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Modeling organ-specific vasculature with organ-on-a-chip devices

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Abstract

Organ-on-a-chip devices, also known as microphysiological systems, have gained significant attention in recent years. Recent advances in tissue engineering and microfabrication have enabled these devices to provide more precise control over cellular microenvironments to mimic the tissue-level or organ-level function of the human body. These more complex tissue models can provide either an improvement in the functional expression and maturation of cells or an avenue to probe biological events and function that would otherwise be difficult to visualize and mechanistically study. This high-value information, when complimented with the existing gold-standards of cell-based assays and animal models, could potentially lead to more informed decision-making in drug development. A prevalent biological component in many organ-on-a-chip devices is an engineered vascular interface that is present in almost all organs of the human body. The vasculature and the vascular interface are particularly susceptible to biomechanical forces, they function as the conduits for inter-cellular and inter-organ interactions, and regulate drug transport. In this review, we examine the various approaches taken to model the human vasculature with an emphasis on the engineering of organ-specific vasculatures, and discuss various challenges and opportunities ahead as the field advances.

Keywords: organ-on-a-chip, vascularization, microfludics, microfabrication

(Some figures may appear in colour only in the online journal)

1. Introduction

The rising cost of drug development is a major healthcare issue. Despite the increasing expenditures invested in drug development, the number of drugs approved annually has been decreasing over the past 20 years [1]. This trend is at least in part due to the lack of tools to dissect and predict the effects of drugs prior to clinical trials. Existing clinical models such as cell-based assay in the format of monolayer culture can be a powerful tool because of its low costs and high-throughput capability and will like to be irreplaceable as an initial screening tool. However, it is clear that cell-based assay even complemented with animal testing fall short in accurately predicting the effects of drugs as the success rate from Phase I clinical trial to market approval is only 9.6% [2]. More advanced tissue model that provides a physiological-relevant microenvironment could drive more matured expression of cellular functions or provide a way to model and probe complex biological events that would otherwise be impossible to dissect or even observe in either monolayer culture or animal tests. Built on this principle, organ-on-a-chip technology emerged from the advancement in micro-fabrication and tissue engineering. Such platforms could provide high-value data that would otherwise be difficult to acquire in the existing drug discovery pipeline. However different models will offer different types of high-value data. Therefore, the utility of organ-on-a-chip devices will vary based on the specific biological question.

For instance, in a lung-on-a-chip device, Ingber's group demonstrated neutrophil transendothelial migration in response to bacterial infection followed by phagocytosis in real time, which is a very complex biological event that would otherwise be difficult to observe directly during pulmonary inflammation and infection [3]. In a liver-on-a-chip device,

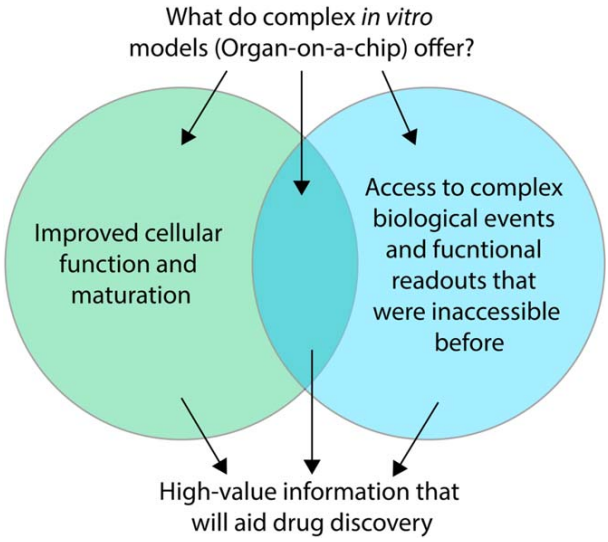

Figure 1. Value proposition of advanced *in vitro* models, organ-on-a-chip.

Bhatia's group showed robust functional improvement in gene expression profiles, phase I/II metabolism, canalicular transport of primary hepatocytes under patterned co-culture of hepatocytes and stromal cells compared to standard monolayer culture [4]. In one case, the organ-on-a-chip device offered a new capability to observe biological events, while in the other case, the organ-on-a-chip device offered more matured cellular functions that could alter the results of drug response (figure 1, table 1). Organ-on-a-chip technology clearly presents an enormous potential. However, translation of this technology will require further refinement in the manufacturing and engineering to address issues in usability, robustness, material compatibility, and end-point analysis. More importantly, extensive biological validation will be expected to justify the additional costs and lower experimental throughputs. Overcoming these barrier to adoption will require collective efforts from the scientific community. In order to move the technology forward, this community will include not only bioengineers, but also clinicians and biologists. Nonetheless, success in this endeavor will lead to enormous economic values as many major organ-on-a-chip companies are starting to explore and expand this market space: US company Organovo (market cap $389 M) endeavors to bioprint liver organoids for drug testing; Wyss Institute startup Emulate raised $45 M to develop lung and gut organ-on-a-chip platforms [1].

A common component of many organ-on-a-chip devices is the blood vessels or the vascular interfaces, which are so prevalent in almost every organ in the body (figure 2). In this review, we specifically focus on the vasculature which functions as both the conduit and the barrier for inter-cellular interaction, inter-organ interactions and drug delivery. Correctly incorporating a perfusable vascular component in an *in vitro* model is a critical aspect of the organ-on-a-chip technology that differs from the conventional monolayer cell culture. Blood vessels are anatomically unique in the sense that they experience a multitude of mechanical factors in their environment. Environmental factors, such as mechanical shear stress, cyclic stretching, flow pattern, and geometrical constraints, have been shown to have profound effects on the permeability, surface activity, and remodeling capabilities of blood vessels. Many engineering approaches have been developed to model the vasculature, but capturing all facets of the vascular physiology in a single model still remains a challenge. Furthermore, blood vessels develop different architectures, flow patterns, anatomical structures, and morphologies in different organs or even in different parts of an organ. These organ-specific differences in the vasculature are closely linked to the specific function of an organ, hence it remains to be seen if reproducing these organ-specific characteristics would lead to improved biological functions and specification as well as capture unique organ-specific biological events (e.g., the presence of tissue-specific oxygen gradients and drug delivery). So far only a few organ-on-a-chip devices have attempted to capture the organ-specific hallmarks of a vasculature while the majority of studies have incorporated the vascular component with only generic functionalities. In this review, we discuss the current state-of-the-art technologies in the modeling of blood vessels with organ-on-a-chip devices, and present a unique focus on the modeling of organ-specific vasculature. Lastly, we discuss future challenges in device development and path to commercialization.

2. Engineering approaches in building blood vessels *in vitro*

2.1. Blood vessel in a channel

Advances in soft-lithography allow us to easily construct optically transparent microfluidic devices containing microchannels in which human endothelial cells can be coated on the inner luminal surface to mimic an artificial blood vessel or a blood vessel network. Within these devices, the microenvironment of the endothelial cells can be fine-tuned by controlling fluid-induced shear stress and geometrical constrains, etc [5, 6]. The effects of fluid-induced shear stress on endothelial cell alignment and elongation, and on the secretion of angiogenic growth factors, etc have been widely demonstrated. Fluid shear forces imposed on the apical surface of endothelial cells are transmitted to the intracellular cytoskeleton through transmembrane mechanosensors, which leads to the morphological adaptation of endothelial cells [7]. These microfluidic devices also facilitated the study of the interaction between the circulating cells in blood and the endothelium. A recent study used a simple microfluidic channel coated with a layer of endothelial cells to examine the role of a glycosaminoglycan-rich layer (presented on the surface of the endothelium) in regulating the surface interaction with blood cells [8] (figure 3(a)). This device offers an important capability to closely examine the distribution of red blood cells (e.g., observation of cell-free-layer) under physiological perfusion inside a micro-capillary mimics (table 1).

Table 1. Representative contributions to the modeling of vascular physiology from organ-on-a-chip devices with a vascular component.

Improved functions	Value added capabilities	References
Mechanical cyclic stretch shows exacerbated nanoparticle infiltration of the vascular-alveolar barrier, more closely resembles the physiological response *in vivo*	Visualizing neutrophil transendothelium migration in response to bacterial infection	[3]
	Observation of cell-free layer under physiological perfusion of blood in engineered microcapillaries	[8]
	Observation of vascular architecture-dependent VWF assembly after vessel injury	[33]
	Visualizing the process of thrombosis that invovles collagen I deposition in blood clots by fibroblasts	[38]
	Visualizing cancer cell extravasation from engineered microcapillaries	[52]
Improved response to known anti-cancer drugs compared to cell-based assays (e.g., mitomycin C, gemcitabine, and vorinostat, etc)	Visualizing vessel regression and inhibition of tumor growth in response to anti-cancer drugs	[50]
	Visualizing vessel dilation and contraction in response to chemical stimuli	[54]
Improved function of hepatocytes in the presence of vasculature (e.g., cytochrome P450 expression, albumin/urea secretion, etc)		[76]
Reduced vascular permeability in blood–brain-barrier with direct neurovascular contact		[95]

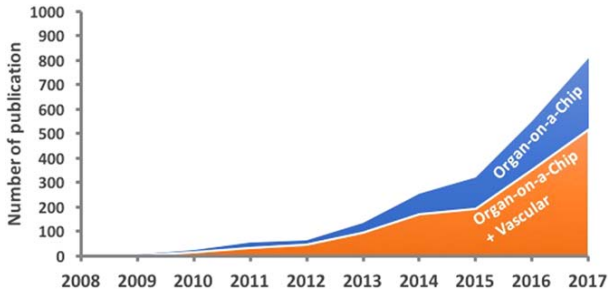

Figure 2. The rise in academic publishing in the field of organ-on-a-chip. The keywords 'organ-on-a-chip' or 'organ-on-a-chip AND vascular' were searched in Google Scholar. The number of publications found from the search for each corresponding year was plotted from 2008 to 2017. The rise in publication on the topic of organ-on-a-chip exemplifies the increasing activity in the field. Vasculature has been mentioned in nearly two-thirds of all organ-on-a-chip publications, indicating the prevalence and the importance of this biological component in organ-on-a-chip models.

In this case, however, microfabrication yielded micro-channels with a square cross-section, and although this does not limit endothelial coverage, it presents a geometrical configuration that deviates from the physiological condition. This issue can be resolved with additional modification steps, including sequentially injecting a silicone oligomer solution and air through the micro-channels to produce a circular cross-section [9]. The macro-architecture of the vascular network can also play a role in regulating the interaction between the endothelium and the circulating cells [10–13]. Microfluidic devices can be used to recapitulate parts of the physiological microcirculation captured from clinical images. The role of bifurcations, tortuosities, and cross-sectional changes of the microfluidic network in the preferential adhesion of circulating cells or drug carriers can be studied on such systems to offer new biological insights [12, 14]. Although growing endothelial cells in microfluidic channels can provide robust control of the vascular structure, vascular permeability is an important parameter that cannot be easily probed in such systems. To overcome this issue, the vascular channels and the parenchymal space can be compartmentalized and connected through permeable barriers composed of channel walls with micro-gaps. This configuration allows diffusion of biomolecules across the vascular barrier to enable the interaction between the endothelium and the parenchymal tissue [15–19].

2.2. Blood vessel on a membrane

Another way of looking at the vasculature is to zoom-in on the vascular interface at where the endothelium can be viewed as a flat cell monolayer. In this format, a number of organ-specific vascular interfaces have been developed (figure 3(b)). Donald Ingber *et al* demonstrated an *in vitro* model that can capture the organ-level functionality (e.g., breathing) of the human lung [3]. This breakthrough involved the culture of endothelial cells and lung epithelial cells on opposite sides of an elastic membrane that was subjected to both fluid-induced shear stress and cyclic stretching, thus mimicking the dilation of the lung alveoli. This platform captured many aspects of the vascular physiology, including the transendothelial migration of circulating immune cells followed by phagocytosis under physiological flow, which would otherwise be impossible to model on a 2D monolayer culture (table 1). The significance of being able to track and visualize these types of complex biological events is that as we look at disease and drug treatments (e.g., inflammatory disease or drugs) we will be able to precisely determine which step breaks down in this sequence of biological process instead of solely relying on

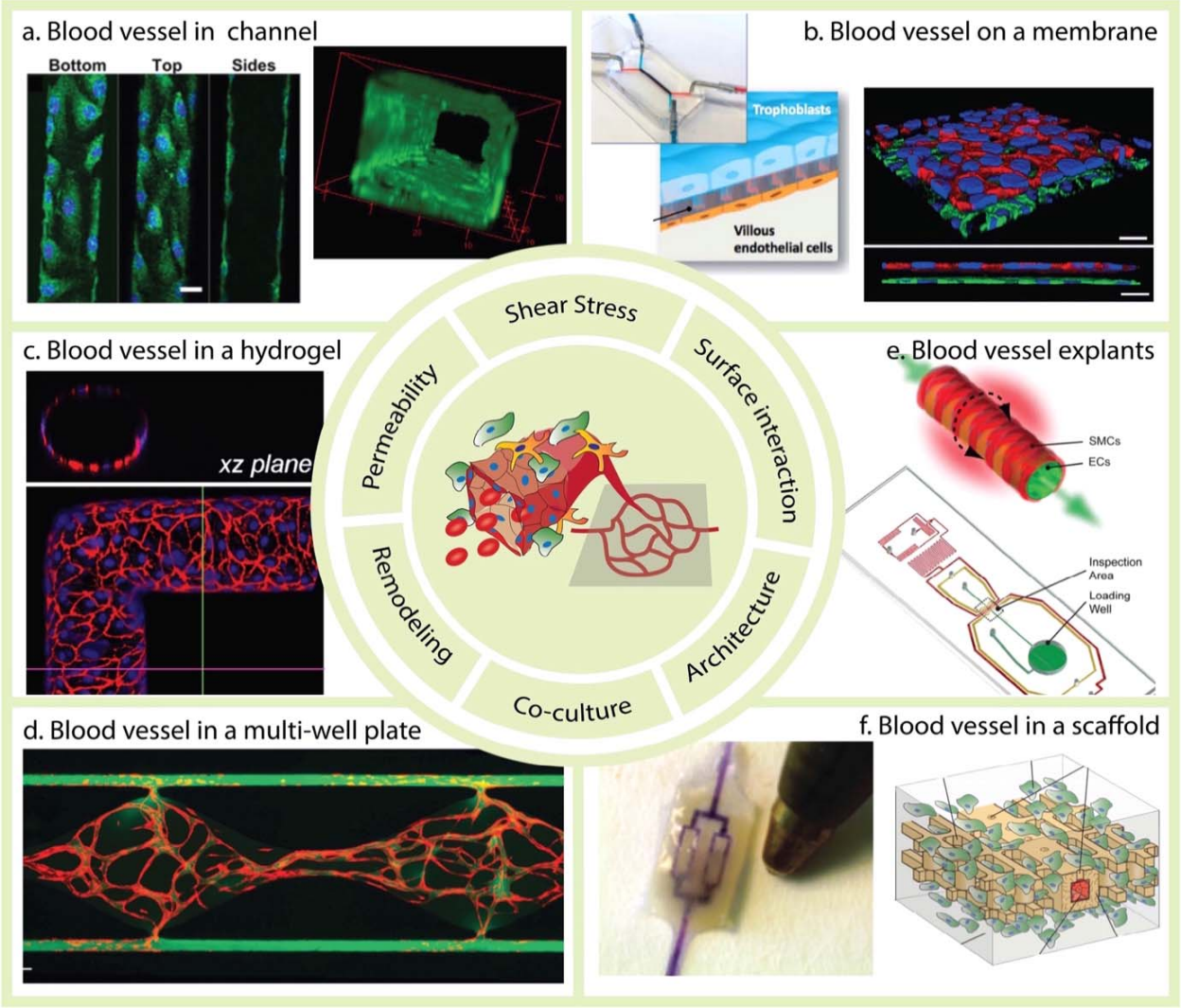

Figure 3. Selected examples of various approaches to create a blood vessel on-a-chip as an *in vitro* model for drug discovery. (a) Human umbilical vein endothelial cells cultured on the inner luminal surface of a PDMS microchannel with a cross-section of 30 × 30 μm. Reproduced from [8]. CC BY 4.0. (b) Human trophoblast and endothelial cell cultured on two sides of a PDMS membrane and stained for E-cadherin (red) and VE-cadherin (green), respectively. Reproduced from [21] with permission of The Royal Society of Chemistry. (c) Human endothelial cells cultured on the inner luminal surface of a microchannel (100 μm in diameter) embedsded inside a collagen I hydrogel matrix and stained for CD31 (red). Reproduced with permission from [29]. (d) Human endothelial colony-forming cell-derived endothelial cells (red) isolated from cord blood formed vascular networks perfused with 70 kDa FITC-dextran (green). Reproduced from [50] with permission of The Royal Society of Chemistry. (e) Illustration of the integration of isolated mouse arteries and microfluidic devices to control and visualize vessel dilation and constriction. Reproduced from [54] with permission of The Royal Society of Chemistry. (f) An engineered AngioChip hepatic tissue with a built-in branched vasculature perfused with a blue dye next to the tip of a ball-point pen to show the scale. [60] 2016 Copyright © Springer Nature.

end results. This will not only allow us to gain confidence in the understanding of a disease but also the mechanism of action of a drug. In this study, the significance of cyclic mechanical strain was also highlighted with the demonstration of elevated nanoparticle uptake across the vascular-epithelial barrier, which is in agreement with animal studies. Building on this seminal work, the Ingber group and others have developed a series of biological models: placenta-on-a-chip [20, 21], glomerulus-on-a-chip [22], blood–brain barrier (BBB)-on-a-chip [23], vessel-on-a-chip [24], blinking eye-on-a-chip [25], and cancer-on-a-chip [26], etc. This expansion of membrane-based devices is a clear indication of the potential to replicate a diverse set of biological environments. To further enable multi-organ integration by potentially linking these individual organ-models together with a continuous vascular interface, endothelialized elastomeric tubing was also developed to facilitate the connections [27].

2.3. Blood vessel in hydrogels

One important concern, however, on regenerating the vascular interface with a membrane-based approach is the

membrane itself. The elastic membrane, with a thickness around 10 μm, is an artificial barrier in between the endothelium and the underlying parenchymal tissue or epithelial cells. However, the basement membrane that separates the endothelium from the parenchymal tissues, which the elastic membrane mimics, has a thickness of no more than 100 nm *in vivo*. Although patterned micro-holes on elastic membranes allow cell migration and molecular diffusion, direct intercellular contact or vascular remodeling can be difficult to establish. This limitation can be overcome with compatible natural hydrogel matrices. Recent advances in hydrogel molding techniques have enabled the fabrication of hollow channels within a soft hydrogel using 3D printing of sacrificial materials [28], soft-lithography [29], etc (figure 3(c)). Culturing endothelial cells within these micro-channels, which are surrounded by compatible hydrogels embedded with parenchymal or stromal cells, allows close contact between the endothelium and the parenchymal tissues to establish a more realistic vascular interface [29, 30]. In addition, the mechanical stiffness of the hydrogel matrix can be fine-tuned to better model the physiological conditions compared to the synthetic materials such as polydimethylsiloxanes (PDMS) and polystyrenes. Within the hydrogel matrices, endothelial cells can readily modify the microenvironment to form circular vessels or vascular branching through angiogenic sprouting [29, 31, 32]. Enabling biological remodeling is as important as controlling the initial biological structure. This type of platform could help visualize and study transient cellular events (e.g., vascular sprouting, regression and rearrangement) that would only happen during tissue growth and remodeling.

Moreover, the emergence of 3D printing and microfabrication makes it feasible to construct complex 3D vasculatures, opening the gateway to engineering large vascularized tissues as well as examining complex blood flow dynamics in 3D [31, 33–37]. For instance, after vessel injury, the endothelium become activated and start to secrete von-Willebrand factors (vWF). Secreted vWF quickly assemble into thick bundles or complex meshes. Using a microfabricated vascular model, the study found high shear stress, sharp geometrical turn, and smaller vessel diameter all induce more vWF assembly [33]. VWF assembly is an important step in the initiation of microvascular thrombosis because the presence of vWF bundles bind platelets, leukocytes and erythrocytes as well as obstruct blood flow [33] (table 1). This study highlights the significant role of vascular architecture in disease modeling. In a different study, the complex interaction among the endothelium, blood, thrombi, and stromal cells in post-thrombotic remodeling was captured in a simple bifurcated vessel that was 3D printed to model thrombosis on-a-chip [38]. By incorporating fibroblasts, endothelium, and blood clots, the study demonstrated the deposition of type I collagen in the blood clot by fibroblasts, which resembles the fibrosis remodeling process *in vivo* (table 1).

2.4. Blood vessel in a multi-well plate

To simplify and accelerate commercial translation, organ-on-a-chip devices would also benefit by adapting the model from a microfluidic setup to a conventional multi-well plate format, which is widely used in the pharmaceutical industry for drug screening. MIMETAS, an organ-on-a-chip company based in Leiden, Netherlands, is leading this effort with their proprietary phase-guided hydrogel patterning technique. On their commercialized microfluidic titer plate, an array of hydrogel-liquid interfaces can be rapidly created in a multi-well plate format with this technique. Endothelial cells [39], intestinal epithelial cells [40], kidney proximal tubular cells [41], and other parenchymal tissues [42–44] have been cultured in this format under perfusion. In contrast to other blood vessel on-a-chip technologies based in microfluidic devices, MIMETAS' OrganoPlate® provides high-throughput experimentation (e.g., 96 vessels in a single plate) with the use of simple gravity-driven flow that requires no bulky external pumps and is highly scalable. However, this platform thus far is limited to modeling tubular vessels without any branched vascular structures. The native vasculature is dynamic and constantly evolves through remodeling and rearrangement, especially in response to disease or tissue regeneration. Based on the intrinsic tendency of endothelial cells to self-assemble into a rudimentary vascular network, a perfusable vascular bed can be engineered in a hydrogel given the right experimental conditions. Several different microfluidic devices have been developed to facilitate anastomoses or the connection of self-assembled microvascular networks with microfluidic perfusion circuits at pre-established hydrogel-liquid interfaces to establish perfusion [45–49]. Without physical constraint on the self-assembled microvessels within the hydrogel, dynamic vascular events, such as angiogenesis and vessel regression, can be studied and visualized in response to drug stimuli under perfusion. This capability is powerful as traditionally only simple cellular function, such as endothelial cell migration, can be studied in a 2D transwell system while more complex vascular remodeling events have to be studied on animals with cranial windows. Organ-on-a-chip platforms based on this principle have also been productized in the multi-well plate format [50–53] (figure 3(d)). In this format, a panel of FDA-approved anti-cancer compounds with different anti-tumor and anti-vessel effects were tested on an array of vascularized tumor models. The platform successfully captured the anti-vascular effects of linifanib, axitinib and sorafenib and the anti-tumor effects of mitomycin C, gemcitabine, vorinostat and tamoxifen, while monolayer cell assay failed to capture the anti-tumor effects of mitomycin C, gemcitabine, and vorinostat at the same dosage (table 1).

2.5. Blood vessel explants

Another important aspect of the vascular physiology is the smooth muscle cells. Many clinical drugs target smooth muscle cells to modulate vessel dilation and contraction. But incorporating smooth muscles in a blood vessel-on-a-chip

model has been challenging, partly due to the complexity involved in reproducing the circumferential alignment and concentric geometry of the smooth muscles. Nonetheless, microfluidic chips have been developed to study explanted small arteries and veins [54, 55], where the vascular explants can be loaded, cultured, and stimulated with drugs inside the chip (figure 3(e)). In this format, vessel dilation and contraction in response to drug stimuli can be visualized in real time. The core of this technology is in the controlled manipulation of organ explants and engineered tissues. Therefore, this technology can potentially integrated with organoid technology and tissue engineering that seek to provide sophisticated human tissue substitutes. For example, stem cell-derived organoids, although structurally sophisticated, may benefit from the dynamic perfusion offered by this type of microfluidic platforms.

2.6. Blood vessel in a scaffold

Many vascular models were developed separately from the parenchymal tissues (e.g., liver, cardiac and skeletal muscles) [29, 56], whereas many parenchymal tissues have been developed in the absence of vasculature [57–59]. There is often a technical gap between these two types of models. But placing a perfusable vascular interface within a 3D functional tissue is a critical step towards high fidelity organ models. Recently, we engineered functional heart muscles and liver tissues with a built-in stable vasculature using a microfabricated bio-scaffold [60] (figure 3(f)). The vascular interface supported by the elastomeric polymer is both permeable and mechanically stable to withstand extensive parenchyma remodeling without collapsing. This feature allows us to effectively place a perfusable vasculature inside a functional tissue with high cell density. Vascular sprouting from the predefined vessel network is also possible. It is important to note that vascular remodeling/sprouting and mass transport in a hydrogel are very different from that of a dense tissue [61]. Therefore, establishing perfusable vasculature within a dense functional tissue at a physiological cell density (10^8 cells cm^{-3}) is a challenge and a physiological hallmark that should not be overlooked.

3. Organ-specific vasculature on a chip

The interactions of blood flow, vessel architecture, endothelium, and parenchymal tissues vary drastically from organ to organ. These differences largely arise from the anatomical and functional differences between the vasculatures in different organs. For instance, the endothelium from different organs display different morphologies. One of the classifications of the vascular system, developed by Majno in 1965, categorized the vascular endothelium into three major groups based on their characteristics of the endothelial lining and the basement membrane: type I vessels have continuous endothelial lining (e.g., skeletal muscle, brain, myocardium, lung, skin); type II vessels have continuous endothelial lining but with fenestrations (e.g., endocrine glands, kidney); type III vessels have discontinuous endothelial lining (e.g., liver, spleen) [62, 63]. These morphological differences impart the vasculature with widely different permeability and barrier functions. In addition, the parenchymal tissue structures provide important physical cues to the development of the vascular networks. For instance, the microvasculature in muscles develops along the muscle fiber whereas the vasculature in kidney glomeruli forms spherical clusters, a striking contrast as a result of different developmental processes [63, 64]. Therefore, to correctly model drug delivery or biological interactions in these organ-specific microenvironments, it is essential to recapitulate the organ-specific vascular architectures and morphologies.

3.1. Cardiac vasculature on a chip

For a typical human at rest, the blood is pumped through the heart at a rate of 6 liter per minute. To support this mechanical demand, the heart developed a muscular wall that is supported by nutrients delivered through a dense vascular network[65]. In fact, in the myocardium, each cardiomyocyte is located no further than approximately 20 μm away from a capillary. The average diameter of capillaries in the adult human is 8 μm [66], and the density of capillary in the myocardium is around 2000 capillaries mm^{-2}. To reproduce this highly organized and dense vasculature, Thomas Eschenhagen *et al* developed a 3D microvessel system with a high vascular density (lumina diameter of 6–8 μm, intervessel distances of 20–60 μm) in a strip of cardiac muscle [67]. A dense capillary network can also be induced with an explanted artery or vein on micropatterned substrates that contain a thymosin β4-hydrogel in the presence of soluble vascular endothelial growth factor. Cardiomyocytes can be seeded on top of the established vascular bed, which can then be perfused to study the impact of the capillaries on the functions of the adjacent myocardial cells (figure 4(a)). Radisic *et al* used the same scheme integrated with cellular processes and lumen propagation to engineer endothelial cell proliferation and capillary outgrowth [68]. Even though the study reproduced aligned blood vessel structures, the model only included a single layer of vascular network physically separated from the cardiomyocyte layer. To establish a multi-layer vessel network, a continuously extruded hydrogel tube that mimics blood vessels can be 3D printed into a 3D lattice matrix, which can be subsequently populated with cardiomyocytes [69]. This approach, although scalable, cannot provide branched vascular structures due to limitation in the 3D extrusion process. Alternatively, we have engineered a 3D branching vasculature with a 3D stamped polymer scaffold that can be populated with a variety of parenchymal cells (e.g., cardiomyocytes and hepatocytes). Different from soft hydrogel materials, the polymer scaffold can provide sufficient mechanical support to the built-in vasculature to support a contractile cardiac muscle [60]. This platform would allow us to deliver drugs indirectly to a beating cardiac muscle through the vasculature barrier, mimicking the actual physiological condition. In a different study that delivered drugs to an engineered liver tissue through a built-in vascular interface using a liver-on-a-chip

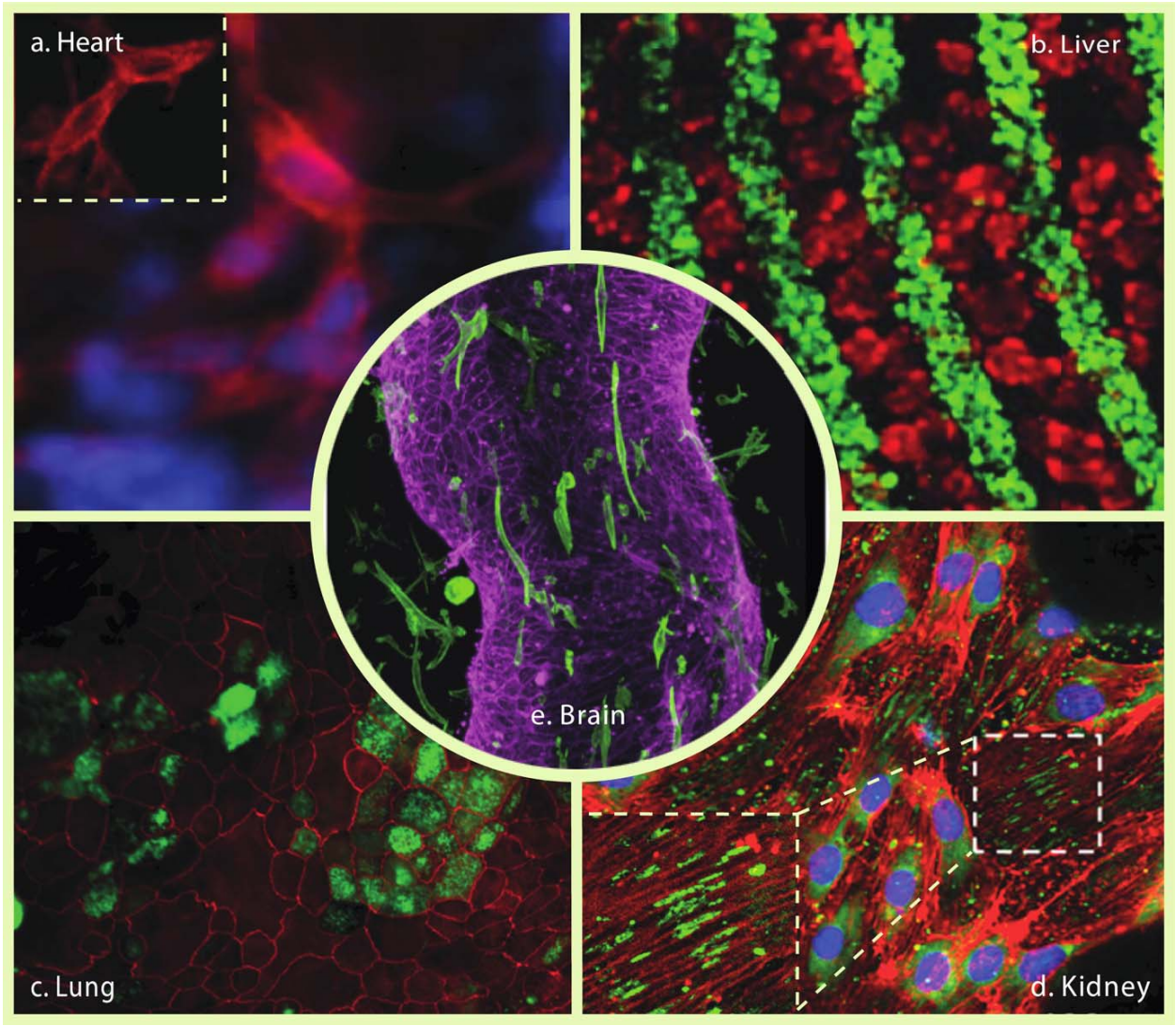

Figure 4. Selected examples of *in vitro* models with organ-specific vasculatures (heart, liver, lung, kidney, and brain) for drug discovery. (a) High magnification image of roponin T immunostained (red) sections of engineered cardiac tissue cultured on top of a Tb4 gel embedded with capillaries, where the cell nuclei are stained with Hoechst dye (blue). Reproduced with permission from [68]. (b) Liver endothelial cells (green) patterned in fibrin hydrogel and integrated with hepatic aggregates (red) to form free-standing liver tissue seeds. From [77]. Reprinted with permission from AAAS. (c) Human airway epithelial cells (Calu-3) cultured on suspended hydrogel and stained with goblet cell marker MUC5AC (green) and ZO-1 tight junctions (red). Reproduced from [83] with permission of The Royal Society of Chemistry. (d) An engineered human kidney microvessel at a junction of a vessel network stained for F-actin (red) and plasmalemmal vesicle associated protein-1 (green). Reproduced with permission from [32]. Copyright © 2016 by the American Society of Nephrology. (e) An engineered brain microvessel composed of human endothelial cells (stained for VE-Cadherin, Magenta) and surrounded by brain astrocytes (stained for F-actin, green). Reproduced from [94]. CC BY 4.0.

device, the results showed a delayed drug response in the presence of the vascular barrier [70]. This study indicates that to better understand the rate of tissue response to drugs, the vascular barrier is a critical parameter to consider even for small molecule drugs.

The endothelium that lines the vascular system has been shown to play a key role in controlling cardiac functions. Regulation of the subjacent cardiomyocytes was first described for the endocardial endothelium, and further established for the vascular endothelium in the myocardial capillaries [71]. While both endothelia have similar impacts on cardiac functions and growth, possibly as autocrine or paracrine modulators, it has been shown that each undertakes different paths in terms of signal processing. In addition, they differ in developmental and morphological features. Past developments in heart-on-a-chip models have focused mainly on the myocardial capillaries, while the endocardial endothelium has yet to be incorporated. The endocardial endothelium plays a critical role in the embryonic development of myocardial cells through the neuregulin growth factor signaling pathway. Endocardial endothelium controls trabeculin expression of the heart by releasing neuregulins as a paracrine [72]. The

modulation of the development of myocardial cells, executed by the myocardial capillaries, occurs much later compared to the endocardial endothelium. These physiological differences can potentially inspire the development of a heart-on-a-chip device with a built-in vascular component. Therefore, to accurately replicate the cardiac physiological environment, specific models that include the endothelium at different regions of the heart need to be developed in the future.

3.2. Liver vasculature on a chip

The hepatic sinusoids and the hepatocytes are the fundamental units that enable the liver to perform metabolism, store glycogen, synthesize proteins, and carry out detoxification. Hepatocytes are responsible for metabolic reactions, and require organized vascular and stromal support [73, 74]. The highly organized hepatic sinusoids are open pore capillaries that have a discontinuous endothelium [75]. This feature of the liver endothelium, together with the presence of the perisinusoidal space (the space of Disse), underlie the efficient mass transport between the blood and the hepatic tissues [73]. To reproduce this intricate tissue organization, multiple types of cells needs to be fabricated in an orderly fashion. An InVERT (Intaglio-Void/Embed-Relief Topographic) molding technique was developed by Stevens *et al* to create an organized hepatic environment with compartments containing different cells *in vitro* (figure 4(b)) [76, 77]. Endothelial/stromal cells and induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells were patterned in PDMS topographic substrates and then transferred into a 3D hydrogel, yielding an engineered tissue with pre-defined microscale architectures. A rudimentary vasculature, for instance, can be patterned into a lattice matrix surrounding isolated islands of hepatocytes. However, with this approach the fabricated vascular system lacks perfusion *in vitro*. Liver-specific vasculature can also be engineered using a decellularized liver matrix. Uygun *et al* have successfully built a transplantable recellularized liver graft that can carry out cytochrome P450 expression, albumin secretion, and urea synthesis [78]. The preserved vascular architecture in the liver matrices can be perfused and re-endothelialized. Decellularized scaffolds can provide direct access to the high-level biological architectures of an organ, which are still largely missing in organ-on-a-chip models. Even though deceullarized matrices are generally used for *in vivo* implantation, they can still play an essential role in an *in vitro* model when used as miniaturized scaffolds or processed hydrogels to overcome the issue of mass production and the high-throughput experimentation required in drug testing applications.

3.3. Pulmonary vasculature on a chip

The minimal functional units for carrying out gas exchange in the lung are the alveoli, which are spherical capsules that have an average diameter of 0.2 mm. A group of alveoli are bundled together around a alveolar duct that has a luminal diameter of 0.3 mm [79]. Around the alveoli, a vascular-ductal complex is formed to facilitate the exchange of oxygen and carbon dioxide between the blood and the air. This blood-air interface, lined with alveolar epithelium and capillary endothelium, can be as thin as 0.5 μm and it directly regulates pulmonary permeability [80]. Moreover, this barrier also needs to withstand mechanical changes in the microenvironment such as transmural pressure. To reproduce this organ-level physiological function, the Ingber group has developed a lung-on-a-chip device that can be populated with human alveolar epithelial cells and human pulmonary microvascular endothelial cells on two sides of an elastic membrane to mimic the alveolar wall [3, 81]. However, in the native lung, instead of an elastic membrane, type I and IV collagen provide the necessary strength to the barrier between the alveolar epithelium and the capillary endothelium. The collagen layer is also an essential component that regulates the rate of oxygen diffusion *in vivo* [82]. Therefore, to better mimic this physiological environment, a recent advance in microfabrication was able to successfully replace the synthetic elastic membrane with a natural collagen membrane [83] (figure 4(c)). Thus far, these devices are designed to recapitulate the alveolar interface in a plane. Future development is needed to capture the organ-specific 3D architecture of the alveolar vascular-ductal complex. For instance, in chronic obstructive pulmonary disease, merging of smaller alveoli to form fewer larger alveoli, trapping of air sac, clogging due to the accumulation of mucus, collapsing of the alveoli are examples of lung damage that will only manifest through the organ-specific architecture of the lung.

3.4. Kidney vasculature on a chip

Nephrons are the minimal functional units in the kidney that filter and reabsorb extracellular fluid. To perform this demanding task, there are about 1.25×10^6 nephrons in each functioning adult kidney [84]. The main components in a nephron are: Bowman's capsule, the proximal tubule, loop of Henle, distal tubule, and the collecting tubule, all of which are surrounded and supported by a dense network of capillaries. This highly organized microvasculature uses up to approximately 20%–25% of the total cardiac output [85]. The glomerulus, which carries out the first round of blood filtration, is composed of an aggregate of microvessels that are surround by a layer of podocytes responsible for the selective permeation of a variety of molecules across this barrier. Musah *et al* developed a glomerulus-on-a-chip device to recapitulate the kidney filtration function in the glomerulus using human iPSC-derived podocytes along with glomerular endothelial cells [86]. When treated with biochemical insults, the model can mimic physiological podocyte injury. A similar membrane-based glomerulus-on-a-chip device, developed by Zhou *et al*, used mouse glomerular endothelial cells and podocytes to study hypertensive nephropathy resulted from mechanical impacts [22]. Another platform, developed by Wang *et al*, used explanted glomeruli from rat kidney in a microfluidic perfusion platform to model diabetic nephropathy by introducing a high glucose medium [87]. Thus far, the focus of these models have been the vascular interface. However, the glomerular vessels have a very unique convoluted 3D

architecture that likely plays a key role in blood filtration. For instance, glomerulosclerosis lead to wide-spread collapse of glomerular capillary loops, which drastically reduces renal function. Modeling this process could require this tissue-level 3D vascular architecture which is yet to be replicated in an organ-on-a-chip system.

To replicate the anatomy of the peritubular capillaries around the kidney tubules, collagen gel can be fabricated with soft-lithography to generate a 3D microvasculature populated with primary human kidney peritubular microvascular endothelial cells (figure 4(d)). In this system, the presence of endothelial fenestration and the variation in barrier permeability can be visualized over the entire vascular network [32]. To replicate the convoluted proximal tubular architecture, hollow channels of pre-designed architecture can be fabricated in a hydrogel with 3D printing of sacrificial materials. The open lumen can be populated with primary human proximal tubule cells [88]. In this perfusable microenvironment, the tubular cells showed remarkable improvements in cell morphology (e.g., polarization, columnar shape, increase in microvilli length and density, etc), as well as in the dose dependent response to Cyclosporine A (nephrotoxin). To reproduce the entire renal tubule and vascular complex in future development, kidney-specific micro-vasculatures will need to be combined with region-specific networks that mimic the proximal convoluted tubule, the loop of Henle and the proximal straight tubule, which collectively contributes to the entire process of filtrate reabsorption.

3.5. BBB on a chip

The endothelium of cerebral blood vessels, along with neurons, astrocytes, and oligodendrocytes, form the BBB, which is the minimal functional unit that regulates the material exchange between the blood and the brain [89]. Wang *et al* developed a microfluidic BBB model for drug permeability screening by culturing hiPSC-derived cerebral vascular endothelial cells and primary rat astrocytes on each side of a porous membrane to mimic the BBB interface. Incorporated with transendothelial electrical resistance sensors, this model can track transendothelial permeability in real time [90]. Even though the porous membrane permits biochemical interaction and crosstalk between the astrocytes and the endothelium, it has been shown that astrocyte end-feet, which physically extend to the cerebral endothelial cells, and pericytes play a key role in maintaining the barrier tightness and permeability [91]. In addition, astrocytes have a group of receptors relevant to the innate immune responses [92], while pericytes participate in immune responses by releasing mediators such as nitric oxide and cytokines [93].

To allow the direct physical interaction between the endothelium and the astrocytes/pericytes, Herland *et al* developed a 3D model by culturing primary human brain microvascular endothelial cells in a hollow cylindrical channel within a collagen hydrogel embedded with pericytes or astrocytes (figure 4(e)). Following inflammatory stimulation with TNF-α, neuroinflammatory response from the 3D BBB-on-a-chip model showed more robust cytokine release profile than the transwell system [94]. Another 3D BBB model with a vascular network in the presence of neural cells was developed by Bang *et al.* This platform can supply the endothelial cells and the neural cells with different media, such as endothelial growth medium and neurobasal medium, respectively. Synaptic structural features and astrocytic contact with the vascular network can also be readily established [95]. Potentially due to the higher degree of neurovascular interfacing and the presence of synapses on this platform, a low vascular network permeability, comparable to values reported *in vivo*, was successfully achieved (table 1). The importance of establishing direct neurovascular contact in enhancing vessel barrier function is a direct evidence of the utility of organ-on-a-chip devices in improving tissue-level function and maturation compared to traditional cell-based assays.

4. Future challenges in device development

4.1. The challenge of material selection in device development

The use of PDMS as an easily moldable material has accelerated the field of organ-on-a-chip and microfluidics in general by providing a means for rapid device prototyping. Its biocompatibility, optical transparency, elasticity, and gas permeability are desirable parameters for cell cultures. However, its hydrophobicity renders it a drug-absorbing material, especially to small hydrophobic drugs, which complicates its use in drug testing. This is especially true for small hydrophobic drugs used in microfluidic channels with a large surface-to-volume ratio. In this case, surface drug absorption can significantly alter the drug concentration in solution. For experiments requiring long term drug exposure, the presence of PDMS can alter the drug concentration [96], even in devices with a low surface-to-volume ratio. As many organ-on-a-chip prototypes move towards commercialization, adapting industrial manufacturing methods with non-absorbent hard plastics, such as polystyrene, becomes essential. Using polystyrene in the prototyping stage can be costly because expensive industrial equipment, such as hot embosser and injection molding system, are required to mold polystyrene. However, this issue can be circumvented by the solvent casting of polystyrene with the use of proper solvent (e.g., gamma-butyrolactone (GBL)) [97]. On the other hand, for devices that require an elastic material, several alternative elastomers that show minimal drug absorption while preserving many desirable characteristics of PDMS are available, such as styrene-ethylene/butylene-styrene (SEBS) block copolymer [98–100] and polyurethane [101]. For drug testing applications, it is crucial to make this transition, especially for blood vessel models that require accurate modeling of mass transport.

4.2. The challenge of clinically relevant cell sources

Human umbilical vein endothelial cells (HUVECs) are commonly used as a general source of human endothelial cells in

many organ-on-a-chip models. However, the organ-specific origin of the endothelial cells can have a profound effect on the biological model [63, 64]. For instance, HUVECs lack the fenestrations present in the microvascular endothelial cells, which is important in the modeling of hepatic sinusoids. Therefore, the choice of endothelial cells will depend on the specific model and the corresponding parenchymal tissues. It remains to be seen if the finely controlled microenvironments offered by the organ-on-a-chip devices can alter the identity of endothelial cells or induce the organ-specific differentiation of endothelial progenitor cells. But prior to that, accurate modeling of organ-specific vasculature will still depend on the availability of specialized endothelial cell sources. Advances in stem biology and primary tissue isolation techniques will provide us with increasingly specialized endothelial cell populations, such as the BBB endothelial cells [102] and the kidney peritubular microvascular endothelial cells [32], which are urgently needed to further advance this field.

4.3. The challenge of sensor integration and device operation

As tissue models become more realistic, experimental readouts can also increase in complexity. Recent advances in tissue clearing techniques have made it feasible to visualize cells deep within a solid organ or even a whole animal [103, 104]. Tissue clearing procedures may become routine for visualizing built-in vasculature in engineered biological models. Built-in electronic sensors may also provide real-time functional readouts on tissue health and conditions [105–107]. This is especially critical if the experimental throughputs of an organ-on-a-chip device are to be increased. Furthermore, device standardization is another important aspect [108]. Ultimately, organ-on-a-chip devices will be operated by non-experts. Therefore, minimizing the learning curve required in device operation will help accelerate device translation. Some organ-on-a-chip companies have approached this issue by adapting their device to the standard multiwell plate format, which can be operated with standard pipetting techniques and is accessible to biologists (e.g., devices from MIMETAS [44], AIM Biotech [109], 4Design Biosciences [48], and AlveoliX [110]). Meanwhile, others are building integrated systems to automate the operation of organ-on-a-chip devices without compromising the complexity of the device itself (e.g., devices from Emulate [3], Quorum Technology [55], and Nortis [111].

5. Conclusions

The specific benefit of organ-on-a-chip devices, especially in modeling blood vessels, will depend on the specific biological questions we seek to answer. For instance, specific organ models will be required to model organ-specific vascular physiology. But considering the multitude of biological factors in the vascular environment that are uniquely present in different human organs, the current 'gold standards' of generalized 2D cell culture and animal models are simply inadequate. Pharmaceutical companies are cautiously optimistic about this technology. By actively collaborating with academic labs, they hope to shape the technology early on in this emerging field to enhance their technical competitiveness in drug development.

Author contributions

DSYL wrote and edited the manuscript. FG edited the manuscript. BZ wrote and edited the manuscript, and supervised the work.

Competing interests

BZ is a co-founder of TARA Biosystems Inc. and holds equity in the company.

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