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REVIEW

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Cardiovascular human organ-on-a-chip platform for disease modeling, drug development, and personalized therapy

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Abstract

Cardiovascular organ-on-a-chip (OoC) devices are composed of engineered or native functional tissues that are cultured under controlled microenvironments inside microchips. These systems employ microfabrication and tissue engineering techniques to recapitulate human physiology. This review focuses on human OoC systems to model cardiovascular diseases, to perform drug screening, and to advance personalized medicine. We also address the challenges in the generation of organ chips that can revolutionize the large-scale application of these systems for drug development and personalized therapy.

KEYWORDS
biomaterials, cardiovascular, organ-on-a-chip, organoid, stem cell

1 | INTRODUCTION

Over 85 million people in the USA are affected by cardiovascular disease (CVD) each year. CVD is associated with over 2000 deaths per day and amounts to $320 billion annually in healthcare costs.[1] Around 60% of CVD-related mortalities are caused by coronary heart disease such as myocardial infarction.[2] Complications such as occlusion and thrombosis occur as a result of CVDs that affect general vasculature, such as atherosclerosis, hypertension, and other vascular cell dysfunctions.[2] Therefore, the development of novel drugs to prevent and successfully treat CVDs is in high demand. Although pharmaceutical R&D expenditures have risen to $50 billion in 2020, there has been a decreasing trend in new drug development in the past five decades, even in light of an exponential increase in demand and technological advancements.[3] For these reasons, advancing drug discovery efforts will ultimately contribute to the development of new treatments for CVDs.

Some of the critical challenges in novel drug discovery and traditional disease models include: (1) accuracy of current CVD models in predicting human physiological responses; (2) difficulty in combating the inflammatory response of metabolite toxicity; and (3) limitations in developing drug screen models for personalized therapy. Further, the process of drug development for CVDs, including regulatory approval and market entry, needs to be more efficient and less cost-prohibitive. Furthermore, developments of in vitro models to predict the efficacy and safety of novel pharmaceutical therapies is needed to overcome these challenges.

A major bottleneck in drug discovery strategies is the development of biomimetic in vitro CVD models to overcome the limitation of traditional preclinical models to fully reproduce human pathological

conditions and complex dynamics.[4] For instance, animal models have different pharmacokinetics than humans, which can result in inaccurate results.[5] These differences necessitate precise and relevant in vitro models with controlled biochemical and physical cues to simulate the tissue environment for development of novel and effective drugs. The organ systems involved in drug testing need to model the physiologically relevant fluid dynamics of the human cardiovascular system to predict accurate novel drug effects in terms of safety and efficacy.[6]

The organ-on-a-chip (OoC) platform is able to recapitulate aspects of the human physiological environment for accurate drug screening and pathophysiological disease modeling, making it an attractive candidate for an in vitro CVD model. Organ chip systems comprised of myocardium, vasculature, or cardiac–vascular interactions have been designed to mimic salient characteristics of cardiovascular tissue.[7] For example, OoC microfluidic devices cultured with cellular populations under physiologically relevant flow shear forces have been successful in mimicking the physiology and pathophysiology of organs.[8]

OoC techniques have been studied to recapitulate the dynamic microenvironment of cardiac and general vasculature. These systems can mimic structural organization, flow shear stress, mechanical tension, transmural force, and electrical activity.[9] Cardiac- and vascular-OoC systems have been developed to model and probe the effectiveness of drugs in treating CVD. This review highlights the recent advancements in the development of the cardiovascular OoC platform for drug discovery and disease modeling.

2 | CARDIAC ORGANOIDS

Conventional 2D cellular culture has been less successful in maintaining native cellular shape and structure, viability, gene expression and lineage differentiation, due to the limitation of cell–cell and cell–microenvironment communication. Conventional 2D cell culture of a single cell lineage fail to mimic multi–cellular interactions and native 3D tissue geometries. New generation 3D cell construct models that mimic the in vivo organ called human cardiac organoids (hCOs) aim to overcome these limitations. An hCO can depict cellular behavior and function better than traditional cell culture models, as the traditional models lack necessary components for self-organization.[10] Exogenous signals are required to induce the initial cell type, and the remaining self-organizing steps need autonomous cues from the system. Specific cues are required to simulate the microenvironment in vivo to maintain cellular survival and for self-assembly into organoid structures. Organoids are organ-like multicellular structures generated by cellular self-assembly in a random or directional manner. Organoids can recapitulate the critical properties of their prototypical organs: (1) multicellularity, (2) similar cellular organization as native organs, and (3) organ function.[10] Three-dimensional ex vivo cellular cultures created through cellular self-assembly are maintained by secreted and/or endogenous growth factors or natural extracellular matrix(ECM) cues and have been shown to reflect the native in vivo environment and recapture some functionality of target organs.[11] Therefore, organoids are a useful tool to study tissue development, homeostasis, pathophysiology, and cellular phenotypic integration.

2.1 | Methods for constructing cardiac organoids

Organoids can be derived from either embryonic stem cells, induced pluripotent stem cells (iPSCs), or adult stem cells through a process very similar to how an organ acquires distinctive organization. Self-organization within the organoid occurs via spatially restricted lineage commitment by activation of a series of signaling pathways mediated by cellular components or extracellular (ECM) and media. The choice of stem cell for OoC generation may be disease or context-dependent. hCOs have been successfully generated by aggregating a cell suspension of human PSC-derived cardiac lineage cells[12,13] in a medium or 3D gel matrix. For cardiac engineering, human PSC-derived embryoid bodies (EBs) attached to hydrogels conjugated with collagen exhibited similar myocardium-like characteristics with vessels compared to suspended state culture.[13] The mechanical properties such as the stiffness of collagen-conjugated hydrogel are similar to the myocardium that can modulate cellular proliferation and differentiation into cardiomyocyte cells. In vivo, the structural organization of cardiac cells can be stimulated by surface modification and electrical stimulation. For example, a bioengineered hCO was developed to understand cell cycle arrest and drug screening and discovery.[13,14] Another example is 3D bioprinting of the heart using human PSC-derived cardiac cells.[15] However, in the absence of an effective electrical conduction system (sinus node), hCOs are incapable of recapitulating electrical properties of the heart. Recently, one group developed a bioprinting technique using Bio3D-derived collagen technology to recapitulate fully functional constituents of the human heart from the scale of fine capillaries to full organs.[16] The fabrication and perfusion of multiscale vasculature and tri-leaflet valves were achieved by controlled pH-driven gelation with a 20-μm filament resolution that created a porous microstructure with rapid cellular infiltration and microvascularization. This technology was based on the FRESH (freeform reversible embedding of suspended hydrogels) bioprinting and could accurately reproduce the primary structural and mechanical characteristics of the human heart, as determined by micro-computed tomography. Human cardiomyocytes bioprinted cardiac ventricles exhibited synchronized contractions, propagation of action potential, and 15% wall thickening during peak systole.

One of the remarkable advances in bioengineering is utilizing organ chips to integrate human tissues and organs for drug screening for safety and efficacy (Figure 1).[17] Even though a whole functional human organ has not been developed yet, these methods for constructing the structure, mechanical properties, and biological characteristics of human tissues can be utilized effectively for investigating pathological progression and evaluating pharmacokinetic design and safety. Despite these advancements, there continue to be challenges, such as large-scale culturing of cardiac cells for 3D bioprinting of the human heart, the generation of mechanically and chemically tunable

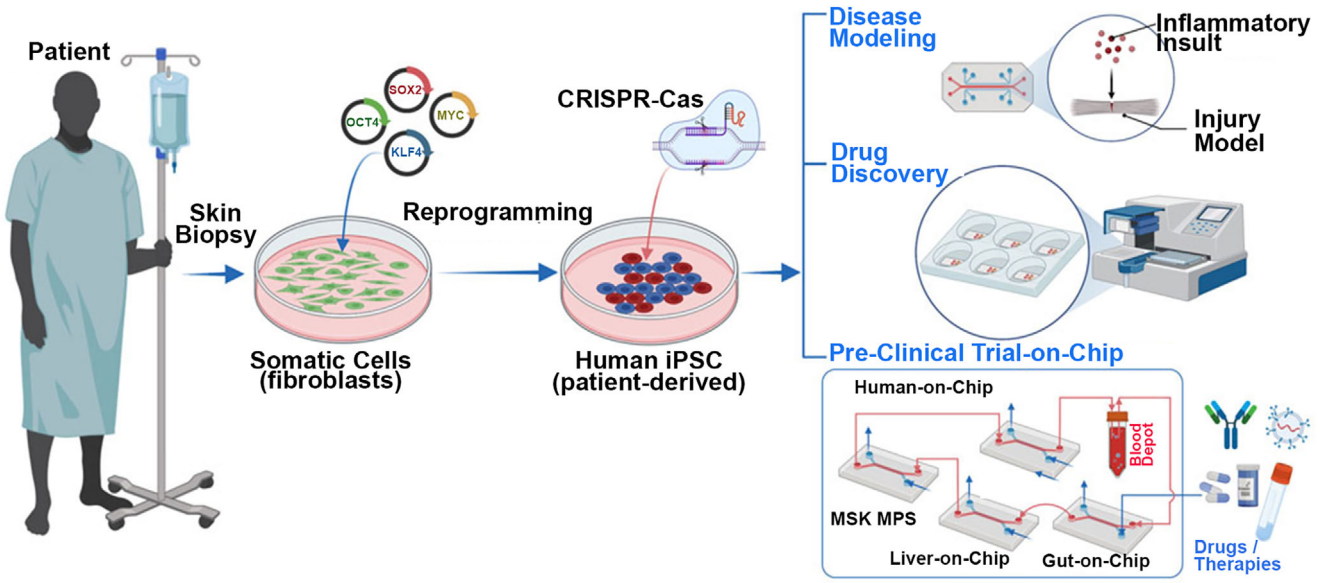

FIGURE 1 Human iPSCs can be derived from patient somatic cells procured through a minimally invasive tissue biopsy and then reprogrammed using pluripotency factors. Human iPSCs can be used for disease modeling, pharmacokinetic and pharmacodynamic screening, and preclinical trials. Adapted with permission from Reference 17.

biomaterials, and optimal manufacturing procedures that are compatible with clinical translation.

3 | VASCULARIZATION OF ORGANOIDS

In vitro 3D organ models utilizing human cell assembly and engineering have attracted interest in the last decade. Despite the success of organoids in recapitulating structural and functional properties of organs, vascularization of 3D organs remains challenging. The absence of oxygen and growth factors, along with the accumulation of metabolic waste, render weaker communication between cells and the microenvironment, causing inadequate growth of vascular networks.[18] As described below, in vitro and in vivo approaches have been employed to address organoid vascularization. In vitro vascularization involves templating and self-organizing methods.[19] Templating methods include bioprinting, hydrogel molding by needles, sacrificial molding, and assembly of patterned hydrogel slabs.[20] Self-organizing methods involve the co-culture of endothelial cells (ECs) and supporting cells in the presence of angiogenic growth factor to promote self-assembly.[21] Transplanting organoids in vivo is another method of achieving vascularization. Each type of organoid vascularization technique is discussed below.

3.1 | Templating method

Templating methods have previously been utilized for vascularization of engineered tissues. It involves templating of structural features in materials on cellularly relevant size scales to generate tissue-like structures with controlled form and function. Microfibrous scaffolds formed by bioprinting of a composite ink and ECs have been studied as a templating strategy, for example. ECs form a confluent layer at the periphery.[22] Cardiomyocytes cultured within a scaffold already containing an endothelial layer generate a functional myocardium with adequate electrical properties.[22] Another strategy is perfusion of scaffold bioprinted with ECs. Bertassoni et al. achieved endothelialization of a hydrogel construct with vascular networks with endothelial layer within fabricated channels.[23] Fully perfusable networks can be formed by removing bioprinted templates, obviating the need for template dissolution. Tissue perfusion can be improved by bioprinting 3D cell-laden vascularized tissues on a chip with prolonged duration of perfusion of >5 weeks. Kolesky et al. co-printed multiple cellular lines of human mesenchymal stem cells (hMSCs) and human neonatal dermal fibroblasts to integrate parenchyma, stroma, and endothelium into a single thick tissue within a customized ECM, followed by perfusion with growth factors.[24] The third strategy is bio-printing of a whole, thick, vascularized tissue in one step, as used by Noor et al. to 3D bio-print using a personalized hydrogel to create a thick, vascularized tissue in one step.[25] This approach yielded thick, vascularized, perfusable cardiac patches that fully resemble immunological, cellular, biochemical, and anatomical patient properties. Biopsy of an omental tissue is derived from the patient. As cells are reprogrammed to become pluripotent stem cells followed by differentiation into cardiomyocytes and ECs, the ECM is processed into a personalized hydrogel. The two cellular types are combined with hydrogels to form bioinks for cardiac tissue and blood vessels. This technique can generate bioprinted volumetric cellular structures including whole hearts and major blood vessels. Together, these results demonstrate the potential of the templating method for engineering personalized tissues and organs to enable drug screening in an appropriate anatomical structure and a patient-specific biochemical microenvironment.

3.2 | Self-organizing method

One of the limitations of the templating technique is the generation of a non-dynamic vessel structure. In contrast, in vitro vascular networks formed by the self-organizing method can closely resemble the native angiogenesis process. Nashimoto et al. formed perfusable self-assembled vascular networks by employing a microfluidic device to induce angiogenic sprouts in a human lung fibroblast spheroid co-cultured with human umbilical vein endothelial cells (HUVECs) and hLFs.[26] The spheroid was introduced to the microfluidic device and HUVECs were seeded into the left and right channels of the well. Soluble angiogenic factors secreted by hLFs formed angiogenic sprouts toward the spheroid and were grown into the perfusable vascular networks that can anastomose to the vessel-like structures in the spheroid. Additionally, oxygen and nutrient exchange was facilitated via vascular networks from cells in the spheroid and nutrients and oxygen were transported to cells in the spheroid.[26] This perfusable vessel model is an effective approach for long-term in vitro tissue culture. The in vitro vascularization methods can have optimum control on the growth of vascular networks and functionality.

3.3 | Vascularization in vivo

To date, transplanting organoids in vivo is the most reliable way to obtain tissue vascularization and vascular function.[27] Van den Berg et al. successfully achieved vascularization of kidney organoids.[28] Transplantation of human pluripotent stem cell-derived kidney organoids under the kidney capsule of host mice resulted in host-derived vascular networks that developed into glomerular structures in the organoids and actively connected to the human-derived plexus. In comparison to control groups, host organoids displayed a greater integrated process of development and maturation. In a similar study, Takebe et al. generated vascularized liver organoids.[29] They cultured human iPSCs-derived hepatic endoderm cells, HUVECs, and hMSCs that self-organized to liver buds, followed by transplantation under the cranial window in mice. Nutrient and oxygen exchange was achieved by human-derived vessels connected to the host vessels to form functional vascularized liver organoids. Achieving spontaneous perfusing capability of blood vessels is a major challenge to form a completely functionalized organoid. This is a goal that yet to be achieved by current approaches for vascularizing organoids in vitro and in vivo.

4 | TRANSLATING CARDIOVASCULAR OOC MODELS

4.1 | Heart-on-a-chip systems in drug discovery

Cardiac contractile function is supported by the parallel arrangement of cardiomyocytes and their interactions with chemical, mechanical, and electrical stimuli.[30] Cardiac tissue is composed of a cellular population of cardiomyocytes, fibroblasts, ECs, and smooth muscle cells (VSMCs), and it remains functional by stimuli that modulates cellular composition and anisotropic organization.[31] The in vitro microfluidic heart-on-a-chip platform provides effective techniques for studying cardiovascular pathology and for designing high-throughput methods for drug discovery.[32]

Recently, myocardial tissue formed in microfluidic organ-on-chip systems has been extensively studied. Poly(dimethyl siloxane) (PDMS) is most commonly used as it is biocompatible and non-toxic, and can be easily customized into a microfluidic platform due to its elasticity and transparency.[33] PDMS alone limits cell growth and attachment due to its hydrophobic nature, and thus surface modification techniques such as attachment of fibronectin have been employed.[33] Annabi et al. coated microfluidic channels with hydrogels such as methacrylated tropoelastin (MeTro) and gelatin methacryloyl (GelMA) to induce on-chip cardiac cell culture.[34,35] MeTro hydrogels had better cellular attachment, proliferation, and beating rate compared to GelMA.[36] Media perfusion through OoC systems can simulate shear stress forces. Therefore, this system can be valuable for studying the impact of biochemical and mechanical cues on cardiac function. However, it remains a challenge for this system to recapitulate the complexity of cardiac ECM composition. Hence, more approaches are needed to identify optimal ECM composition and subsequent organization for generating a heart-on-chip model.

In engineering cardiac tissues, critical components include myocardial cells and tissue, scaffold, and ECM. Two factors critical in heart-on-chip devices are electrical simulation and integration of structural and functional cardiomyocyte anisotropy, which mediates electrical propagation of action potentials and cardiac muscle contraction.[37] Grosberg et al. utilized a muscular thin film (MTF) technique with cardiomyocyte anisotropy to design a heart-on-a-chip model.[38] PDMS films were cultured with rat ventricular cardiomyocytes and printed with fibronectin to form a MTF platform with platinum electrodes. One of the critical factors in fabrication of heart-on-a-chip device is electrical stimulation and cardiomyocyte anisotropy. Propagation of the electrical signals followed by depolarization of the anisotropic and elongatedcardiomyocytes results in regular and synchronized cardiac muscle contraction. The external electrical stimulation and anisotropic organization of cardiomyocytes can generate a more physiologic in vitro heart tissue. The electrical and mechanical functioning of cardiac tissue is prominently affected by anisotropic alignment of cardiomyocytes and ECM. Electrical stimulation of the cardiomyocytes generated electrical signals in vivo that contributed to enhanced cellular alignment, growth, and functionality of cardiac tissue.[39] This approach was successful in measuring contractile function and action potential morphology of MTFs. Therefore, this technique could be effective for screening the impact of a drug treatment on cardiac microtissues in the presence of hydrodynamic and electrical stimuli.[40] In another MTF-based heart-on-a-chip system, the impact of isoproterenol on cardiac tissue contractility was measured for high throughput screening of drug treatment.[41] In addition, Au et al. engineered in vitro cardiac microtissues using a cell culture chip with electrical stimulation and topographical

signals[42] that resulted in enhanced cellular alignment along the grooves and maturation of sarcomeric α-actinin. This approach was effective in measuring the impact of different factors on cellular response.

Fabrication of heart-on-chip systems utilizes cardiac tissue perfusion to generate complex tissues of cardiac bundles with microvessels. Xiao et al. developed a perfusable heart-on-a-chip model with a poly(tetrafluoroethylene) tube with aligned and elongated cardiomyocytes along the tubing.[43] This platform was also used to study the role of nitric oxide exposure for 24 h, with results showing a decreased cardiomyocyte beating rate compared to control group. These studies highlight the possibility of heart-on-chip as effective biomimetic platforms for drug development and impact on cardiac tissue functionality.

4.2 | Cardiac disease models

Tissue-engineered and OoC platforms have been applied to in vitro cardiac disease modeling. OoC-based cardiomyopathy models of rhythm disorders and dilated cardiomyopathy have been studied using iPSC derivative cells.[44–47] In addition, 2D tissue-engineered heart models for cardiac conditions including MI and arrhythmia have been successfully utilized.[48] Microfluidic heart-on-chip systems for disease modeling and drug development are further discussed below.

Mechanical stress and chronic conditions of hypertension causes hypertrophy, fibrosis, and heart failure.[49–51] Mechanotransduction, a process of a series of mechanical changes that translate into biochemical and cellular modifications, can partially induce hypertrophy and heart failure.[52] McCain et al. developed a hypertrophic cardiomyopathy model using an MTF failing myocardium-on-a-chip platform where remodeled tissue pathology and mechanical cycle stretch resulted in disorganization of cardiomyocytes.[53,54] Heart failure-on-chip models could be useful for understanding pathological cardiac remodeling and for drug screening potential novel treatments. Ren et al. developed a PDMS-based microfluidic system for a heart-on-chip model of hypoxia-induced myocardial injury using an oxygen consumption blocking agent, FCCP (cyanide *p*-trifluoromethoxyphenylhydrazone).[55] The cardiomyocytes exhibited morphological alterations such as shrinkage and intracellular actin bundle disassembly in the hypoxia conditions, with cardiomyocyte apoptosis observed during myocardial ischemia (MI).

Stem cell transplantation for MI cardiac repair has gained attention in the last decade, with different cellular populations of cardiac progenitor cells, myoblasts, and iPSCs.[56] He et al. developed a microfluidic system to study the repair of hypoxia-injured cardiomyocytes in a co-culture model of skeletal myoblasts and rat cardiomyocytes.[57] This microfluidic system was designed to mimic the in vivo transplantation process and to monitor the interactions between skeletal myoblasts and hypoxia-injured cardiomyocytes in a spatiotemporally-controlled manner. A myocardial hypoxia environment was created using an oxygen consumption blocking reagent, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, and interactions between the skeletal L6 myoblasts and hypoxia-injured myocardium H9c2 cells were studied. Results showed that cell–cell interactions could result in skeletal myoblasts repairing hypoxia-injured H9c2 cells. This approach could be effective for studying myocardial repair processes and for in vitro screening of drug-induced cardiotoxicity.

Myocardial tissue disease models have been developed using hydrogels and microfluidic systems.[58] Calcific aortic valve disease (CAVD) is an actively regulated degenerative disease. In vitro systems have the limited ability to recapitulate the 3D geometric complexity and cellular organization of clinical lesions. 3D culture systems have been studied that recapitulate valve interstitial cell (VIC)-induced matrix calcification and valve endothelial (VEC)-interstitial collaborative interactions that can modulate the risk and complexity of pathogenesis within mechanically stressed and pro-inflammatory environments.[58] Chen et al. developed a biomimetic microfluidic platform to study physiological and pathological interactions between valvular ECs (VECs) and valvular interstitial cells (VICs) under static and dynamic fluid flow conditions.[58] Fluid shear stress induced VEC stimulation on fibronectin-coated PDMS microchannels and activated VICs into α-smooth muscle actin-expressing myofibroblasts with enhanced ECM synthesis, one of the hallmarks of valvular pathological remodeling.[59,60] Therefore, this platform could be efficient for screening drug molecules in pathological aortic valve disease.

Recent advances in stem cell research and regenerative medicine have shown great potential in patient-specific iPSC-derived cardiomyocytes to treat cardiac disease and drug development.[61,62] Wang et al. developed a Barth syndrome (BTHS) in vitro disease model using iPSCs, MTFs, and a microfluidic system.[63] BTHS patient-specific iPSCs differentiated into patient-specific iPSC-derived cardiomyocytes were cultured on MTFs to elicit BTHS cardiomyopathy pathophysiology. BTHS cardiomyopathy on-a-chip platform could be utilized to screen pharmacological agents for therapeutic treatment. In vitro heart-on-chip systems have been utilized as human physiologically relevant models for potential drug development.

5 | VASCULATURE-ON-A-CHIP SYSTEMS

5.1 | Recapitulating vascular environment

Vessel homeostasis is maintained by hemodynamic factors. Microfluidic platforms have been designed to understand the effect of hemodynamics in vitro by studying the vasculature in physiological and pathological mechanisms.[64–67] Recapitulating the human vascular environment in vitro is challenging, as vessels such as arteries, arterioles, veins, venules, and capillaries differ in structural and cellular composition. Furthermore, flow-induced forces exert biophysical stimuli on vessels. For example, ECs lining the vessel lumen are exposed to flow-induced pulsatile wall shear stress, transmural pressure, and cyclic mechanical stretching for vessel relaxation.

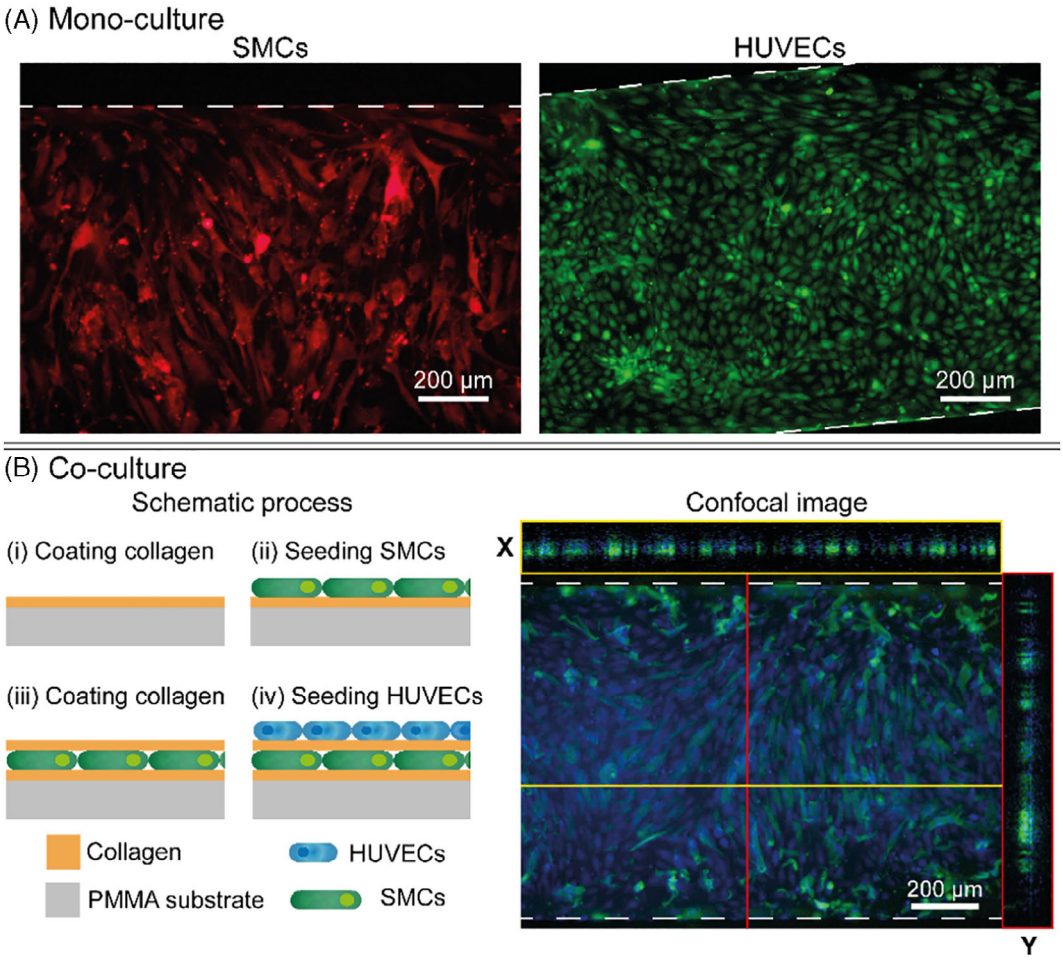

FIGURE 2 Vascular cell-on-a-chip system. (A) Fluorescence images of the monoculture model of the smooth muscle cells (SMCs) (red) and human umbilical vein endothelial cells (HUVECs) (green) in different microchannels. (B) Schemes illustrating the process of a layered co-culture model of the SMCs and HUVECs (left). Confocal image (right) of the co-culture model of the SMCs (blue) and HUVECs (green) with the vertical (red) and horizontal (yellow) cross-sections of the microchannel. The white dashed lines indicate the microchannel area. Reproduced with permission from Reference 68.

Vasculature-on-chip systems have been applied toward drug screening and the development of potential therapeutic treatments for vascular diseases. Le et al. designed a vascular cell-on-a-chip system using human SMCs and HUVECs cultured inside a PMMA (poly(methyl methacrylate) microdevice.[68] The microchannels were collagen-coated for enhanced cellular attachment. Additionally, SMCs and HUVECs were cultured on bare PMMA channels and PAA (poly(acrylic acid))-coated PMMA channels without the collagen-coated layer as controls. The results revealed the low cell adhesion capacity of PMMA and high cell toxicity of the PAA-coated layer. Despite the high cytotoxicity, the PAA-coated layer was designed to engraft collagen type I to form a stable collagen-coated layer inside the microchannels. Figure 2 shows a confluent culture of SMCs and HUVECs inside the microdevice after 48 h of incubation, as indicated by the red and green fluorescence signals, respectively, which confirms the successful coating of collagen inside the microchannels. Furthermore, this study explained the role of PAA as an adhesion promoter for grafting biological molecules to develop physiological microenvironment for OoC applications.

Microfluidic systems with 3D hydrogel platforms under physiological shear stress and fluid flow levels have been extensively studied. Kim et al. studied vasculogenesis and angiogenesis in a microfluidic system made of PDMS and fibrin matrices[69] to generate perfusable 3D microvessels as well as tumor vasculatures in vitro based on the spatially controlled co-culture of ECs with stromal fibroblasts, pericytes, or cancer cells. The microvessels exhibited the morphological and biochemical markers of in vivo blood vessels with barrier function and long-term stability. In addition, the production of increased nitric oxide by the vascular networks versus static conditions was measured. This platform is useful for studying applications in vascular physiology and developing vascularized organ-on-chip and human disease models for screening therapeutic drugs. Other systems have been designed to generate vascular networks to study vascular biological mechanisms.[70,71] Morgan et al. studied blood–vasculature interactions in tissue engineered type I collagen constructs cultured with ECs in a system of microvascular endothelialized structures.[72] In another study, fibrin-based techniques have been employed and

studied in conjunction with distinct cellular types. Hasenberg et al. developed vascular (capillary) networks in fibrin gels on multi-organ chips.[73] These capillary networks displayed stability in serum-free media, an essential factor studied in drug development systems.

Bioprinting techniques have gained immense attention in engineering vascular networks. Bertassoni et al. bioprinted vascular networks in UV-cross-linkable GelMA hydrogels with precise control of microarchitectures to mimic distinct vascular regions.[70] This approach allows for the design of 3D constructs to screen drug compounds on a large scale and in a wide range of vascular flow settings. This platform has several advantages: (a) it allows for the generation of 3D vascular system with a wide of hydrogels, (b) it has fine-tuned control over mechanical properties, and (c) it promotes ECM signals for targeting specific vascular regions. Therefore, these systems have been successful in mimicking vascular microenvironments for drug development.

While 2D models are simple, recapitulating the complex cellular interactions is beneficial for optimal tissue and organ function. In contrast, animal models can mimic integrated 3D functions of the body but exhibit severe limitations, such as cardiac function, liver metabolism, and immune response. These considerations, along with advancements in microfabrication and stem cell biology, have spurred the creation of new model systems referred to as microphysiological systems (MPSs) to simulate the 3D multicellular human tissues.

Tissue chips integrate microfluidic channels and chambers on a single platform for nutrient exchange and to remove waste from cells and organoids.[74] In comparison to traditional tissue culture wells and plates, tissue chips provide a far more dynamic and flexible environment to recapitulate a 3D multicellular human tissue and to line multiple organoids (e.g., gut and liver) in series or in parallel, thus referred to as MPSs.[74] This platform can be designed to have multiple organ systems with organ-specific microvasculature that are physically coupled to allow exchange of cells and fluids. Pathological conditions such as inflammation, autoimmune diseases, allogenic transplants, infections, immune cell therapies, and cancer metastasis require inter-organ exchange of fluid and cells can be modeled using integrated systems.[74] This platform has also been used for efficacy and toxicity testing of different therapeutic drugs such as anti-cancer agents. Finally, tissue chips are often made of optically clear materials such as PDMS and therefore enable clear 3D visualization of dynamic events with high spatiotemporal resolution.

5.2 | Vascular patterning

Spatially organized vascular networks of hollow microconduits can be created using 3D printing, soft lithography, or a combination of microfabrication strategies.[75–80] An endothelial tube is formed as a monolayer on the inner surface of the conduit. Early techniques generated the conduit using needles etched into soft biomaterials such as collagen[75] (Figure 3A). Vessels generated remained stable and functional for 2–3 weeks and demonstrated the initiation of angiogenesis into the surrounding soft biomaterial. One of the limitations of this approach is the placement of the needle into collagen gel, impacting throughput and the network geometry by being limited to a single straight tube. These challenges were circumvented by using soft lithography, where a polymer cast forms an interconnecting network of channels in a transparent polymer such as PDMS over an SU-8 master mold. The surface is then coated with ECs into the microfluidic lines (Figure 3B,C). This technique is highly reproducible in creating a network of endothelial-lined channels that precisely mimic in vivo vascular patterning. However, one of the challenges is that the network is not dynamic, as ECs cannot penetrate the surrounding polymer, which is metabolically inert. Therefore, the network fails to adapt to the changing metabolic needs of the surrounding matrix.[76,77] Additionally, it is challenging to create 3D networks, as the network of channels is confined to one plane. The third limitation is that the cross-sectional shape of the microvessels[76–78] is a square or a rectangle instead of a circle. Such non-physiological geometries discourage the growth of a continuous endothelial monolayer, as ECs tend to avoid sharp geometrical transitions and create in vitro artifacts. As an example, rectangular microvessels sprout mainly at the corners of the vessel, which is not physiologically relevant.[79] Therefore, these existing strategies are not sufficient to create organized vascular networks.

Traditional 3D in vitro platforms to create microvessels employ either a single suspension of ECs or endothelial-coated microbeads embedded into the ECM gels of collagen, fibrin, or Matrigel.[80] The microvessels with lumens are effective models of vasculogenesis and angiogenesis. However, the vessels had limited stability and lacked functional perfusion capacity through the vascular lumen for nutrient delivery or waste removal from surrounding tissue. The advancement of microfabrication and microfluidics has led to many novel techniques to create perfused microvascular vessels and vessel networks. Microfluidics-based techniques generate perfusable vascular networks by either vascular patterning (i.e., ECs populating a patterned network of microfluidic channels) or by self-assembly (i.e., stimulation of a microvessel network to self-organize through either angiogenesis or vasculogenesis to achieve a stable anastomosis with adjacent microfluidic channels).[81] Microfluidics-based techniques are primed for creating effective and spatially organized vascular networks.

5.3 | Vascular disease models

Vascular models have been extensively studied to recapitulate human vascular geometries and structures subjected to physiologically relevant fluid flow and shear forces. For example, Rosano et al. mapped a mouse cremaster muscle to generate out of PDMS a vascular network mold.[82] This approach is advantageous to recapitulate the complex vascular structures and geometries for modulating cellular responses to pharmacological treatments in disease models such as atherosclerosis or thrombosis.

Models to study vascular mechanisms such as angiogenesis have been studied, in addition to recreating complex vascular structures and geometries.[83] For example, Galie et al. studied the impact of shear

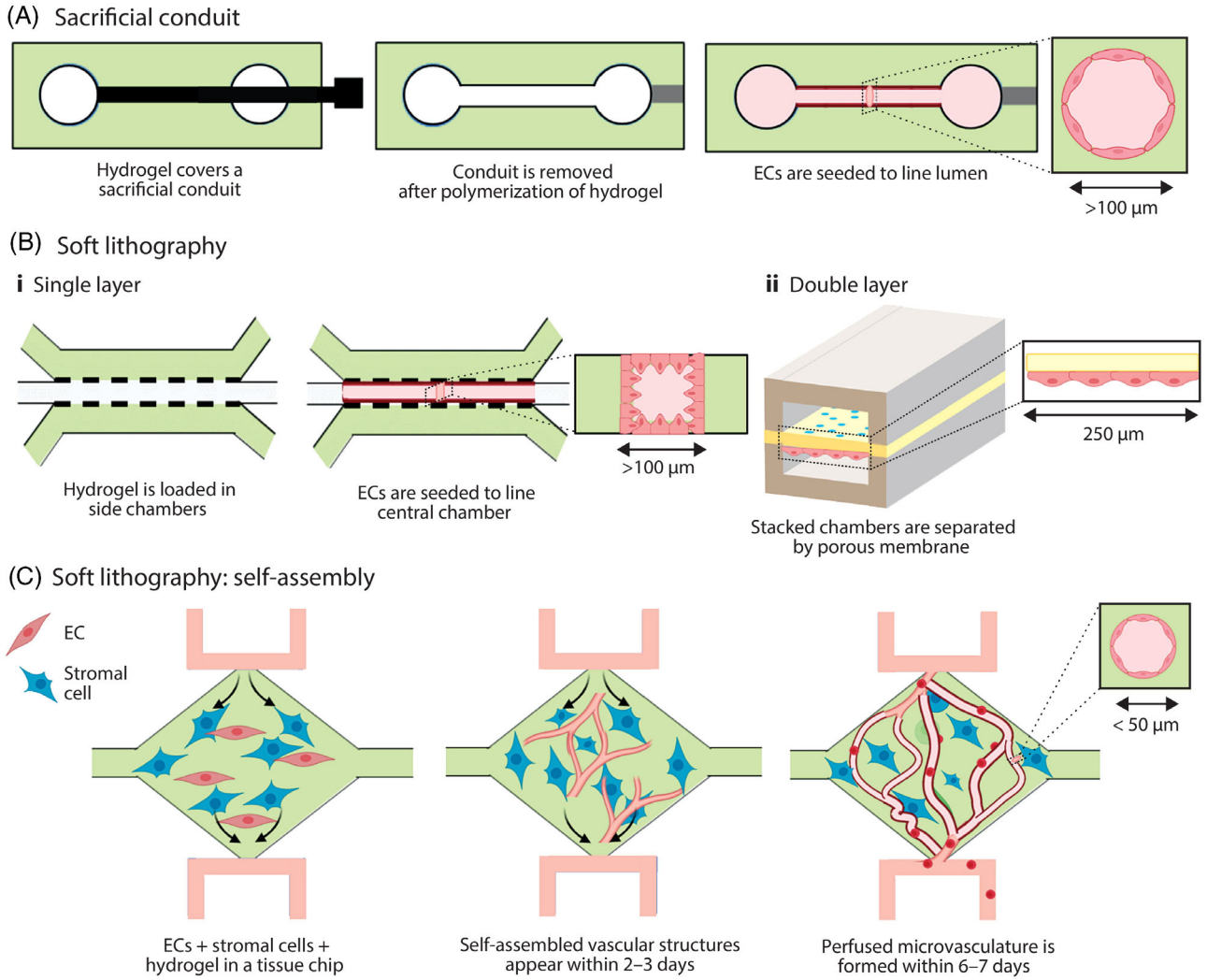

FIGURE 3 Vascularization strategies. (A) A single channel mimicking a vessel can be created by placing a hydrogel around a sacrificial conduit (e.g., needle etching), followed by coating the microconduit with endothelial cells (ECs). A cross-section of the vessel is shown at the far right of panel A. (B) Hollow EC-coated conduits can also be made using a microporous membrane to separate (i) matrix-filled chambers in a single-layer design or (ii) two adjacent fluid-filled chambers in a double-layer device. (C) Alternatively, ECs (red) and stromal cells (blue) can be mixed with a hydrogel and seeded in a device created using soft lithography under controlled interstitial flow (black arrows) and growth conditions to allow a vascular network to self-assemble. Vascular structures appear within 2–3 days, which then connect to form an interconnected, branched, and perfused microvasculature. A cross-section of the vessel is shown on the far right of panel C. Reproduced with permission from Reference 75.

stress and transmural flow (>10 dyn/cm^2) in endothelial sprouting, with narrow vessel regions of high shear stress promoting further sprouting.[84] In another study, Buchanan et al. studied tumor angiogenesis in a co-culture model of a breast cancer cell line MDA-MB-231 with ECs to understand the impact of high wall shear stress on downregulation of tumor genes such as *MMP9*, *HIF1*, and *VEGFA*.[85] Jeon et al. developed a breast cancer 3D microfluidic model under differing microenvironments.[86] The blockage of A_3 adenosine receptors in cancer cells resulted in enhanced extravasation, an effective platform for drug screening for personalized therapies.

Microfluidic systems have been generated to study diseases such as thrombosis, occlusion, and stenosis.[87–91] Li et al. designed a microfluidic system to study the effect of pharmacological drugs and shear rates on stenosis and thrombus formation over a range of shear stresses, and the authors reported longer occlusion rates with low shear stress.[88] Korin et al. engineered microparticles incorporated with tissue plasminogen activator (tPA) that were responsive to shear stress levels. In regions of high shear stress levels in stenotic regions, the microparticles became disrupted, forming smaller nanoparticles that enabled local tPA delivery to the stenotic regions for rapid thrombolysis.[91] Vascular thrombotic and stenotic models have been studied for therapeutic drug development.[92,93] For example, Muthard et al. developed a microfluidic thrombogenesis system to model the effects of wall shear stress and trans-thrombus pressure.[89] This platform could be used to assess thrombotic areas to understand hemodynamics of pressure gradients in the vessel wall. These examples highlight the application of microfluidic approaches to study or treat thrombosis, occlusion, and stenosis.

6 | OOC MODELS FOR DRUG DEVELOPMENT AND SCREENING

The drug development pipeline could benefit from an in vitro "body-on-a-chip" model that links multiple OoC tissue models, such as liver, heart, lung, stomach, muscle, brain, bone, pancreas, and kidney, thereby obviating the need for an animal model. Multi-organ-on-chip platforms could be applied to predict drug toxicity as a result of tissue-–tissue cross-talk with circulating microfluidics. Recent studies have developed multi-tissue systems for drug development and screening. For example, Hickman et al. recently designed a multi-tissue model consisting of liver, skeletal muscle, and neurons to understand the effect of pharmacological drugs such as Tegafur (5-fluorouracil) on cellular toxicity.[94,95] In another example, Agrawal et al. developed a multi-organ "human-on-chip" microfluidic system of liver, heart, and skeletal muscle to recapitulate human physiology in vitro, with recirculating media for nutrients and drug transport through target organs[96] The multi-organoid model was generated using a single microfluidic device with a central chamber with multiple acellular and cell-laden hydrogels. Additionally, Ma et al. designed a multi-organ 3D microfluidic system of liver and glioblastoma tissues to study cellular resistance to cancer therapeutics.[97] Finally, Atala et al. developed a three-chip organ system of liver, heart, and lung-on-chips to study tissue cross-talk-influenced drug responses that can potentially impact cardiac cell death and contractile function.[98] These examples demonstrate that multi-organ-on-chip systems can recapitulate aspects of in vivo functionality to accurately predict therapeutic drug response.

7 | CHALLENGES AND FUTURE PROSPECTS

Advancements in human organ chips have led to success in the development of disease models and drug development that was challenging to accomplish with traditional culture systems or non-dynamic MPSs. Although 3D organoid cultures have the advantages of patient-specific cells and compatibility with high-throughput assays, OoC devices are an advantageous platform for incorporating physico-mechanical cues such as shear stress and can respond to drugs with predictive ability of human drug pharmacokinetics. OoC devices have also been shown to mimic human pathophysiology and can be used to study therapeutic responses better than animal models. Healthy and pathophysiological human OoC models can be customized with patient donor cells from diverse genetic sub-populations or co-morbidities to better replicate the diversity in clinical data. Furthermore, human OoC models are an effective platform to analyze complex drug disposition, pharmacokinetic/pharmacodynamic properties, and inter-organ interactions for optimizing dosing regimens or predicting clinical efficacy.

One of the challenges to organoid maturation is lack of functional vascularization with perfusing capabilities close to humans. New bioengineering approaches can allow long-term culture of the organs with efficient mass transfer and biochemical signals for maturation. It is also important to establish a system for cytokine induction to promote in vivo vascularization. Vessel maturation can be enabled by biophysical factors (e.g., interstitial flow, intraluminal flow, and matrix stiffness) and biochemical cues [e.g., vascular endothelial growth factor (VEGF)] provided to the ECs from the surrounding matrix and stromal cells.

There are several challenges to be addressed for the successful vascularization of OoC devices. The small size of the chip restricts the formation of large, complex blood vessel networks, which limits the scalability of OoC devices. Next, the use of organ-specific ECs or pluripotent stem cell derivatives is desirable for patient-specific modeling of the target organ. Another challenge is the precise simulation of the internal environment, such as shape of the micro-flow model, hydrogel properties, the chemical gradient of cytokines, and mechanical properties that impact vascularization in OoC devices. For vascularized multi-OoC devices, blood vessels that connect tissues and loading sensors on the blood vessels are helpful to track the biological activity on the chip.

A limitation of hCOs is their inability to analyze diverse cellular populations of the native myocardium. For example, the role of inflammatory cells is critical in early biological responses after myocardial infarction prior to fibrotic response. Current hCOs do not have the capability to simulate an immune response in an infarction model. Additional challenges need to be addressed to recapitulate structural and functional aspects of tissues in vivo, such as the scale-up of cells for organoid culture, integration of multi-disciplinary areas of bioengineering and materials science for generation of precise complex organs, and the need for endogenous factors such as cytokines and their interplay between cells and ECM. Continued optimization of vascularization is critical toward the development of maturation of functional organoids. These challenges need to be addressed to advance the success of OoC devices to accurately model diseases, cardiac development and drug discovery and screening for personalized medicine.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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