in the clinic. MPS should be compared not only to current *in vitro* models, as many often are, but also to the *in vivo* systems or pathology the component is attempting to model. Clear markers of improvement and success should be determined prior to readout. Computational models should be developed to compare measurements in MPS to *in vivo* systems. Programs such as the National Institute of Health (NIH) MPS Program run by the NIH, the Defense Advanced Research Projects Agency (DARPA) and the U.S. Food and Drug Administration (FDA) not only provide funding for MPS research but also provide a set of standards and compounds for the testing of new models (60). This set of standards involves minimal functional requirements for each system and a set of training compounds based on known organ-specific toxicities in the human population (60). These types of standards should be further developed and applied to all projects regardless of funding for effective development of new systems (60).

In addition to standards for testing new systems, it is imperative to also consider the analysis of these systems and how data for this analysis will be collected. Common methods such as staining or anti-body based assays are often destructive and time-consuming (61). MPS must move toward real-time, non-destructive read-out capabilities for



**Figure 4** Thrombosis-on-a-chip [adapted from (22)]. (A) Schematics and (B) photographs showing the sacrificial bioprinting method to fabricate thrombosis-on-a-chip. (A) i,ii) bioprinting of a pluronic template; iii) dried template is placed on a PDMS support; iv) the mold is filled with GelMA followed by crosslinking; v) dissolution of the sacrificial channels and frame to produce; vi) the final construct with hollow channels; (B) photographs showing the experimental depiction of the corresponding steps of the sacrificial bioprinting process illustrated in (A); (C) endothelialization of the hollow microchannels inside the GelMA construct for (i) a linear and (ii) bifurcating microchannels; (iii) CD31 (green) and nuclei (blue) staining of the confluent layer of HUVECs. PDMS, poly(dimethylsiloxane); GelMA, gelatin methacrylate; UV, ultraviolet light; HUVECs, human umbilical vein endothelial cells.

active monitoring and technologies with realistic scalability. Advances in soft sensor technologies and developments of bioinks for fabrication of integrated electrode arrays, such as the ones in Lewis *et al.*, demonstrate an ideal approach to MPS design. More advancements in bioinks and incorporation of these soft sensor technologies are needed

for the advancement of MPS.

## Conclusions

Current methods of *in vitro* testing are highly ineffective and costly. MPS provide great promise to improve the

effectiveness and decrease the cost of this testing. For truly advanced *in vitro* models, however, advanced fabrication methods must be implemented to recapitulate complex *in vivo* environments. Extrusion biofabrication platforms allow for the complexity, versatility and reproducibility necessary to fabricate truly physiologically relevant models. These systems have the potential to save billions of dollars and minimize exposure of harmful treatments to both animals and humans. Through automated fabrication via extrusion biofabrication, advancements in MPS have the potential to advance the rate of discovery of therapies to improve human health, hopefully creating a pace of advancement closer to Moore's law.

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## Footnote

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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