Cardiovascular Organ-on-a-Chip Platforms for Drug Discovery and Development

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Abstract

Cardiovascular diseases are prevalent worldwide and are the most frequent causes of death in the United States. Although spending in drug discovery/development has increased, the amount of drug approvals has seen a progressive decline. Particularly, adverse side effects to the heart and general vasculature have become common causes for preclinical project closures, and preclinical models do not fully recapitulate human *in vivo* dynamics. Recently, organs-on-a-chip technologies have been proposed to mimic the dynamic conditions of the cardiovascular system—in particular, heart and general vasculature. These systems pay particular attention to mimicking structural organization, shear stress, transmural pressure, mechanical stretching, and electrical stimulation. Heart- and vasculature-on-a-chip platforms have been successfully generated to study a variety of physiological phenomena, model diseases, and probe the effects of drugs. Here, we review and discuss recent breakthroughs in the development of cardiovascular organs-on-a-chip platforms, and their current and future applications in the area of drug discovery and development.

Key words: cardiovascular, drug discovery, heart-on-chip, organ-on-a-chip, vasculature-on-a-chip

Introduction

CARDIOVASCULAR DISEASES (CVDs) are the most frequent causes of death in the United States.[1] In 2011, 800,000 people were killed by CVDs; approximately 60% of those deaths were attributable to coronary heart disease (such as myocardial infarction [MI]) and hypertensive heart disease.[1] CVDs also include diseases affecting the general vasculature, such as atherosclerosis, hypertension, and other vascular cell dysfunctions that can result in occlusion and thrombosis.[2,3] Over the years, these cardiovascular complications have been treated with numerous interventional[4] and pharmacological agents.[5–7] Several drugs have been developed against heart and vasculature diseases, but the increasing number of patients affected by them motivates a strong need for new and improved treatments. However, drug discovery and development has seen a decline each year in approved cardiovascular drugs.[8]

The cost of developing a new drug varies approximately between $0.8 and $1.2 billion U.S. dollars.[9] After an intensive and costly research period, new drug candidates are subjected to clinical trials to assess safety and efficacy, before reaching regulatory decision by the U.S. Food and Drug Administration (FDA). However, despite extensive preclinical laboratory and animal testing, high rates of potential drug candidates still fail in clinical trials, especially during later

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stages of development.[8,10] For instance, in the year of 2000, clinical safety (such as toxicity) and efficacy issues accounted for approximately 30% of drug project failures.[11] Between 1991 and 2000, the rate of clinical trial success in cardiovascular drug development was as low as 20%.[11] Moreover, over the past five decades, adverse side effects have been a major reason for cardiovascular drugs being rejected or retracted from the market.[12–14] This can be partially attributed to the lack of effective and predictive preclinical models. Although conventional two-dimensional (2D) cultures and animal (disease) models have successfully contributed to the development of a wide range of therapeutics, many of these models do not adequately mimic human *in vivo* conditions. For example, there are essential genetic and metabolic differences between human and animals that can lead to significant pharmacokinetic mismatch.[15] These differences motivate the development of better and more predictive human-based *in vitro* models to assess the safety and efficacy of potential new drugs. Ideally, these models should consist of platforms in which biochemical, physical, and electrochemical factors can be integrated and controlled in order to recapitulate the dynamic microenvironment of tissues *in vivo*.

Several studies have been able to microengineer biologically relevant heart and general vasculature tissue models *in vitro*.[16,17] The development of these three-dimensional (3D) engineered microtissues can potentially be used for *in vitro* drug testing, disease modeling, and biological mechanistic studies.[18–20] Moreover, these models exhibit the physiological characteristics of myocardium and blood vessels because of the presence of tissue-like properties such as multiple cell interaction (e.g., paracrine signaling and cell–cell signaling), cell–extracellular matrix (ECM) interactions, and mechanical stimulation (Fig. 1). Importantly, in the past few years, novel microfluidic platforms have been introduced and have led to the creation of biomimetic cardiovascular tissues *in vitro*.[21–25] These microfluidic organs-on-a-chip models can recapitulate important organ-level functions, multicellular microarchitecture, and environment dynamics (Fig. 1), thus providing a technological platform capable of accelerating cardiovascular drug development. Therefore, these engineered and novel heart- and vasculature-on-a-chip
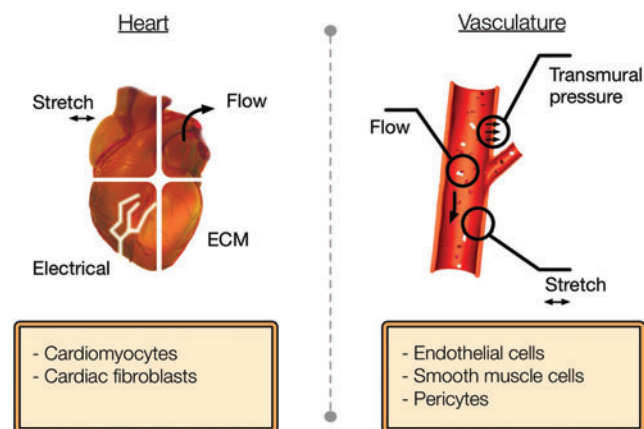

FIG. 1. Key design parameters required to mimic a heart and general vasculature-on-a-chip model. Color images available online at www.liebertpub.com/aivt

systems could contribute to the development of suitable high-throughput platforms for drug development and disease modeling of major CVDs such as MI, hypertension, heart failure, and atherosclerosis.

Here, we discuss the challenges for the validation of cardiovascular organs-on-a-chip platforms and their potential applications on the pharmaceutical industry drug development pipeline. Furthermore, we describe and discuss the advances of the past decade in the development of heart- and vasculature-on-a-chip platforms to study heart and general vasculature physiology and disease, pinpointing their successes and shortcomings. Additionally, we identify opportunities for *in vitro* disease modeling and drug development.

Challenges and Translational Opportunities

Need for cardiovascular organ-on-a-chip models

CVD is the leading cause of death in the United States.[1,26] Approximately 85.6 million people suffer from a CVD, resulting in 2,150 deaths per day in the United States alone and costing annually $320 billion and predicted to continue to rise.[1] As a result, there is a need for development of novel drugs either for prevention[27] or for treatment of CVD.[28] Despite such a demand, the number of new pharmaceutical compounds approved per amount of dollars spent on research and development (R&D) has halved approximately every 9 years since 1950.[29] Although most of the scientific and technological fields have seen exponential advances, the past 60 years of drug development has not followed that trend.[29] However, spending continues to increase, with a reported $40 billion expenditure in 2014 in pharmaceutical R&D.[30] Together, this suggests that there is a need for a disruptive technology that allows for a more predictive and efficient drug discovery/development process.[8,31,32] Such technology would have the potential to lead to the discovery of new and improved drugs to treat CVD, while reducing the current patient burden.

Some of the key challenges that current drug discovery models face have been previously summarized by Pound et al.[33] Most of these drawbacks fall into one main problem—positive/negative findings in animals do not necessarily link to positive/negative findings in humans.[34,35] Below, we have listed some of the shortcomings associated with the current paradigm of drug development, as appeared in the literature:

1. CVD animal models are poor predictors of human responses.[36]
2. Adverse effects caused by systemic toxicity or toxicity of metabolites are organism dependent.[37]
3. Some drugs can benefit a certain group of ethnicity, sex, age, and/or genotype. Such phenomenon is not necessarily captured in a limited clinical trial study. For example, it is known that the average volunteer for clinical trials is a midaged female.[38,39] This does not recapitulate the multitude of possible users of a particular drug.
4. The current paradigm is lengthy and costly.[40]

For all of the reasons mentioned above and others discussed in the literature, the process of drug development in the cardiovascular arena, obtaining regulatory approval, and achieving market entry is becoming less efficient and more costly. In fact, industry faces an increasing FDA regulatory scrutiny,[9]

while less drugs are being allowed to the market. However, this is not unfounded: regulatory agencies are requiring more evidence of safety to avoid postmarket issues, because clinical trials do not offer a complete measure of protection.[41,42] Together, the above-described reasons highlight the role of new models to better predict safety in humans.

Some researchers underscore that inefficiency in the drug discovery pipeline could perhaps be attributed to current cardiovascular models. In fact, these models are not entirely capable of mimicking human conditions and dynamics. For example, a survey showed that industries were interested in adopting human tissue-based approaches for drug testing, despite the fact that they were not widely used (Fig. 2A).[31] Interestingly, costs were not seen as a major issue toward adopting such models, while 50% reasoned that human models were more relevant.[31] A study analyzing AstraZeneca's drug pipeline from 2005 to 2010 showed that 82% of drug project closures in the preclinical phase was because of safety issues.[8] Interestingly, among the organ systems involved in the safety failures, the cardiovascular one accounted for as much as

17% failures, the highest of all the organ systems analyzed.[8] The solution could be the introduction of a paradigm shift using a disruptive technology that presents a physiologically relevant *in vitro* model of the human heart and general vasculature system.[43] In contrast to preclinical stages, the project closures in clinical phase are mainly because of efficacy issues. Remarkably, in 40% of the cases of lack of clinical efficacy (Fig. 2B), the reason is no target linkage to disease or no validated models available, whereas other aspects are related to results not fitting preclinical evidence.[8]

Organs-on-a-chip platforms have gained wide scientific attention because of their potential in achieving a physiological resemblance *in vitro*, with a hope to potentially change the way scientists and industry can test the effect of drugs on human cells and organs. Indeed, pioneering work has focused on mimicking cardiovascular tissue *in vitro*.[44–46] These organs-on-a-chip platforms have thus far been able to make interesting additional *in vitro* models. However, to date, it is still difficult to claim that the associated studies have created examples that are adopted by industry, as means toward
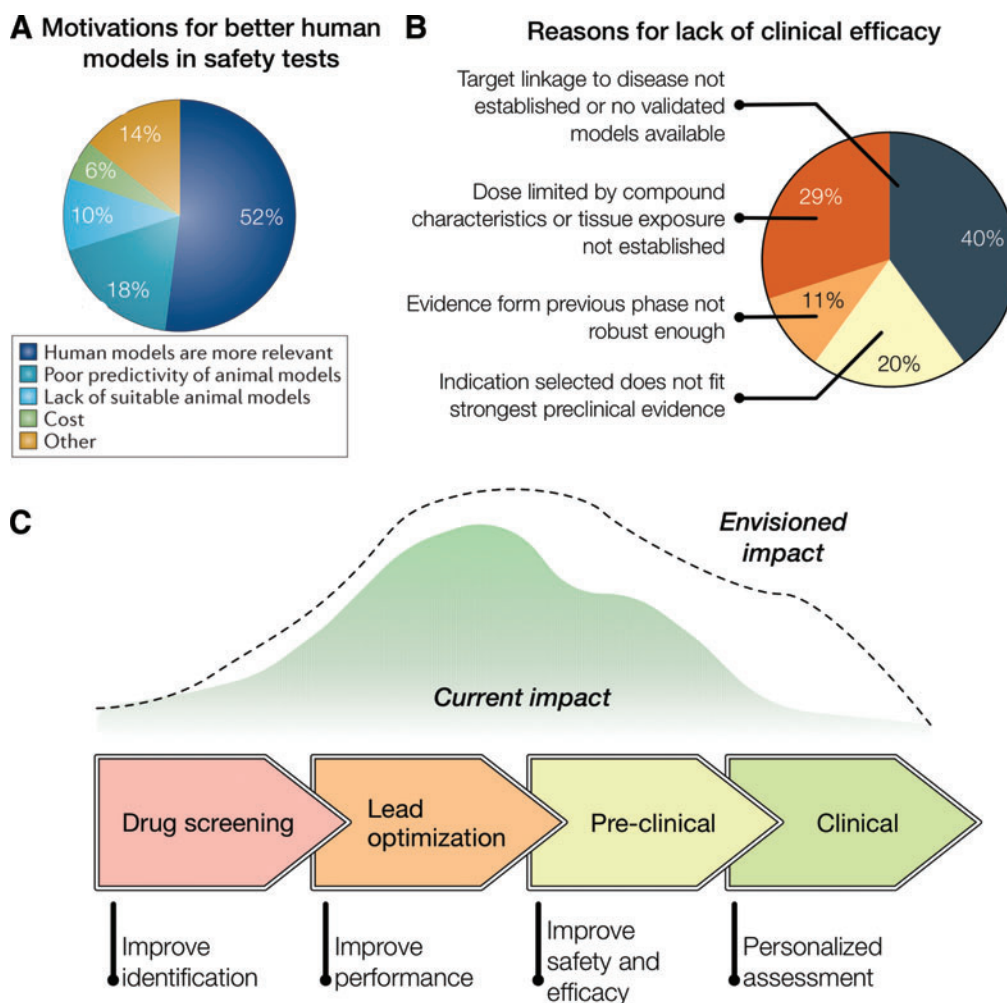

FIG. 2. **(A)** Survey on the motivations behind adopting human tissue-based approaches for safety pharmacology studies.[31] **(B)** AstraZeneca's small-molecule projects from 2005 to 2010. the terminated projects were analyzed to understand the causes for failure and identify potential predictors of success (total of 28 projects).[8] **(C)** Impact of organs-on-a-chip technologies on the drug development pipeline. [**(A)** adapted from Holmes et al.[31]; **(B)** adapted from Cook et al.[8]]. Color images available online at www.liebertpub.com/aivt

cost-effective development of new drugs that would not have been possible otherwise. Even though such models have produced exciting scientific studies, there has not been a major reception for them by the industry. Furthermore, focusing on scalability and ease of handling of the technology would accelerate the adoption of such platforms.

The needs of the industry can broadly be divided into a need for better *in vitro* models predicting the behavior of animal and human organs (i.e., heart), and human organ systems (i.e., heart–vascular interactions). While actual proof of clinical predictability is necessary to make an impact and engage with regulatory agencies, scientific studies to date have demonstrated that their platforms could be potentially capable of performing a high-throughput screen.[47,48] In the path toward achieving broad application by the pharmaceutical industry are two key challenges: guaranteeing clinical relevancy and reproducibility.

Translating cardiovascular organ-on-a-chip models

Once the advantages over current technologies/models are proven, the following step is to have a commercialization strategy. Aside from the academic examples mentioned above, it is also worthwhile considering some of the ventures established toward commercializing and implementing the associated technologies. There are a number of enterprises aiming at commercializing products that encompass single and multiple organs-on-a-chip.[49] These companies have mainly developed products for research laboratories, but ongoing efforts are being made to apply such technologies at a broader scale in the pharmaceutical industry.

The market need from pharmaceutical industry is expected to be high, mainly because of the ever-decreasing number of new drug approvals and the challenges faced with preclinical models. However, these *in vitro* platforms would require regulatory approval for cardiovascular applications, either for heart- and vasculature-on-a-chip systems alone, or for a potential combination of both. An opportunity in this arena is to show comparable results to animal tests, followed by a strong demonstration of reproducibility and clinical significance. Another path for application of cardiovascular organs-on-a-chip could be direct regulatory endorsement. The current platforms required by FDA for such assessments have remained limited to petri dish, animal studies, and limited clinical trials—models still lacking the level of accuracy and predictability required. Here, the dialog with regulatory agencies such as FDA would be a starting point, in order to establish standards expected for such platforms from the regulatory point-of-view.

Some of the open challenges that are being addressed by research teams and companies are related to standardization, optimization, and scaling up for manufacturing (discussed in detail by Vladisavljevic et al.[50]). While the scientific community is geared toward innovating and creating new platforms, the growth of companies in this area could lead to their optimization for standardization, reproducibility, and scaling-up. These key challenges, once tackled, will allow the widespread usage of cardiovascular organ-on-a-chip for drug discovery, while securing regulatory endorsement. The impact of such technologies for drug discovery and development is expected to be vast (Fig. 2C). Current opportunities exist for improving performance at later stages of lead optimization, and safety and efficacy at early stages of preclinical studies. Furthermore, we envision that such technologies would be able to provide significant impact on preclinical stages in the near future, and personalized medicine approaches at a later stage.

Heart-on-a-Chip Platforms in Drug Discovery

Mimicking cardiac environment

Cardiac tissue contractile function is mediated through cardiomyocytes (CMs) that are organized in parallel arrays of myofibril bundles. The contractility of CMs is mediated through chemical, mechanical, and electrical stimuli.[51,52] In addition to CMs, cardiac tissue is composed of fibroblasts and microvessels, which consist of endothelial cells (ECs) and vascular smooth muscle cells. Cellular composition and anisotropic organization, together with continuous exposure to multiple dynamic stimuli, plays a critical role in maintaining cardiac physiological function.[53–55] Microfluidic *in vitro* heart-on-a-chip platforms can provide precise individual control over each these essential factors, allowing for novel opportunities to study cardiac physiology, pathology, and pharmacology. In particular, these platforms may hold great promise for the development of high-throughput assays that could be valuable in drug screening and toxicity studies.[21,22,24,25,56]

Recent advances have enabled the engineering of physiologically relevant microscale myocardial tissues by using microfluidic organ-on-a-chip systems. Such systems are often fabricated using poly(dimethyl siloxane) (PDMS) because of its biocompatibility, nontoxicity, and low cost.[57,58] In addition, PDMS is an elastomeric and transparent material that can be rapidly prototyped and customized into a microfluidic system by using soft and photolithography techniques.[57] However, the hydrophobic nature of PDMS limits the attachment and spreading of cells.[58] This challenge can be addressed by treating the PDMS surface with proteins (e.g., fibronectin) that facilitate cellular attachment.[58] Recently, Annabi et al.[59] developed a technique to overcome this challenge by coating microfluidic channels with hydrogels (Fig. 3A), which provided a suitable environment for on-chip cardiac cell culture. Specifically, methacrylated tropoelastin (MeTro) and gelatin methacryloyl (GelMA) facilitated cellular adhesion inside the microfluidic channels,[59] which was also reported in other cell types.[60,61] The seeded CMs revealed not only an increased attachment, but also proliferation and beating rate on MeTro hydrogels compared to GelMA (Fig. 3B).[62] This was mainly attributable to the presence of cell-interactive amino acid sequences and the more elastic nature of tropoelastin compared to GelMA. In addition, continuous perfusion of medium through the heart-on-a-chip platform provided the possibility to mimic blood flow-induced shear stress on myocardial tissue. Therefore, this platform could potentially be useful to study biomimetic cues on cardiac function. The results of this study suggested that tropoelastin-based hydrogels might offer a suitable microenvironment for proper functioning of CMs *in vitro*. Important properties of tropoelastin that support this are the tunable elasticity and biocompatibility. Moreover, this microfluidic system could be used to study safety and efficacy of drugs under physiologic-like conditions.[62] However, this simplified ECM approach might not recapitulate the complexity of cardiac ECM composition. Further
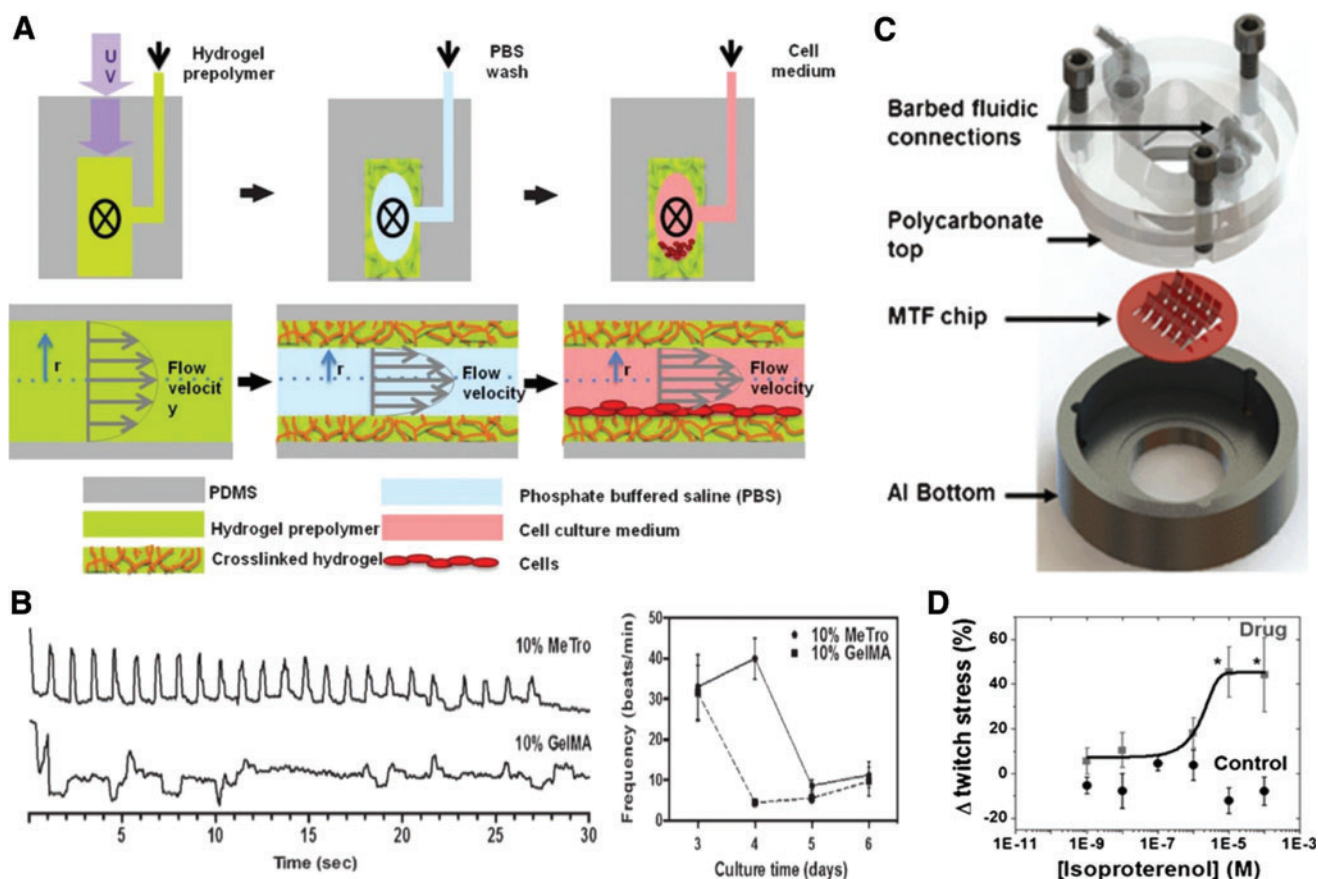
FIG. 3. **(A)** Schematic of a PDMS-based microfluidic heart-on-a-chip model developed to culture cardiomyocytes. These microfluidic channels were coated by gelatin- and tropoelastin-based hydrogels to induce cellular attachment. **(B)** The effect of 10% (w/v) tropoelastin and 10% (w/v) gelatin-based hydrogels on the beating pattern (left) and beating frequency (right) of cardiomyocytes (CMs) inside microfluidic microchannels. **(C)** The schematic of a heart-on-a-chip microdevice designed for pharmacological testing. **(D)** The effect of isoproterenol on the contractility of CM-seeded muscular thin films, showing an increase in beating rate compared to control. Adapted with permission from Annabi et al.[59] and Agarwal et al.[69] Color images available online at www.liebertpub.com/aivt

challenges include identifying the minimal design criteria for ECM composition and organization that are required to properly mimic the heart, which might, among others, require the screening of different ECM compositions in a high-throughput manner.

Although native myocardial cells, scaffold elasticity, and (natural) ECM proteins are critical to engineering biomimetic cardiac tissues *in vitro*, additional components of native heart tissue are required to recapitulate proper cardiac tissue function. Here, we will highlight some of these factors and discuss their use and application in the fabrication of heart-on-a-chip devices.

One of the factors important for the fabrication of heart-on-a-chip devices is electrical stimulation and CM anisotropy. *In vivo*, myocardial pumping arises from the electrical stimulation of CMs by automated pacemaker cells in the sinoatrial node. These electrical signals propagate through the left and right atria to go to the atrioventricular node and eventually reach the right and left ventricles. As a result, the anisotropic and elongated CMs are depolarized, which results in regular and synchronized cardiac muscle contraction. Consequently, external electrical stimulation and an anisotropic organization of CMs may contribute to the development of more physio-

logic *in vitro* heart tissue. Anisotropic alignment of CMs and ECM significantly affects electrical and mechanical functioning of cardiac tissue.[63,64] Integrating structural and functional anisotropy used in cardiac tissue engineering[65] into heart-on-a-chip devices could result in better contractile force generation and better electrical propagation of action potentials.[65] Grosberg et al.[66] used a muscular thin film (MTF) technique to design a heart-on-a-chip with anisotropically organized CMs. MTF platforms were created by microcontact printing fibronectin patterns on a thin, deformable PDMS film, later seeded with primary neonatal rat ventricular CMs. Additionally, the self-organized CMs on the functionalized surface were electrically stimulated by platinum electrodes. By electrically stimulating CMs on the chip, the *in vivo* generation of electrical signals by pacing cells could be recapitulated. Moreover, electrical stimulation of cells increased the cellular alignment, differentiation, and functionality of engineered cardiac tissue.[67] This study showed that contractile behavior and electrophysiological properties such as the action potential morphology of MTFs could be measured successfully. Consequently, these measurements could be used in the evaluation of pharmacological intervention on the contractile function of multiple cardiac microtissues. Using a dose-

range experiment, the effect of epinephrine concentrations on the frequency of contractions was calculated based on the MTF contractility stress profiles. This demonstrated that MTFs in a microfluidic chip can be used to assess contractility in the presence of drugs or physiologic-like cues such as hydrodynamic, mechanical, and electrical stimuli. Although this 2D monolayer of CMs provides an opportunity to analyze deformation of cardiac microtissue in a 3D manner, it is not able to recapitulate the 3D microenvironment of myocardial tissue. A solution to this challenge could be found by combining cellular encapsulation and microengineering strategies to form aligned 3D microtissues.[68]

A similar MTF approach was used to create an aluminum-based microdevice for high-throughput pharmacological testing of isoproterenol (Fig. 3C).[69] This platform provided the advantage of a semiautomated fabrication method that could be scaled up and automated for higher throughput screening of drugs. Moreover, a metallic heating element, electrodes, and a transparent top were incorporated in the device for simultaneous temperature control, electrical field stimulation, and optical contraction analysis, respectively. The microfluidic device's high-throughput capacity was leveraged to test the effect of isoproterenol on the contractile stress of MTFs (Fig. 3D). In this experiment, a series of increasing drug concentrations were tested within the same microdevice. The MTFs showed an increase in contractility stress when compared to control. Furthermore, by using this system, the same level of isoproterenol potency *in vitro* was comparable to the reported values for rat hearts.[69] This microdevice enabled the incorporation of an MTF-based heart-on-a-chip and allowed automatic fluidic control, wash-in and washout after each drug dosage. In addition, this system was shown to be suitable for simultaneous analysis of cardiac tissue contractility during drug compound testing, thus demonstrating its ability to perform rapid and accurate drug analysis. Consequently, this microdevice could potentially improve pharmacological *in vitro* studies on a higher throughput manner that is not feasible with *in vivo* studies.

A cell culture chip to form *in vitro* cardiac microtissues through the application of electrical stimulation and topographical cues was created by Au and colleagues.[70] Here, neonatal rat CMs were seeded and cultured onto polystyrene cell culture chips with integrated topographical microgrooves. CMs showed attachment and alignment along the grooves. In addition, electrical stimulation of the CMs increased the cellular maturation in terms of sarcomeric protein (such as sarcomeric alpha-actinin) and gap junction protein expression. This work highlights the importance of combining several different factors toward achieving an *in vivo*-like cellular response.

Another factor important for the fabrication of heart-on-a-chip device is cardiac tissue perfusion. The complex architecture of myocardial tissue, consisting of cardiac bundles and associated microvessels, can be more accurately recreated by fabricating *in vitro* systems that can recapitulate cardiac tissue perfusion. Xiao et al.[71] designed a more physiologically relevant heart-on-a-chip by creating a perfusable cardiac microtissue with a poly(tetrafluoroethylene) microtubing to induce CMs to align and elongate along the tubing template. This system demonstrated the feasibility of mimicking cardiac tissue by showing spontaneous beating, expression of sarcomeric troponin-T, and gap junction markers (connexin-43). In addition, the functionality of this system as a platform for pharma-

cological studies was examined by testing the system with nitric oxide (NO). After exposure to NO for 24 hours, the beating rate of the CMs was significantly decreased in comparison with the untreated control. These results indicated that this platform could serve as suitable biomimetic *in vitro* system for both drug development and drug-induced changes in beating behavior.

Cardiac disease models

Many stem cell-based, tissue-engineered, and organ-on-a-chip systems for *in vitro* modeling of cardiac disease have been developed. Cardiac diseases such as rhythm disorders[72,73] and dilated cardiomyopathy[74] have been studied by using induced pluripotent stem cells (iPSCs) in monolayer disease models. In addition, cardiac conditions, including MI and re-entry arrhythmia, have been successfully re-created in 2D tissue-engineered heart models.[34] Although these models were able to represent specific phenotypic disease characteristics, they still lack the incorporation of proper environmental factors such as 3D cell–ECM and cell–cell interactions. To overcome this hurdle, several biomaterials have been mixed with different cell sources (e.g., iPSCs, rat neonatal ventricular cells) toward the development of 3D structures of cardiac disease models.[35,75–77] Recent advances in the field of microfluidics have allowed researchers to construct novel heart-on-a-chip systems for disease modeling and drug testing. Next, we will summarize some of the recent findings on the development of these microfluidic systems.

Several chronic CVDs, such as hypertension and aortic valve stenosis, can cause pressure overload in the left ventricle and lead to cardiac hypertrophy and fibrosis.[78] Increased mechanical stress during disease is a critical factor that drives the initiation of hypertrophy, fibrotic remodeling, and heart failure.[79,80] These dynamic changes are partly attributable to mechanotransduction, a process in which external mechanical forces are translated into internal chemical and electrical cellular responses.[81] Therefore, it is important to develop an *in vitro* heart-on-a-chip model that can provide insight into the pathophysiology of this phenomenon. McCain et al.[48] successfully applied an MTF platform toward the creation of a failing myocardium-on-a-chip.[82] Mechanical cyclic stretch of the MTFs resulted in pathological tissue remodeling, with several genetic indicators of pathological remodeling being upregulated. Furthermore, by the application of transverse stretch, structural organization of micropatterned CMs was disrupted, resulting in disorganized CMs similar to hypertrophic cardiomyopathy (Fig. 4A).[48,82] Further development of similar heart failure on-a-chip models could potentially aid in a better understanding of cardiac remodeling, while providing valuable tools for pharmaceutical industries.

Ren et al.[83] fabricated a heart-on-a-chip to study hypoxia-induced myocardial injury. By designing a PDMS-based microfluidic device with four functional units, the interface of myocardial tissue and microcapillaries was mimicked *in vitro*. The four units consisted of a central cell culture chamber and two lateral channels that were separated from each other by micropillar arrays, representing the interface between the blood vessel and cardiac tissue (Fig. 4B). To mimic hypoxia-induced conditions, a specific oxygen consumption blocking reagent, FCCP (cyanide *p*-trifluoromethoxyphenylhydrazone), was introduced into one of the side channels (Fig. 4C).[83]
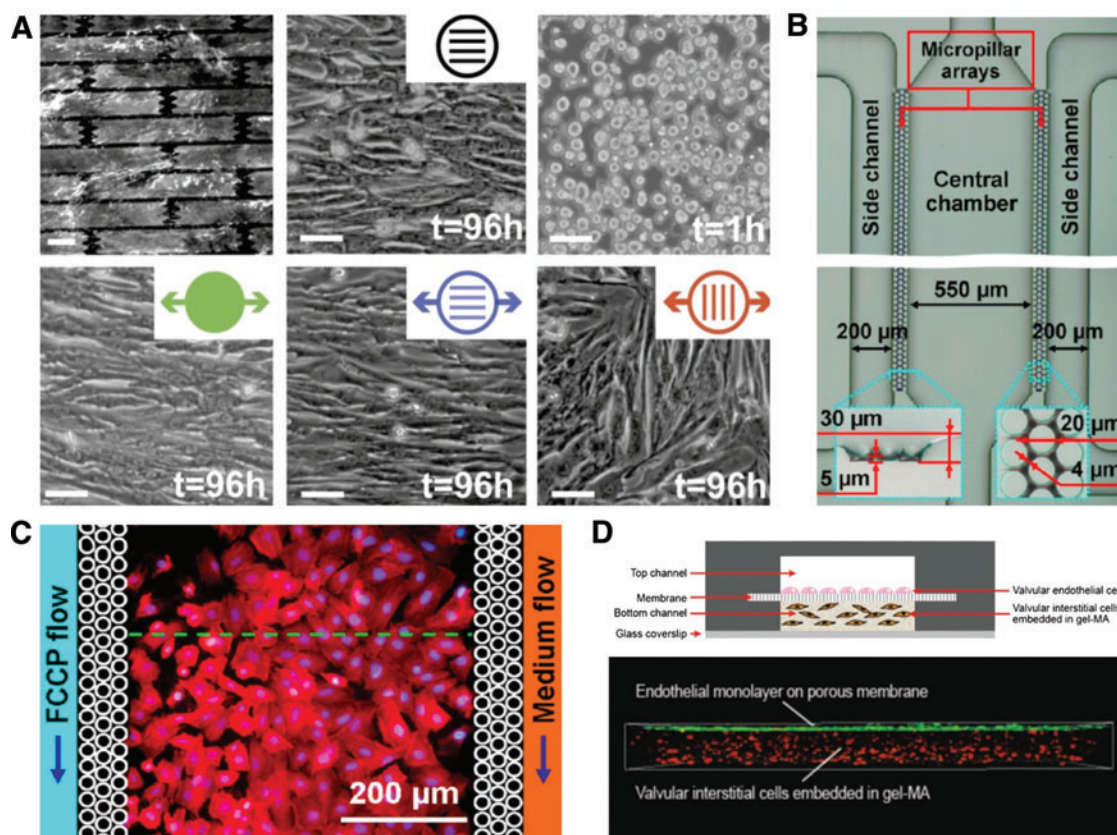
FIG. 4. **(A)** The effect of transverse mechanical stretch showing a disorganized architecture of cardiomyocytes cultured on muscular thin films (scale bar = 50 μm).[48] **(B)** Schematic illustration of a microfluidic heart-on-a-chip model that ressembled the native interface of myocardial cells and microcapillaries. Reprinted with permission from Ren et al.[83] (copyright 2013 American Chemical Society). **(C)** Representation of fluorescence images of cytoskeletal changes in cardiomyocytes under hypoxic conditions, which were induced by the introduction of an oxygen consumption blocker in one of the microfluidic side channels. Reprinted with permission from Ren at al.[83] (copyright 2013 American Chemical Society). **(D)** Schematic representation of a bi-compartmental co-culture of valvular endothelial (VEC) and valvular interstitial cells (VIC) (top panel). VECs were embedded on a thin and porous membrane on top of a VIC-loaded gelatin methacryloyl (GelMA) (bottom channel).[86] Color images available online at www.liebertpub.com/aivt

CMs presented normal attachment, spreading, and proliferation under normoxic conditions, but underwent morphological changes such as shrinkage and disassembling of intracellular actin bundles after induction of hypoxia. During myocardial ischemia (or MI) there is an increased CM apoptosis, a feature that was accurately recapitulated within this device.

Various (stem) cell transplantation therapies for cardiac repair after MI have gained considerable attention in the past several years.[84] Many groups have explored the potential of numerous cells types such as embryonic stem cells, cardiac progenitor cells, skeletal myoblasts, and iPSCs.[84] To study the interactions as well as the potential role of skeletal myoblasts in the repair of hypoxia-injured CMs, a comparable microfluidic device was created in a follow-up study by He et al.[85] In this system, skeletal myoblasts and rat CMs were co-cultured in separate chambers that were connected through permeable microvalves. The results of this study demonstrated that skeletal myoblasts were capable of repairing the hypoxia-injured CMs through cell–cell interactions.

Hydrogels and microfluidic systems that are developed to mimic myocardial tissue could also be leveraged to mimic valvular tissue and disease.[86] Chen et al.[86] used a multicompartmental approach to design a valvular and vascular compartment in a 3D microenvironment, allowing cells to communicate through paracrine signaling (Fig. 4D). A thin porous membrane separated the cells and enabled the paracrine communication between valvular endothelial cells (VECs) and valvular interstitial cells (VICs). Fibroblast-like VICs were encapsulated in GelMA hydrogels and VECs seeded on fibronectin-coated PDMS microchannels. In addition, VECs could be stimulated physiologically by the introduction of flow-induced shear stress. Activation of quiescent VICs into alpha smooth muscle actin (α-SMA)-expressing myofibroblasts are thought to be one of the hallmarks of valvular pathological remodeling, because of their increase in ECM synthesis and contractile features.[87,88] Myofibroblasts have the ability to increase calcium deposition by differentiating into osteoblast-like cells.[89] Consequently, myofibroblasts play an important role in calcific aortic disease and valvular fibrosis.[90,91] A study by Chen et al. showed that VECs are able to inhibit VIC activation under both static and dynamic fluid flow, which is in line with *in vivo* conditions.[86] This biomimetic microfluidic system

could be used to study biological and pathological VEC-VIC interaction, as well as drug-associated toxicity. In addition, this platform may be an appropriate testing model for potential drug candidates in treatment of aortic valve disease.

Recent developments in stem cell biology have further enabled the use of patient-specific iPSC-derived CMs for generating *in vitro* models of cardiac disease.[92] These advances may lead to the creation of physiologically and genetically relevant models of cardiac disease for drug discovery and drug toxicity.[93] By using iPSCs, MTF, and microfluidic technologies, Wang et al.[94] successfully engineered the cardiomyopathy of Barth syndrome (BTHS) in an *in vitro* disease model.[94] iPSCs were generated from patients with BTHS and subsequently differentiated into patient-specific iPSC-derived CMs. In this study, the pathophysiology of BTHS cardiomyopathy was demonstrated by seeding iPSC-derived CMs on MTFs. The BTHS-derived cardiac microtissues were shown to have impaired sarcomeric structures as compared to control. Additionally, MTFs from BTHS iPSC-CM revealed a significantly lower contractile performance compared to controls under the same conditions. Moreover, this ''BTHS cardiomyopathy on-a-chip'' was used to test potential therapeutic options (e.g., pharmacological and genetic modifications), indicating its suitability for identifying new therapeutical targets.

All together, these studies point toward a better understanding of CM physiology under a range of dynamic conditions. The new *in vitro* models of heart-on-a-chip present a strong step toward testing cardiotoxicity in human-relevant models, but models of disease are still an area filled with challenges and opportunities. In particular, we propose that heart disease-on-a-chip models represent a high-value area for new drug screening platforms.

Vasculature-on-a-Chip Platforms in Drug Discovery

Mimicking vascular environment

It is challenging to mimic the vascular environment of the human body *in vitro*. Arteries, arterioles, veins, venules, and capillaries are all part of the vascular system, but differ in the structural and cellular compositions. In addition, blood vessels are subject to a range of biophysical stimuli because of the pulsatile nature of blood flow. ECs lining the lumen of vessels experience flow-induced pulsatile wall shear stress and transmural pressure. ECs and vascular smooth muscle cells (VSMCs) both experience cyclic mechanical stretching, which causes the vessels to increase in diameter in response to blood flow. Hemodynamic parameters contribute to the maintenance of homeostasis in the vessel wall, with several microfluidic models studying the effects of hemodynamics *in vitro*.[95–98] Because the vascular system is constantly under these biophysical stimuli (Fig. 1), microfluidic technologies are an advantageous way to recapitulate these forces. Recently, several microfluidic-based platforms have been described for distinct vascular applications,[97] including the formation of tissue-engineered vascular networks, angiogenesis, and vascular disease models. The end goal of such models is to gain mechanistic and contextual insight on the vascular biology processes, while providing a superior platform for drug discovery and development.

The use of hydrogels in microfluidics is a simple platform to provide relevant 3D matrixes to vascular cells, while main-

taining the ability to apply shear stress. Kim et al. used the flexibility of PDMS to generate a microfluidic device (Fig. 5A) capable of containing fibrin matrices.[99] The multichannel device enabled the study of vasculogenesis and angiogenesis, and the fibrin matrix was able to support cell growth. Kim and colleagues were able to show the formation of a perfusable vascular network, and the maintenance of barrier function. Additionally, the vascular networks under flow exhibited an increased nitric oxide production compared with static conditions[99] (Fig. 5B, C). Similar methodologies have been used with different hydrogels[100–103] with the end goal of creating vascular networks *in vitro* to study different aspects of vascular biology. Morgan et al.[102] used type I collagen gels with embedded cells and hollow channels that were later seeded with ECs. This simple system allows for the creation and design of defined microvascular endothelialized geometries that are useful for the study of permeability and blood–vasculature interactions in a tissue-engineered construct.[102] In another example, Wang et al.[103] utilized sodium alginate as artificial templates to cast a hydrogel mixture containing gelatin, agarose, and collagen. The resulting matrices were seeded with HUVECs, and displayed good barrier properties and response to flow. Other fibrin-based approaches have been pursued in combination with other cell types.[101,104] Hasenberg et al.[101] aimed to incorporate capillary networks in fibrin gels for multi organ-chip constructs; these vascular networks were stable in serum-free media, which is an important aspect in designing such systems for drug development. Schimek et al.[105] designed a similar microfluidic chip, which focused on designing an on-chip peristaltic micropump to provide the vascular network with pulsatile shear stress.

Others have used different fabrication techniques such as bioprinting to produce vascular networks.[100] In one example, Bertassoni et al. utilized bioprinting of template sacrificial layers to generate vascular networks in hydrogels (Fig. 5D). Combined with UV-crosslinkable GelMA hydrogels, this bioprinting methodology allows an on-demand precise control of microarchitectures, yielding potentially complex structures that can mimic different vascular regions (Fig. 5E, F) observed in the human body.[100] This bioprinting approach can generate 3D constructs in a fast, controllable, and inexpensive way, which can be useful for testing drug compounds in a wide range of vascular flow settings and on a large scale. These technologies[106] aim to create 3D vascular beds utilizing a wide range of hydrogels. This allows the fine-tuning of mechanical properties and ECM cues to target the specific regions of interest in the vascular system. These systems can have the potential to provide meaningful impact on drug discovery by mimicking *in vitro* vascular tissue microenvironments.

Conversely, some studies have used microfluidics in combination with *ex vivo* vascular segments.[47,107] Gunther et al.[47] generated a small but complex microfluidic device that captured a mouse mesenteric artery segment and held it in place. Once this segment was fixed, it could be imaged and exposed to drugs through the lumen or external wall (Fig. 5G, H). The contraction or dilation of the artery segment was then imaged upon drug exposure. The exposure to phenylephrine in this system resulted in a segment contraction in a dose-dependent way (Fig. 5I). Although this represents a major step forward in microfluidics for vascular drug
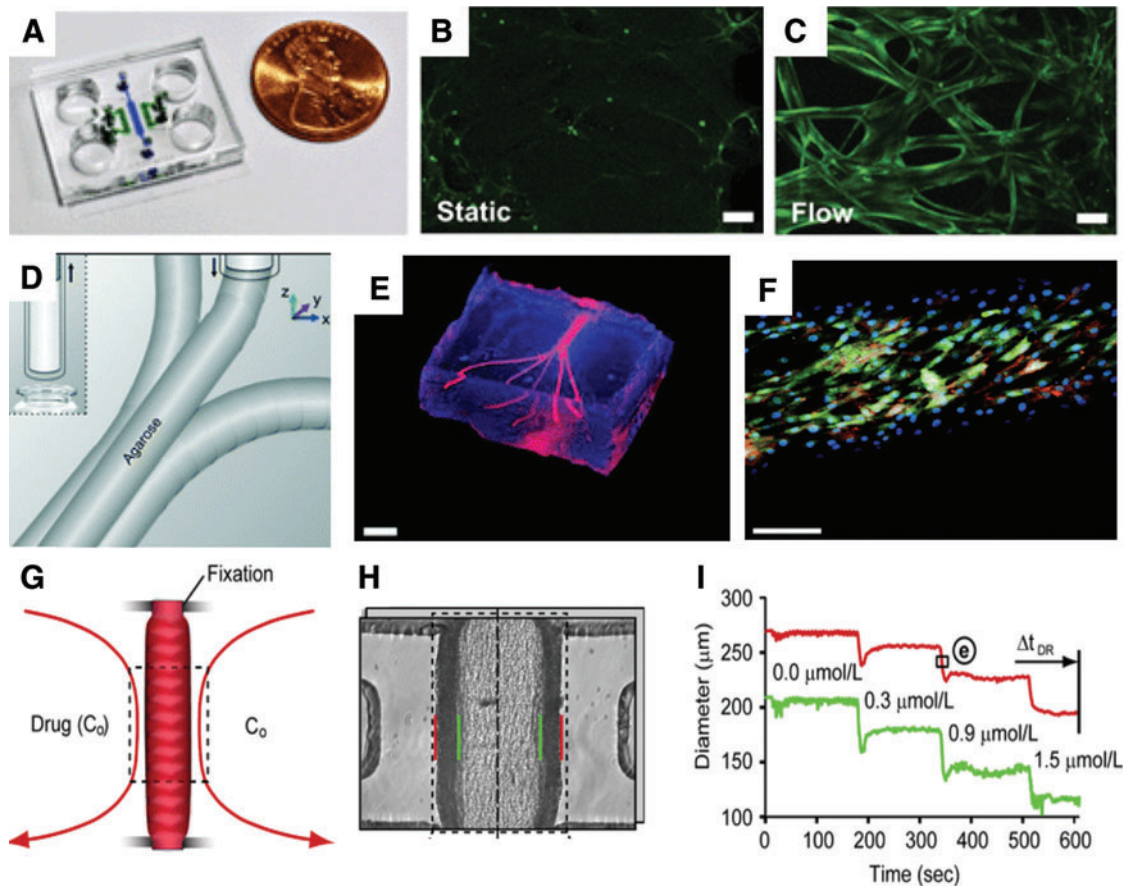
FIG. 5. *In vitro* microfluidic models to study vascular networks and vascular functions. **(A)** Microfluidic chip for forming vascular networks and to study angiogenesis. **(B, C)** Nitric oxide production in static and flow conditions inside the device A (scale bar = 50 μm). **(D)** Bioprinting methodology to produce vascular networks with agarose sacrificial layers. **(E)** Bioprinted network (scale bar = 3 mm). **(F)** Bioprinted vascular network with ECs (green, GFP; blue, DAPI; red, CD31; scale bar = 250 μm). **(G)** Artery-on-a-chip model where a small mouse artery segment is held in place and perfused through the lumen and outside walls. **(H)** Microphotograph of artery-on-a-chip model (red, outer side wall; green, inner side wall). **(I)** Response of the artery-on-a-chip to phenylephrine. [**(A–C)** adapted with permission from Kim et al.[99]; **(D–F)** adapted with permission from Bertassoni et al.[100]; **(G–I)** adapted with permission from Gunther et al.[47].] Color images available online at www.liebertpub.com/aivt

discovery and development, the usage of animal vessels limits its application and translational relevance to humans.

Vascular disease models

Rosano et al.[108] mapped a mouse cremaster muscle and used it to build a PDMS mold of the vascular network. This biomimetic vascular model can be analogous to human vascular structures, possessing different geometries and regions with different shear stresses. Such models can comprise complex bifurcation and geometric intricacies inherent to vascular networks that change the way cells interact with drugs. Importantly, they can potentially be applied to generate individualized vascular networks and simulate scenarios of thrombus or atherosclerosis formations.

Besides recreating vascular structures *in vitro*, researchers have also pursued models to study angiogenesis.[109,110] Galie et al.[110] highlighted the influence of shear stress in angiogenic sprouting. Both wall shear stress and transmural flow in the endothelium above a certain threshold (>10 dyn/cm^2) induce endothelial sprouting. Local narrowing of blood ves-

sels induces high shear stress regions, which can trigger further sprouting. Such information can be valuable in the context of pharmacological interventions that promote or inhibit angiogenesis. In a cancer setting, vascular microfluidic tools can help identify molecules inhibiting tumor angiogenesis and help elucidate the relationship of tumor extravasation in the vasculature. In order to better understand the early phase of metastatic processes, some microfluidic models have been designed to study the process of tumor extravasation.[111–113] Buchanan et al.[111] used co-cultures of MDA-MB-231 and ECs to study tumor angiogenesis. Interestingly, high wall shear stress had protective effects, downregulating several genes important for tumor angiogenesis such as *MMP9*, *HIF1*, and *VEGFA*. Jeon et al.[112] used a microfluidic device to develop a 3D model of metastatic breast cancer extravasation in different environments. Using a bone- and muscle-mimicking environment, extravasation rates were found significantly increased in the bone-mimicking models. This microfluidic model elucidated the effects of different microenvironments and their effect in cancer cell extravasation. Additionally, the blockage of A$_3$ adenosine receptors in cancer
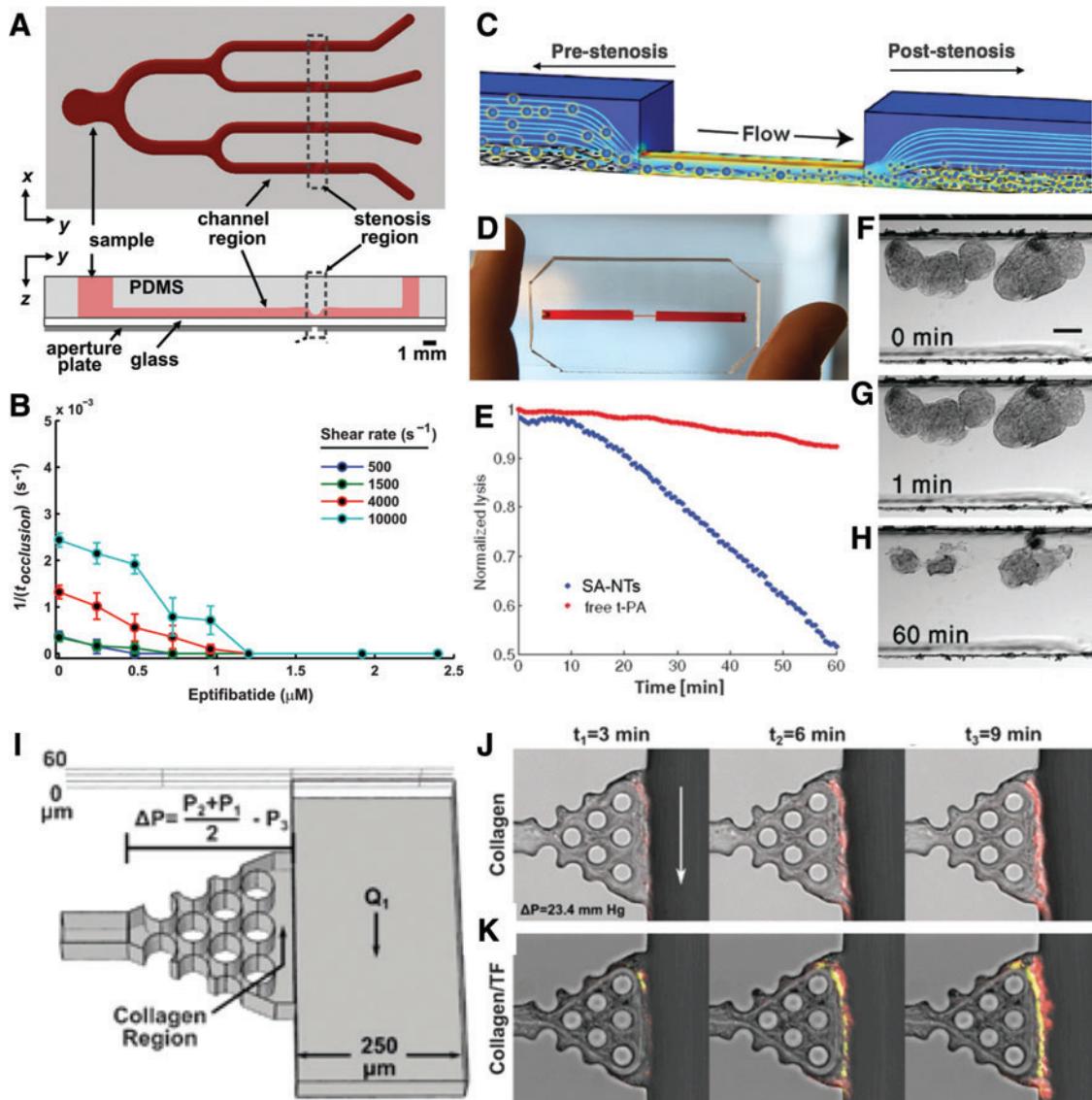
FIG. 6. Vascular models to study stenosis and thrombosis *in vitro*. **(A)** Microfluidic model of stenosis to study the effect of drugs on occlusion. **(B)** Effect of drug epitifibatide concentration on dissolving clots over time. **(C)** Microfluidic stenosis model used to test shear-activated microparticle formulation. **(D)** Photograph of the microfluidic device. **(E)** Exposure of clots to free or encapsulated t-PA (shear-activated microparticles). **(F–H)** Time-dependent thrombolysis of clots upon exposure to encapsulated t-PA. **(I)** Microfluidic device to study thrombosis *in vitro* containing a flow region (Q1) and a collagen region where pressure gradient is varied (ΔP). **(J, K)** Thrombus formation on collagen and collagen/TF hydrogels (white arrow indicates flow direction). [(A, B) adapted with permission from Li et al.[115]; (C–H) adapted with permission from Korin et al.[118]; (I–K) adapted with permission from Muthard et al.[116].] Color images available online at www.liebertpub.com/aivt

cells resulted in increased extravasation, enabling a deeper mechanistic insight into cancer extravasation, while also providing a valuable platform to test new pharmaceutical agents.

Several microfluidic platforms have been used for *in vitro* drug screening[114] and for the development of drug delivery systems.[24] Microfluidic models can be applied to study thrombosis,[115,116] occlusion,[117] and stenotic regions.[118] Li et al.[115] designed a microfluidic system (Fig. 6A) to study stenotic regions and thrombus formation under different shear rates. Low shear rates lead to longer occlusion times. However, the administration of increasing concentrations of the antiplatelet eptifibatide (Fig. 6B) in high shear stress

did not reduce the occlusion times compared to no drug. This highlights the need of studying drug effects, *in vitro*, together with relevant biophysical stimuli such as shear rates. Another system that mimics a stenotic region (Fig. 6C, D) was used to design a "smart" drug.[118] Blood vessels are often narrowed in thrombotic regions. At this site, wall shear stress can rapidly increase by two orders of magnitude. Korin et al.[118] engineered microparticles that were responsive to shear stress that would thus breakup into smaller nanoparticles when exposed to the shear stresses observed in stenotic regions. By incorporating tissue plasminogen activator (tPA) in the nanoparticles, Korin et al. were able to

show local delivery and rapid thrombolysis (Fig. 6E–G). This innovative platform illustrates how microfluidic biomimetic vascular systems can be used in the context of drug development. In this field, researchers have used the physical properties of the stenotic regions to target drugs, thus avoiding the need for systemic delivery of tPA and the resultant adverse side effects. Other vascular models have been used to study drug carriers and physical characteristics.[24,119,120] Thrombotic and stenotic models are closely related; Muthard et al.[116] engineered a microfluidic system (Fig. 6I) to probe the effects of wall shear stress and transthrombus pressure gradients in the thrombogenesis. In this system, a side flow region was filled with collagen gel with the pressure across it controlled by computer. The collagen area was exposed to the main fluidic channel and visualized directly on the device. As expected, the perfusion of whole blood induced thrombus formation at the collagen site (Fig. 6J, K). Interestingly, by varying the pressure gradient in the trans-thrombus collagen area, the authors observed a decrease in thrombin with higher pressure-gradients. This device can act as a useful tool to assess thrombotic areas and the hemodynamics of pressure gradients in the vessel wall.

Vascular permeability and dynamic condition models

Studies have also been conducted to evaluate the involvement of ECs in wound healing.[121] In order to better understand the vascular wound healing processes, Franco et al.[121] focused on the combined effect of flow and topographical cues on endothelial migration. A device was fabricated combining 1-μm-grated surfaces and a fluid flow parallel to these gratings. Interestingly, ECs cultured in such surfaces exhibited a much faster migration velocity compared to flat surfaces under the same flow rate. The research highlights the role of surface modifications and topographical micro-

architectures, particularly ones mimicking the basal matrix, in wound healing. Therefore, in certain settings, wound healing might benefit from approaches that change topography, in addition to pharmacological interventions.

In a recent study by Arends et al.[122] the authors developed a microfluidic device to probe the diffusive transport of analytes through basal lamina interfaces. By using ECM gels made from Engelbreth–Holm–Swarm sarcoma of mice, the authors were able to study permeability and the accumulation of different molecules at the interface. The accumulation of molecular at the ECM gel interface was charge-selective, and the results were corroborated in vivo. The system provided a platform to screen drugs and evaluated how changes in properties could affect interactions with the vascular basal lamina. Several other systems have been developed to study the permeability changes in the vasculature.[123–127] Microfluidic collagen gels were fabricated[123,125] and seeded with ECs to study barrier functions and permeability. Chrobak et al.[123] showed that cells formed a strong barrier in these gels and responded to inflammatory stimuli by rapidly decreasing barrier permeability and increasing leucocyte adhesion. Price et al.[125] demonstrated that low flows were related to higher permeability, whereas higher flows promoted a stronger barrier function, while increasing the lifespan of the endothelial constructs in vitro. This demonstrates the effects of biophysical parameters such as shear and transmural pressure in mediating barrier functions. Additionally, Lee et al.[124] designed a simple microfluidic device to generate a tubular, perfusable microvessel network. Similarly, Chrobak et al.[123] found that microvascular networks were responsive to inflammatory stimuli such as histamine and TNFα.[124] Furthermore, U87MG cancer cells were used to mimic high-permeability conditions seen in neoplastic settings. A drug administered in vivo—bevacizumab—was able to reinstate permeability and improve barrier functions in agreement with other in vivo data.[124] A more complex system
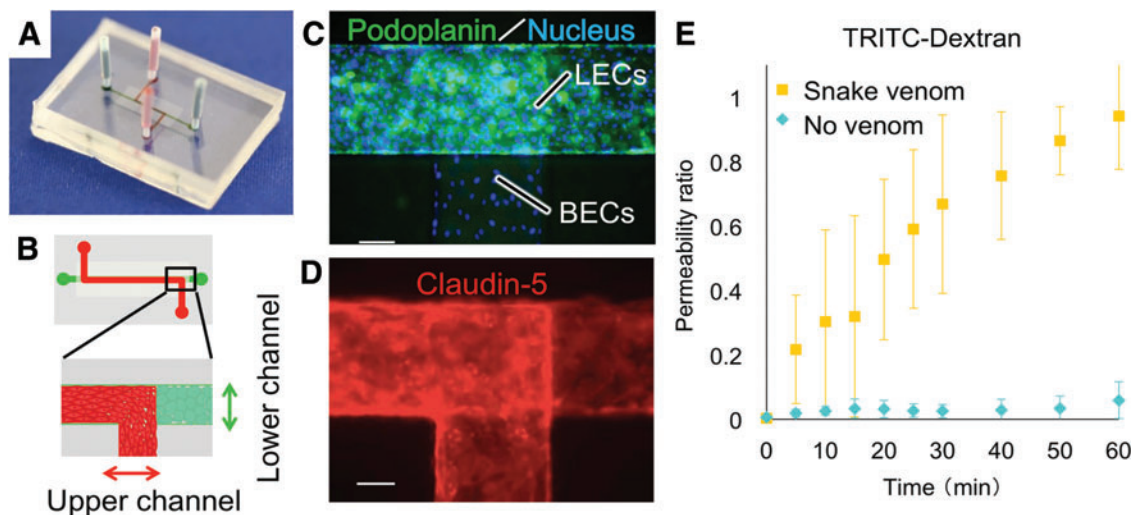

FIG. 7. Microfluidic model to study vascular permeability. **(A)** Photograph of microfluidic dual-channel model to study permeability under shear stress; **(B)** Schematic of the microfluidic device with the upper channel containing blood endothelial cells (BECs) and the lower channel containing lymphatic endothelial cells (LECs), separated by a porous membrane. **(C)** Immunostaining for LECs marker podoplanin (green) and DAPI (blue). **(D)** Immunostaining with endothelial-specific marker claudin-5. **(E)** Changes in permeability induced by exposure to habu snake venom. Adapted with permission from Sato et al.[126] Color images available online at www.liebertpub.com/aivt

envisioned by Sato et al.[126] focused on barrier function and the vascular permeability of ECs and lymphatic ECs (LECs). The microfluidic chip (Fig. 7A) consisted of two channels separated by a porous membrane (Fig. 7B). LECs (Fig. 7C) and ECs (Fig. 5D) were cultured and maintained in different channels. With flows mimicking that of blood and lymphatic vessels, transport across and from EC channel to LEC channel was observed. In accordance to the aforementioned reports, histamine stimulated also the increase in permeability, and the authors further tested the system with habu snake venom (Fig. 7E), showing its effect on the barrier function.[126] With the development of vascular permeability assays, it is essential to track changes in permeability and other parameters in real time. Young et al.[127] developed a microfluidic system and technique to measure real-time endothelial permeability by using laser-induced fluorescence. Additional work developed by Li et al.[128] generated a vascular lumen integrated with a nanowire array that was capable of measuring nitric oxide produced by ECs. Such systems could pave the way to the application of different permeability and barrier function assays within ECs.

In addition to a considerable amount of research on vascular networks and fluid flow, some studies have incorporated additional biophysical hemodynamic parameters.[129,130] Shao et al.[129] generated a microfluidic chip that applied pulsatile and oscillatory shear stress to ECs, and Zheng et al.[130] focused on the combined effects of shear and stretch. Together, these works highlight the need to incorporate complex dynamic conditions in order to fully mimic the vascular microenvironment.

There has been an increasing amount of work developed to recreate vascular networks *in vivo*. By combining biomaterials, microfluidics, and microarchitectures, we expect to be able to mimic the vascular wall in different settings, modeling disease, and ultimately generate better *in vitro* models for drug discovery and development.

Conclusions

Mimicking the cardiovascular system is challenging because of its high-level dynamics such as blood flow, stretching, and electrical stimulation. Several approaches have produced a wide range of microfluidic-based organs-on-a-chip to study different aspects of the cardiovascular system. Some of these have been used, in an academic context, to test and design different drugs and formulations. Although they present a tremendous leap forward for scientific knowledge, further work is required to translate and apply such technologies in drug discovery and development in industry.

Toward the translational pathway, a few startup companies have emerged, having raised significant amounts of funds and starting collaborations with industry partners. Here, we highlighted also the importance of regulatory agencies in the process of identifying new models and addressing concerns of reproducibility and standardization across the industry.

Although promising, cardiovascular organs-on-a-chip have not revolutionized the drug discovery process as of yet, and key challenges still lay ahead. Partnering industry with multidisciplinary academic teams can leverage the translational potential and help accelerate the development of technology. In short, the current progress and opportunities discussed highlight that cardiovascular organs-on-a-chip models still hold great promise for streamlining the drug discovery process.

Author Disclosure Statement

No competing financial interests exist.

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