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Microfluidic chips for the endothelial biomechanics and mechanobiology of the vascular system

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Abstract: Endothelial cells arranged on the vessel lumen are constantly stimulated by blood flow, blood pressure and pressure-induced cyclic stretch. These stimuli are sensed through mechanical sensory structures and converted into a series of functional responses through mechanotransduction pathways. The process will eventually affect vascular health. Therefore, there has been an urgent need to establish *in vitro* endothelial biomechanics and mechanobiology of models, which reproduce three-dimensional structure vascular system. In recent years, the rapid development in microfluidic technology makes it possible to replicate the key structural and functionally biomechanical characteristics of vessels. Here, we summarized the progress of microfluidic chips used for the investigation of endothelial biomechanics and mechanobiology of the vascular system. Firstly, we elucidated the contribution of shear stress and circumferential stress, to vascular physiology. Then, we reviewed some applications using microfluidic technology in angiogenesis and vasculogenesis, endothelial permeability and mechanotransduction, as well as the blood-brain barrier under these physical forces. Finally, we discussed the future obstacles in terms of the development and application of microfluidic vascular chips.

Introduction

The vascular system, composed of arteries, capillaries, and veins, is essential in maintaining the physiological activities of the human body. Arteries carry oxygenated blood to various organs. This blood contains high-concentration oxygen, abundant nutrients, and immune cells. The veins then transport the deoxygenated blood to the heart, and the two kinds of vessels are connected by capillaries (Aird, 2004; Alitalo *et al.*, 2005; Chiu and Chien, 2011). Despite their unique functions, the inner surface of each vessel is comprised of endothelial cells (ECs) that are directly exposed to the blood. As a result, ECs are constantly affected by hemodynamic forces, including the wall shear stress (WSS) and cyclic circumferential stress (Hahn and Schwartz, 2009; Chatterjee, 2018; Campinho *et al.*, 2020). More detail on the hemodynamics in vasculature was reviewed recently by Secomb (Secomb, 2016). Besides, ECs produce a variety of molecules and hormones that play vital roles in vascular homeostasis, local cellular growth, and inflammatory responses (Baratchi *et al.*, 2017). Based on these physiological features, ECs were sometimes considered to be a dependent organ and used to study vascular physiopathology.

Cells convert mechanical stimulus into electrochemical activity, which is called mechanotransduction. In blood vessels, vascular cells convert cell-generated forces or mechanical stimuli from the extracellular environment into biochemical signals and induce downstream cellular responses in the vascular system (Wang, 2017). Specifically, it has key effects on angiogenesis as well as vascular integrity and remodeling in capillaries. And for arterial and venous ECs, it is crucial to several cellular activities such as regulation of the cytoskeleton structure, cell differentiation and permeability, the expression of cytokines and adhesion molecules, and the communication between cells (Zhou *et al.*, 2014; Gordon *et al.*, 2020).

There were multiple *in vitro* macro-fluidic systems designed to recapitulate significant characteristics of *in vivo* flow environment. These systems were mainly modified from parallel-plate flow chamber (Frangos *et al.*, 1985; Wang *et al.*, 2016) and cone plate viscometer (Dewey *et al.*, 1981;

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Spruell and Baker, 2013). In these platforms, ECs directly exposed to shear stress and molecular signaling and gene expression of ECs under flow shear stress could be analyzed. Compared with conventional *in vitro* macro-fluidic systems, the fabrication, mechanical control and chemical analysis of the microfluidic systems were much more efficient and economical (Young and Simmons, 2010). For instance, the microfluidic systems with superior microfabrication technology brought achievable models, which improve our understanding of molecules and cells in the vascular system in various mechanical environments (Lee *et al.*, 2018; Skorupska *et al.*, 2018; Castiaux *et al.*, 2019). These microfluidic systems were able to provide precise fluidic control because the micro-channels can be designed with great flexibility and microfluidic flows are laminar (Duncombe *et al.*, 2015). Besides, molecule and cell collection are easier in these microsystems, which brought great convenience for cell collection, cell sorting, and high throughput analyses (Chen *et al.*, 2008; Halldorsson *et al.*, 2015).

Here, we elucidated the contribution of shear stress and stretch to ECs. Cellular responses, including the adaption of the cytoskeleton, the secretion of mechanical responsive molecules, and cell-cell communication, were described. Then, we introduced two categories of recent microfluidic chips mimicking the mechanical environment of vessels: independent vascular systems with only ECs, and organ-on-a-chip models incorporated with ECs, which focused on organ-specific microvascular function. We also provided some microfluidic chips for vascular diseases such as atherosclerosis. Finally, we discussed the limitation and future perspectives of vascular microfluidic chips.

The Hemodynamics and Mechanotransduction of Endothelial Cells in Blood Vessels

The physiological function of ECs could be affected by the blood flow (Fig. 1). It is one thing to know that shear stress can influence ECs through plenty of mechanical responsive protein factors on the cytoskeleton, and quite another to know when ECs are exposed to different mechanical stimulate, specific mechanotransduction will be triggered. Those physical stresses will be transformed into biological signals secreted by ECs. It has been proposed to many mechanotransductions, including ion channels, platelet-endothelial-cell adhesion molecule-1 (PECAM-1), vascular endothelial cadherin (VE-cadherin), and Piezo 1, function in sensing blood flow. This section summarizes the hemodynamic forces in blood vessels and endothelial mechanotransduction pathways, including ECs cytoskeleton, ion channels, junctional proteins, and some membrane proteins.

The complex hemodynamic forces acting on endothelial cells in blood vessel

Blood flow and cyclic stretch are typical forms of hemodynamics in vessels, which have two main characteristics. Firstly, they are time-dependent and change with the progress of the cardiac cycle. Secondly, it is also spatially variational because of the complex geometry features of blood vessels such as branching, curvature, taper, and torsion.

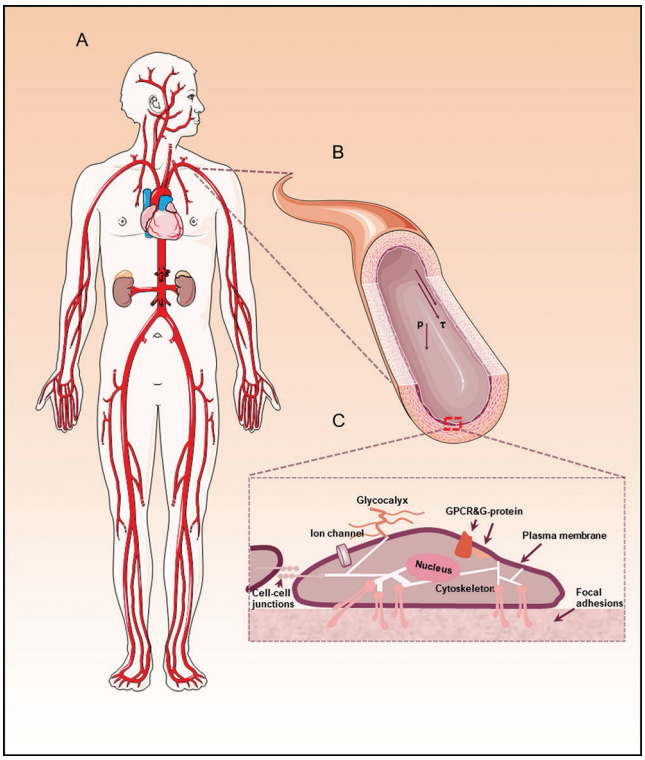

FIGURE 1. The hemodynamics and mechanotransduction of endothelial cells.
(A) Arteries in the human circulation system. (B) The hemodynamics on endothelial cells. Pressure (p) is a common physical stimulus that can result in circumferential stretching of the ECs. Wall shear stress (τ) is exerted longitudinally along the direction of blood flow (Hahn and Schwartz, 2009). (C) The mechanotransduction of ECs: Many mechanotransductions including the plasma membrane, membrane-embedded G-proteins and G-protein-coupled receptors, glycocalyx, ion channels, focal adhesions, junction proteins, and nucleus, were reported to function in sensing blood flow (James and Allen, 2018).

Blood flows and their patterns

Patterns of the flows in straight and bifurcating vessels are different. In straight vessels, the flow pattern is laminar, and the WSS on the ECs is largely unidirectional. In contrast, the flow pattern at curvatures of the vessels is usually disturbed and bidirectional. The flow through the branch vessels results in flow separation with transient flow reversals and occasional turbulence (Davies, 2007). In recent years, helical flow patterns in the vascular system induce great attention (Morbiducci *et al.*, 2011; Gallo *et al.*, 2012; de Nisco *et al.*, 2020). The helical flow is characterized by high velocity and high shear stress, which is regarded as a physiological form of blood flow (Liu *et al.*, 2015; Baratchi *et al.*, 2020). This kind of flow is considered to be atheroprotective because it can prevent the accumulation of low-density lipoproteins, enhance blood perfusion and oxygen transport, and reduce the adhesion of blood cells on the arterial wall (Liu *et al.*, 2009; Liu *et al.*, 2010). Interestingly, the helical flow can improve the hemodynamic performance of vascular devices (Liu *et al.*, 2016; Zeller *et al.*, 2016; Zhang *et al.*, 2018).

Cyclic stretch

Cyclic stretch (CS), sometimes called circumferential strain resulted from blood pressure, manifests the expansion and contraction of

the vessel wall. Similar to the blood flow, the stretching magnitude is implicated in the systolic and diastolic blood pressure, which are related to the heart cycle frequency (Kaunas *et al.*, 2005). The range of strains varies in different blood vessels. For instance, the arterial strain is 9%–12%, the pulmonary artery strain is 6%–10%, the carotid artery strain is 1%–2%, and the femoral artery strain is 2%–15% (Anwar *et al.*, 2012; Jufri *et al.*, 2015). However, in pathological conditions, such as hypertension, strains can reach as high as 20% (O'Rourke, 1995). Besides, it is also reported that the lag of temporal phase angle exists between the WSS and the CS, this is because of the viscoelastic mechanical properties of the vessels (James and Allen, 2018).

Endothelial mechanotransduction

The ECs can sense physical forces, which interact with the surrounding environments. Then, the stimuli are further transduced into biochemical signals via mechanotransduction pathways. Cytoskeleton can act as key mechanosensory molecules to transduce mechanical stimuli from the endothelium to the nucleus. In addition, multiple mechano-sensors on the membrane of vascular ECs membrane have been reported, such as integrins (Israeli-Rosenberg *et al.*, 2014), ion channels (Delmas and Coste, 2013; Leckband and de Rooij, 2014), junctional proteins (Chen and Tzima, 2009), PECAM-1 (Chen and Tzima, 2009; Conway and Schwartz, 2015; Wang *et al.*, 2015), caveolae (Gilbert *et al.*, 2016; Shihata *et al.*, 2016), membrane lipids and protein(Panciera *et al.*, 2017), glycocalyx (Florian *et al.*, 2003; Zeng *et al.*, 2018; Weinbaum *et al.*, 2020), primary cilia (Luo *et al.*, 2014), etc. Here, we firstly show some recent research of cytoskeleton in response to blood flow. Then, we emphatically discuss the mechanotransduction of ion channels, junctional proteins, and some membrane proteins.

Cytoskeleton in response to blood flow

The cytoskeleton consists of microtubules, actin, and intermediate filaments. These components concatenate different areas of the ECs and play a role in transmitting forces to the basal or lateral areas. These forces come from the apical domain of the cell, which also directly senses shear forces (Huber *et al.*, 2015). The response of ECs to the flow was blocked when actin, microtubules, or intermediate filaments were inhibited (Acharya and Yap, 2016). Furthermore, there was a theory about the cytoskeletal flows: The spatial gradient of motor contractility or cytoskeletal polymerization may induce flow. This theory has been used to study cell division, migration, polarization (Hannezo and Heisenberg, 2019). Besides, the actin polymerization at the leading edge of ECs may be made answerable for the retrograde flow of the actin cytoskeleton. Then, this process will create friction with the extracellular matrix (ECM) and then produced the force to help cell migration (Recho *et al.*, 2013; Barnhart *et al.*, 2015; Bergert *et al.*, 2015). The friction between cells and ECM generates from adhesion receptors, which are the main molecular link between ECs and ECM. The adhesion receptors also act as bidirectional hubs transmitting signals between ECs and the surrounding environment. Interestingly, when exposed to blood flow, actomyosin may reorganize its own network. This process is regulated by the adhesion strength and/or orientation of actin filaments and myosin motors (Allen *et al.*, 2020).

Mechanically activated ion channels

When ion channels sense the stress, the proteins allow the passage of ions selectively. Therefore, it can help maintain the equilibrium of electric potential. Earlier studies have shown that flow can activate ion channels, including the transfer of Na^+, Ca^{2+}, K^+, Cl^- (Barakat *et al.*, 2009), and stretch can activate ion channels, including the transfer of Na^+, Ca^{2+}, and K^+ (Liu *et al.*, 1998). Of course, they activate immediately and independently when the flow starts (Barakat *et al.*, 2009). To be specific, when the shear force is less than 0.01 Pa, the K^+ channels are activated. When the shear force is 0.03 Pa, the Cl^- channels are activated (Lieu *et al.*, 2004; Gautam *et al.*, 2006). Meanwhile, the activation of K^+ and Cl^- channels are also affected by the oscillating flow frequencies below the threshold frequency. In recent years, the Piezo1 channel, a kind of mechanically activated Ca^{2+} ion channel, was found to be able to sense flow shear stress (Coste *et al.*, 2010; Ranade *et al.*, 2014; Volkers *et al.*, 2015). It is suggested that the Piezo1 channel is regulated by various mechanical stimuli (Nourse and Pathak, 2017). For example, the detection of Piezo1 showed that it changed during exercises because of increased blood flow (Rode *et al.*, 2017; Beech, 2018). Furthermore, Piezo1 may regulate the migration of cells. In a developing vasculature, Piezo1 mediates the migration of ECs through vascular endothelial growth factors (Li *et al.*, 2014). The released Ca^{2+} in ECs would activate endothelial nitric oxide (NO) synthase, which is necessary for the production of NO. It is a key signaling molecule with a plethora of biological functions (Tejero *et al.*, 2019). In blood vessels, NO produced by ECs would diffuse rapidly to smooth muscle cells. Eventually, it will cause vasodilatation (Maiorana *et al.*, 2003). The NO is considered a hallmark of endothelial dysfunction because NO-mediated vasodilation is induced by blood flow shear stress (Davignon and Ganz, 2004). The formation, transport, and consumption of NO in blood vessels are controlled by the local blood flow pattern, hemoglobin in red blood cells, and NO scavengers in the arterial wall (Qian *et al.*, 2020; Su *et al.*, 2020).

Junctional proteins and some membrane proteins

ECs mainly adhere to the extracellular matrix through focal adhesion of transmembrane. Specifically, the tripeptide alanine-glycine-aspartate is the most characterized integrin-binding ligand. It exists widely in fibronectin, vitronectin, and many other molecules (Ruoslahti, 1996). In addition, focal adhesions involved in cellular migration are dynamic structures. When applied with force, they will reshape and strengthen through actin connections (Geiger *et al.*, 2009). In addition, ECs connect and communicate with neighboring cells through cell connections. For example, the adherent junction such as VE-cadherin is recognized as a mechanosensory. It was first found that VE-cadherin plays a role in the incorporation of vascular endothelial growth factor receptor 2 and PECAM-1 (Tzima *et al.*, 2005). Notably, PECAM-1 is considered a mechanosensor that performs tyrosine phosphorylation under the action of a force from neighboring cells (Li *et al.*, 2005; Collins *et al.*, 2012).

Microfluidic Chips for the Endothelial Biomechanics and Mechanobiology

With the increasing development of manufacturing and processing techniques, microfluidic devices can be designed

flexibly. The width of the microchannel can range from the nanometer to a few hundred micrometers, and the length can reach several centimeters (Skorupska *et al.*, 2018). Microfluidic devices have many advantages that they can carry out experiments with higher throughput, less reagent consumption, design flexibility and adaptability of experiments under different conditions (Kang *et al.*, 2008). Therefore, microfluidic systems have been made some significant breakthroughs in conventional biological research. They can be applied in multiple experiments, including cell culture, intercellular interaction, cell lysis or cell sorting, and the mimic of different types of tissues (Yum *et al.*, 2014; Zhang *et al.*, 2018; Li *et al.*, 2019). In particular, microfluidic systems have been widely used in biomechanics research (Kurth *et al.*, 2012). Several studies have explored the impact of WSS on cells, and the stimuli can influence cytoskeleton transformation and cell development (Toh and Voldman, 2011; Kurth *et al.*, 2015). Additionally, microfluidic platforms can also apply other forces, such as stretch and compression, on target objects (Dongeun *et al.*, 2010). As a result, microfluidic systems are introduced to mimic the mechanical environments in vessels to conquer the limitation of *in vitro* vascular system studies. Besides, it can reproduce a pathological environment for cells and be applied in studies of vascular diseases, such as atherosclerosis (AS). A summary of microfluidic chips for the endothelial biomechanics and mechanobiology is provided in Tab. 1.

Microfluidic chips for endothelial permeability and mechanotransduction

With the transport barrier formed by blood vessels, cells and nutrients are selectively exchanged to meet the needs of metabolism and homeostasis of surrounding tissues. In addition, whether the ability to regulate vascular permeability is normal or not is fundamental to the judgment of cardiovascular function. Therefore, barrier dysfunction is a hallmark of many cardiovascular diseases (Hahn and Schwartz, 2009; Dongaonkar *et al.*, 2010). Besides, these large amounts of chemical signals were found to affect the degree of permeability (Mehta and Malik, 2006). Blood flow plays a significant role in the guiding of permeability of ECs (Tarbell, 2003). Given the significance of vascular permeability, a variety of microfluidic chips were developed to investigate the selective permeability of the endothelial cells (Shao *et al.*, 2010).

To be specific, Lee *et al.* designed a novel microfluidic system (Fig. 2A) to form multiple perfusable 3D microvessels with reliable functional barrier properties (Lee *et al.*, 2014). Using this platform, the barrier function measured is similar to the barrier function measured in the *in vivo* experiment, but the permeability coefficient measured in the *in vivo* experiment will be relatively low. On the one hand, vessel networks in physiology are bifurcating while previous microfluidic models were limited to the design of the channel, which was often a single channel or two parallel vessel analogs (Bischel *et al.*, 2013; Akbari *et al.*, 2017). Therefore, Akbari et al (Akbari *et al.*, 2018)considered the hemodynamic characteristics of the bifurcated vessels (Fig. 2B). Their model reproduced the state of blood flow at bifurcated vessels and the structure between endothelium and the extracellular matrix (ECM). This model also systematically studied the effect of local hemodynamics on changes in endothelial permeability in the bifurcating vessel structure. They also studied the influence of NO in the guiding of permeability of ECs under shear stress using the device. On the other hand, assessments of barrier function in these microfluidic vascular models were usually limited to fluorescence-based diffusion permeability measurements. To overcome this problem, a recent model established by Wong *et al.* (2020)

TABLE 1

Summary of microfluidic chips for the endothelial biomechanics and mechanobiology

Chip function	Cell type	Targeted application	References
Permeability	LFs HUVECs	- measuring permeability - assessing drug effects on permeability of microvessels	(Lee *et al.*, 2014; Akbari *et al.*, 2018; Wong *et al.*, 2020)
Mechanotransduction	rMSCs HAECs HUVECs	- studying effects of WSS, CS and their combination on cytoskeleton reorganization - showing Piezo1 channels as sensors of shear stress and determining the vascular structure in fetal development and adults. - applications for quantitative measurements of agonist-induced real-time changes in Ca^{2+} and NO production	(Estrada *et al.*, 2011; Zheng *et al.*, 2012; Li *et al.*, 2014; Xu *et al.*, 2016; Jin *et al.*, 2020)
Angiogenesis vasculogenesis	iPSC-ECs ECFC-ECs LFs NHLF HUVECs	- providing evidence that iPSC-ECs self-assemble into vascular networks - investigating the role of IF during vasculogenic formation and angiogenic remodeling of microvascular networks - studying the regulation of IF and morphogenetic ladder on angiogenesis	(Kim *et al.*, 2016; Zanotelli *et al.*, 2016; Shirure *et al.*, 2017; Zhao *et al.*, 2020)

Note: LFs, Lung Fibroblasts; HUVECs, Human Umbilical Vein Endothelial Cells; rMSCs, Rat Mesenchymal Stem Cells; HAECs, Human Aortic Endothelial Cells; iPSC-ECs, Induced Pluripotent Stem Cell-Derived Endothelial Cells; ECFC-ECs, Endothelial Colony Forming Cell-Derived Endothelial Cells; NHLF, Normal Human Lung Fibroblasts; CS, Cyclic Stretch; PEG, Polyethylene Glycol; IF, Interstitial Flow.

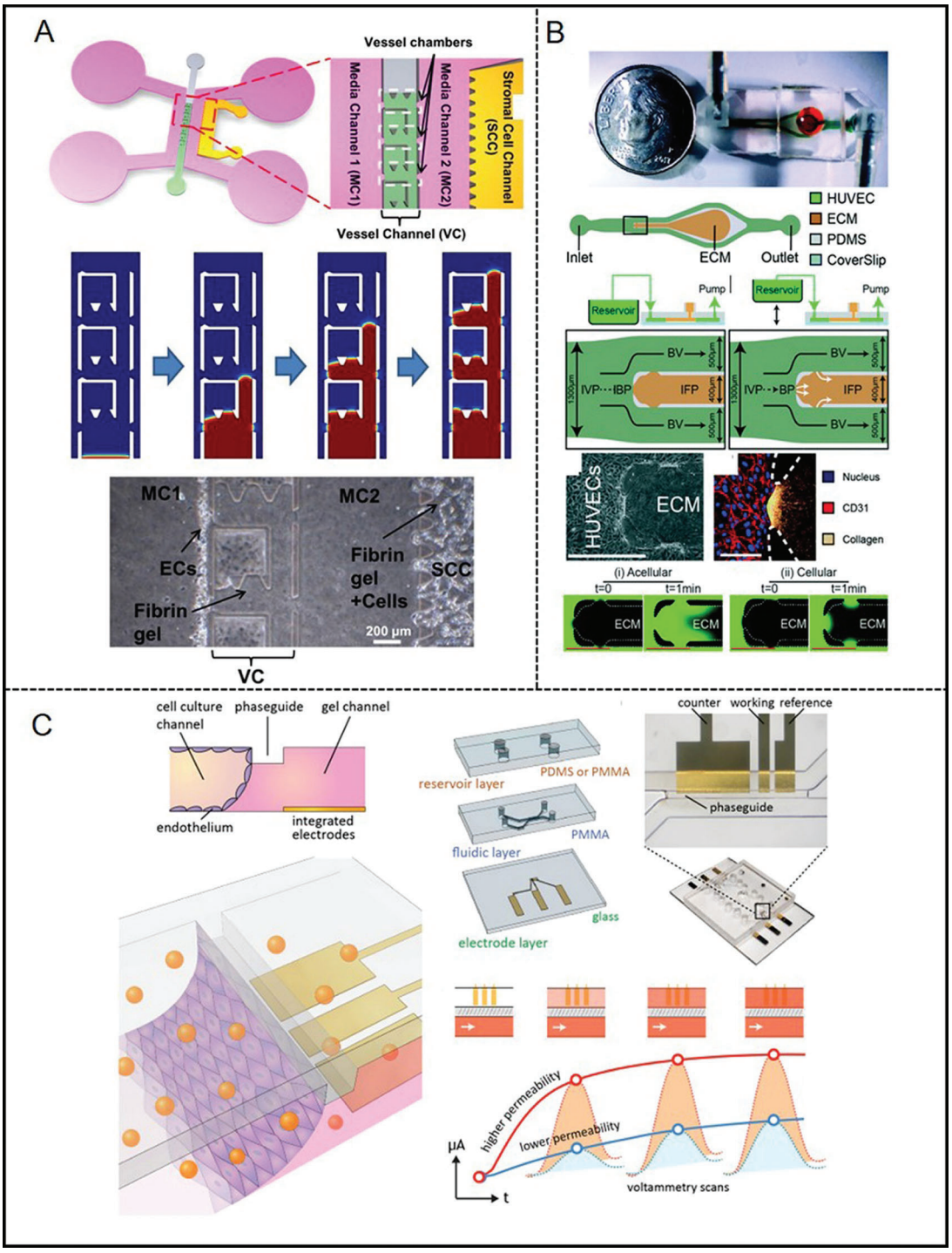

FIGURE 2. Examples of microfluidic vascular system for endothelial permeability developed by (A) Lee *et al.* (2014), (B) Akbari *et al.* (2018), (C) Jin *et al.* (2020).

measured the permeability of ECs by using an electroactive tracer on a 3D hydrogel-based microfluidic vascular model (Fig. 2C). They measured the permeability of EC under the condition of the cell-sensing mechanical stimulation and after exposure to a permeability mediator.

In addition to studying endothelial permeability, microfluidic systems have shown tremendous potential in studying the mechanotransduction of ECs. The first related vascular chip, a silicon platform with varying widths of microchannels, was fabricated by Gray *et al.* (2002) using deep reactive ion etching method. The result showed that the elongation of ECs progressively increased as the channel width decreased. ECs were planted in a 200 mm wide microchannel and formed a monolayer after being sheared by 20 dyne/cm^2 shear stress for 16 h. Besides, they considered the magnitude and waveform of shear stress similar to physiological conditions. Although the laminar-steady flow is simple and easy to implement in a chip, but importantly, the comparison between laminar flow and static culture can directly show the effects of shear stress on the cells.

Because the blood flow in the body is pulsatile, other work adds various types of unstable flow to the microfluidic devices. The dynamic effects of pulsatility on cell growth, such as oscillatory and non-reversing pulsatile flows, were tested. Multiple parameters, including flow amplitude, frequency, and reversal degree, could be applied and

manually controlled. These applications also showed the flexibility of microfluidic platforms (Vedel *et al.*, 2010; Mohammed *et al.*, 2019). For example, the microfluidic system with pulsatile flow described by Mohammed *et al.* (2019) can study the mechanobiology of human aortic endothelial cells (Mohammed *et al.*, 2019). To achieve the simplification of microfluidic technologies and minimize their reliance on supporting equipment, they integrate microfluidic channels with miniaturized pumping units and flow sensors. However, the flows mimicked in those chips still have relatively large differences in comparison with the *in vivo* hemodynamic microenvironment of blood vessels. To deal with the problem, Zheng *et al.* (2012) developed a microfluidic vascular chip (Fig. 3A). This platform can provide WSS and CS for cultured vascular ECs, which are also the two main mechanical stimulations of the cardiovascular system (Zheng *et al.*, 2012). The chip contains a microchannel layer, an elastic membrane, and a stretch layer. The cells planted on the elastic membrane can be stimulated by WSS and CS simultaneously or independently as the membrane deforms under the action of perfusion. Furthermore, Estrada *et al.* (2011) fabricated a microfluidic model (Fig. 3B) to culture ECs under physiological flow patterns (Estrada *et al.*, 2011). The system is designed to use a variety of adjustable analog controls, including compliance, resistance, foldable pulse chamber, and control valve, to accurately simulate hemodynamic waveforms.

Given the significance of Piezo1 for ECs, Li *et al.* (2014) designed microfluidic chambers applying shear stress on ECs. They concluded that the calpain activation will enhance the entry of calcium ions under the action of shear stress. This entry process is regulated by the Piezo1 channel and has a significant impact on endothelial cell organization and alignment. Additionally, Xu *et al.* (2016) presented detailed procedures of cultured microvessel network development

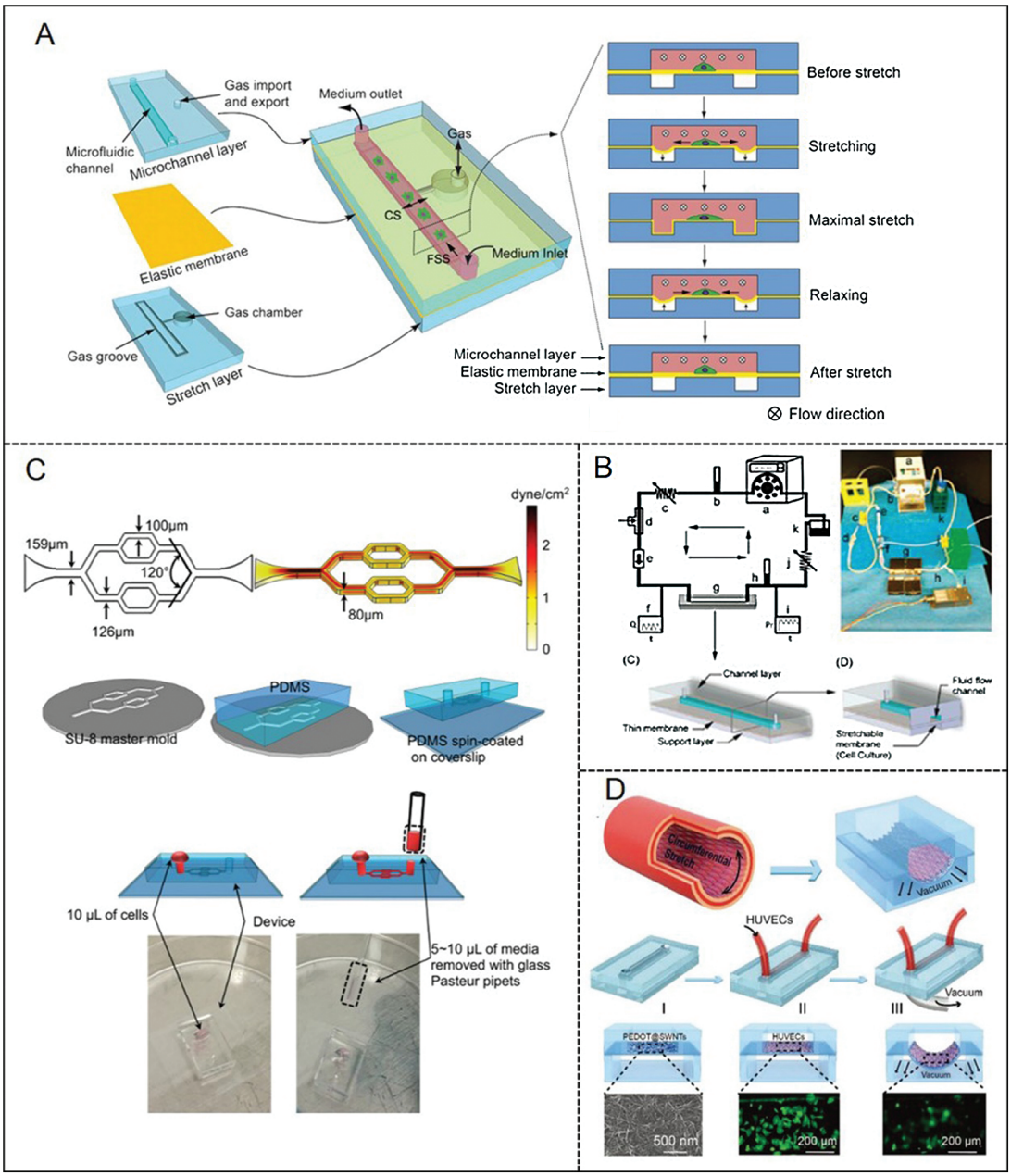

FIGURE 3. Examples of microfluidic vascular system for endothelial mechanotransduction developed by (A) Zheng *et al.* (2012), (B) Estrada *et al.* (2011), (C) Xu *et al.* (2016), (D) Jin *et al.* (2020).

(Fig. 3C). The device possessed long-term perfusion capability and quantitatively measured the agonist-induced changes of Ca^{2+} in EC. They also measured the production of NO in real time. Although microfluidic chips are now integrated for multiple purposes, one of the most important applications is to monitor *in-situ* mechanical force trigger signals in real time during vascular mechanical transduction is still a problem to be solved. Recently, the microfluidic vascular chip designed by Jin *et al.* (2020) is helpful to overcome the challenges (Fig. 3D). The microfluidic vascular chip contains a flexible and stretchable electrochemical sensor, which was constructed using conductive polymer-coated carbon nanotubes. The material has good mechanical compliance and real-time electrochemical performance. Moreover, they also studied the effect of cyclic CS on ECs and detected NO signals and reactive oxygen species under different conditions.

Microfluidic chips for angiogenesis and vasculogenesis

Vascular development involves a coordinated process of ECs differentiation, proliferation, and migration. It includes two main processes: Angiogenesis and vasculogenesis. Vasculogenesis is in the early stage of embryonic development. It is a process in which angioblasts differentiated from mesoderm cells gather to form primary capillary plexus (Semenza, 2007). Subsequently, the vascular network is reconstructed during the vascular maturation process, new blood vessels sprout from the existing vascular network and grow to the surrounding tissues. This process is called angiogenesis (Carmeliet, 2000; Feige *et al.*, 2014). Angiogenesis usually occurs during normal embryonic development, but it also occurs during the regeneration of damaged tissues. In addition, it involves the sprouting or division of pre-existing angiogenesis, which is an important natural process in the development and wound repair process, especially in the development of cancer (Adams and Alitalo, 2007; Herbert and Stainier, 2011). In this process, the pivotal molecular mechanism in maintaining vasculature homeostasis is the vascular endothelial growth factor pathway (Olsson *et al.*, 2006). Moreover, both intrinsic molecular pathways and extrinsic stimuli can influence vascular development. Among *in vitro* models, microfluidic technology also showed excellent performance in reproducing angiogenesis that complex vascular systems were fabricated with fluidic perfusion tightly controlled. The advantages of microfluidic technology also provide a feasible method in studying the effects of hemodynamic forces on cells, cell interactions, and the effects of biochemical/biophysical stimulation on angiogenesis.

In *in vitro* experiments, a hydrogel was used as a significant component in vascular formation instead of ECM to better reproduce the three-dimensional (3D) *in vivo* niches of vascularization (Lewis and Gerecht, 2016). Moreover, it becomes possible to recreate the angiogenesis process (Bersini and Moretti, 2015). The microfluidic system well defined the cell patterns, biochemical gradients, and dynamic physical stimulation in microchannels to achieves angiogenesis. The flow channel connecting the microfluidic channel to one or more chambers is usually composed of different 3D matrix microstructures. The materials are selected to emulate the ECM and thereby promote the attachment and sprouting of ECs (Cochrane *et al.*, 2019). For instance, Zanotelli *et al.* (2016) designed a 3D microvascular model (Fig. 4A) on a tri-channel microfluidic system. The vascular channel is filled with polyethylene glycol hydrogel, and then human-induced pluripotent stem cell-derived endothelial cells are encapsulated in the hydrogel. The hydrogel contained cell-adhesive and MMP-degradable peptides, which could be beneficial for the generation of lumenized vascular networks under flow conditions. Moreover, interstitial flow (IF) is significant for angiogenesis for two reasons. Firstly, Changes in IF can affect pathological and physiological angiogenesis. Second, both IF and morphogen gradients (such as VEGF) may stimulate and guide the growth of new blood vessels (Shirure *et al.*, 2017). Kim *et al.* (2016) investigated the individual and combined effect of IF and proangiogenic factors (Fig. 4B). As long as IF exists, no matter how to change the flow direction, it will significantly promote the angiogenesis of the microvascular network. Subsequently, Shirure *et al.* (2017) also presented similar results in terms of the effect of IF on angiogenesis. They developed an *in vitro* microfluidic platform (Fig. 4C) that can model 3D angiogenesis in the tissue microenvironment. Their results show that under physiological IF (0.1–10 μm/s) conditions, morphogen gradients were eliminated within a few hours. In addition, the results of angiogenesis show that the opposite direction of IF is more conducive to angiogenesis.

Based on microfluidic techniques, our group developed a microfluidic sprouting chip (Fig. 4D) and an automatic system to control the circulatory perfusion system and to simulate the process of neovascularization (Zhao *et al.*, 2020). The microfluidic chip contained an endothelial cell culture channel (having flow shear stress control), a liquid channel (supporting medium and VEGF), and a wide central hydrogel channel (mimicking the ECM) separating two liquid channels. The result showed that high shear stress prevents the initiation of new blood vessel formation and stabilizes the ECs layer. This process is regulated by the mechanical transduction of the heparan sulfate proteoglycan coating on ECs.

Microfluidic chips for endothelial vascularization in blood-brain barrier

The blood-brain barrier (BBB) contains peripheral cells, pericytes, and astrocytes and forms a strictly regulated neurovascular unit (NVU) to control the dynamic balance of the central nervous system to maintain normal brain functions (Arvanitis *et al.*, 2020). The physical barrier has the function of restricting the passage of ions and hydrophilic agents. It is formed by ECs connected by tight junction proteins (such as closed zonule 1) (Wong *et al.*, 2013; Serlin *et al.*, 2015). The microfluidic BBB models replicated realistic dimensions and geometries, so the endothelium can be directly exposed to physiological fluid flow. Using "BBBs-on-chips", researchers can detect the expression of specific markers, including adhesion proteins and tight junction proteins, to lay the foundation for further understanding of the blood-brain barrier mechanism. Besides, the permeability of the cell barrier could be investigated (van der Helm *et al.*, 2016).

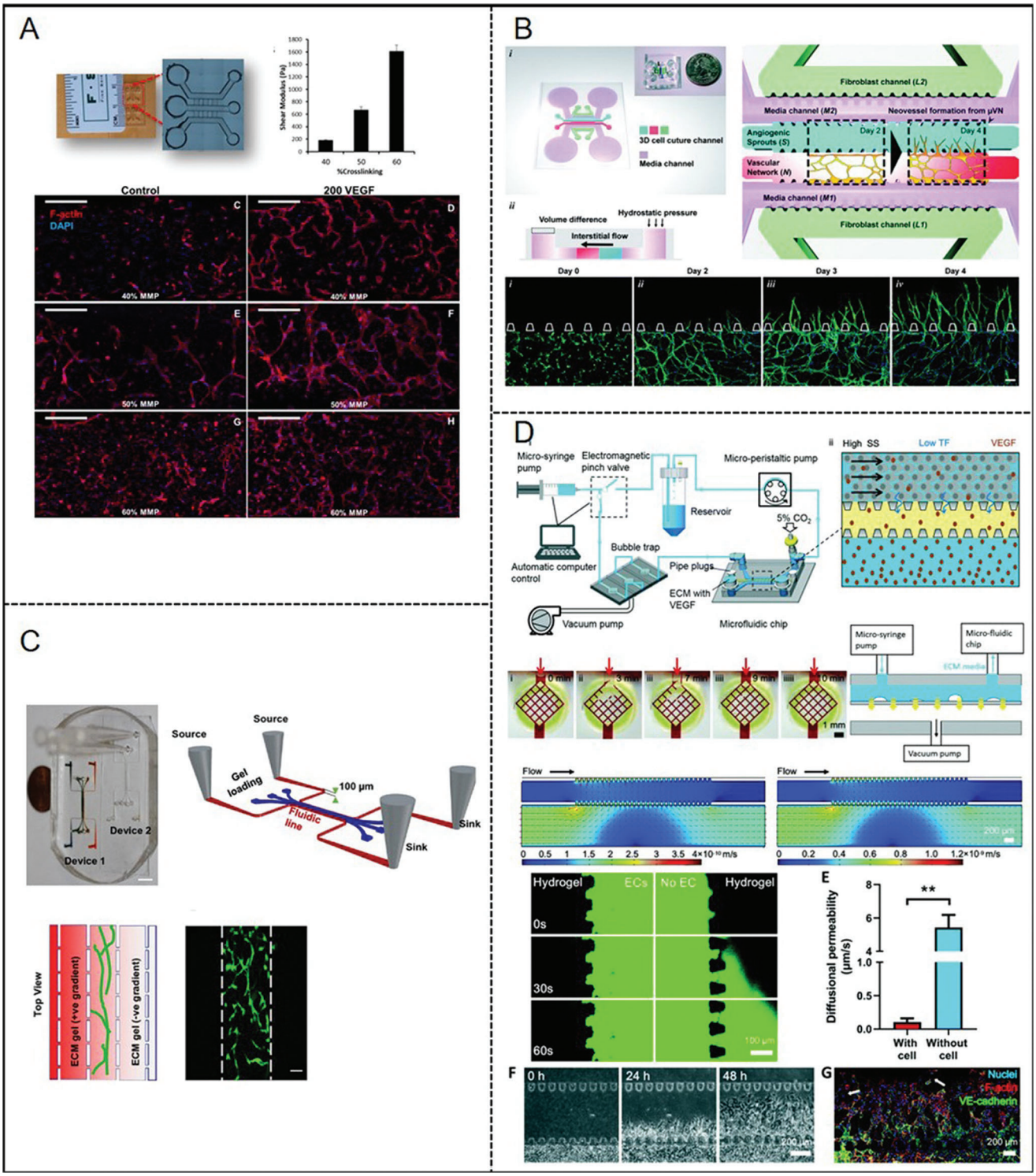

FIGURE 4. Reported examples of microfluidic vascular systems for angiogenesis and vasculogenesis developed by (A) Zanotelli *et al.* (2016), (B) Kim *et al.* (2016), (C) Shirure *et al.* (2017), (D) Zhao *et al.* (2020).

Brown *et al.* (2015) reported a microfluidic brain vasculature chip (Fig. 5A). The system consists of the vascular and brain chambers and they are closely opposed to each other and separated by a thin polycarbonate (PC) membrane (0.2 μm pores). They cultured primary microvascular endothelial cells, derived from the human brain, on the upper membrane, implanted a gel containing astrocytes under the membrane, and performed blood flow. The amount of ZO-1 was semi-quantified by immunofluorescence. In addition, it was observed that the actin filaments were rearranged along the flow direction, and the percentage of actin filaments was quantified. Their work also confirmed that permeability is related to the active transport of ascorbic acid. Besides, based on the effect of astrocytes in a 3D collagen matrix to modulate brain endothelial barrier function, Sellgren *et al.* (2015) developed a novel microfluidic device. Their device is composed of two upper and lower polydimethylsiloxane (PDMS) micro-molded channels and a middle nano-porous membrane. The membrane used commercial polyester (PE) and

polytetrafluoroethylene (PTFE) nanoporous membranes (pore size 0.4 μm, TClear 3450, Corning, and BGCM00010, Millipore). They incorporated a 3D matrix for supporting astrocytes on the lower membrane and planted ECs on the upper membrane at a perfusion condition. Similar to the foregoing work, Walter *et al.* (2016) designed a barrier-on-a-chip device (Fig. 5B), which consisted of two PDMS parts with channels. The middle layer is a porous PET membrane with 23 mm thick and 0.45 mm pores. Co-culture of 2 or 3 types of cells (ECs, pericytes, and astrocytes) were available with the flow of culture medium. The whole-cell layer was observed, and the monitoring of transcellular electrical resistance in real time was applied as well. Subsequently, Maoz *et al.* (2018) considered the interaction of different cells so that the effect of individual cell types or sub-compartments on NVU function would not be missed. They constructed three chips, with a brain chip in the middle and a BBB chip on each side (Fig. 5C). Then, they cultured human brain microvascular endothelial cells on the lower surface of the BBB chip membrane. Besides, in order to simulate the outer wall of brain microvessels, primary brain microvascular pericytes were dispersed in astrocytes and cultured on the upper surface of the membrane. Additionally, it is also a very meaningful work to apply the BBB system to drug development. Ahn *et al.* (2020) presented a microfluidic microphysiological platform (Fig. 5D) to mimic the key structure and function of the human BBB. Comparing to previous models, it can achieve the reproduction of BBB-specific endothelial characteristics. In addition, they used microfluidic technology to combine HDL mimetic nanoparticles with apolipoprotein A1 (eHNP-A1) to reconstruct the physiological condition of discoidal HDL. Finally, they

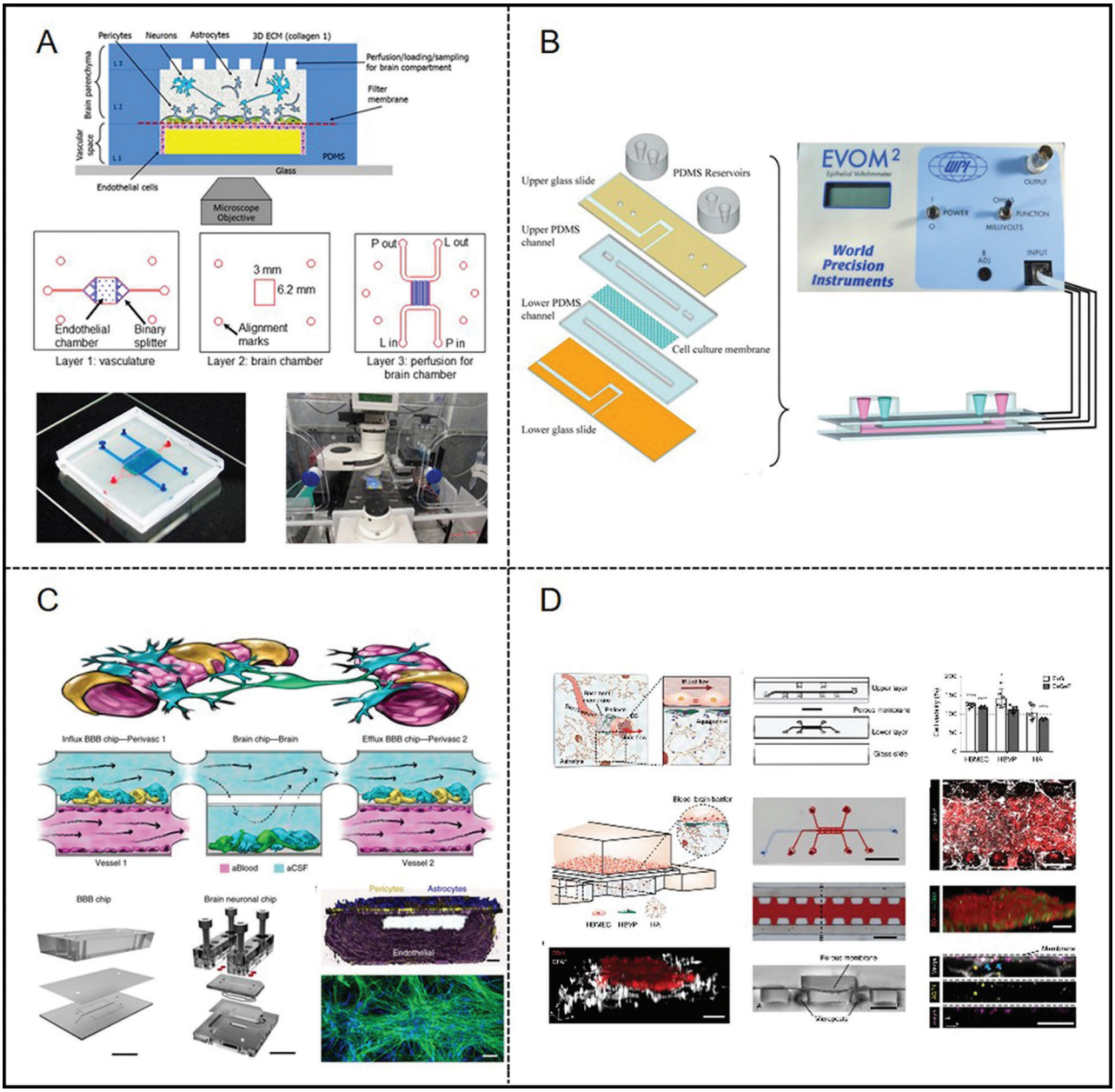

FIGURE 5. Examples of microfluidic vascular system for blood–brain barrier developed by (A) Brown *et al.* (2015), (B) Walter *et al.* (2016), (C) Maoz *et al.* (2018), (D) Ahn *et al.* (2020).

confirmed that eHNP-A1 entered the brain blood-brain barrier with a relative accumulation of about 3%.

Microfluidic chip for atherosclerosis

It is known that AS is a chronic cardiovascular disease that is closely related to the arterial microenvironment. Hemodynamics also affects the process of AS (Zaromitidou *et al.*, 2016). Recently, AS chips containing several cell types and flow patterns have been developed to reproduce the various characteristics of atherosclerotic diseases, including mechanical strain (Zheng *et al.*, 2016), monocyte phenotypes (Foster *et al.*, 2013), disturbed flow (Patibandla *et al.*, 2014), platelet aggregation (Westein *et al.*, 2013), and thrombosis or leukocyte–endothelial interactions at the atherosclerotic plaque (Venugopal Menon *et al.*, 2018). For instance, Zheng *et al.* (2016) reported a microfluidic model (Fig. 6A) to reconstruct early-stage AS to investigate physiological or AS-prone hemodynamic conditions. In addition, they designed a microfluidic chip that can integrate FSS and CS and study the behavior of ECs under physiological and AS-prone mechanical conditions so as to simulate the mechanical properties of blood vessels. Besides, Venugopal Menon *et al.* (2018) innovatively introduced a 3D stenosis vessel model to research the effect of hemodynamics on the interaction between

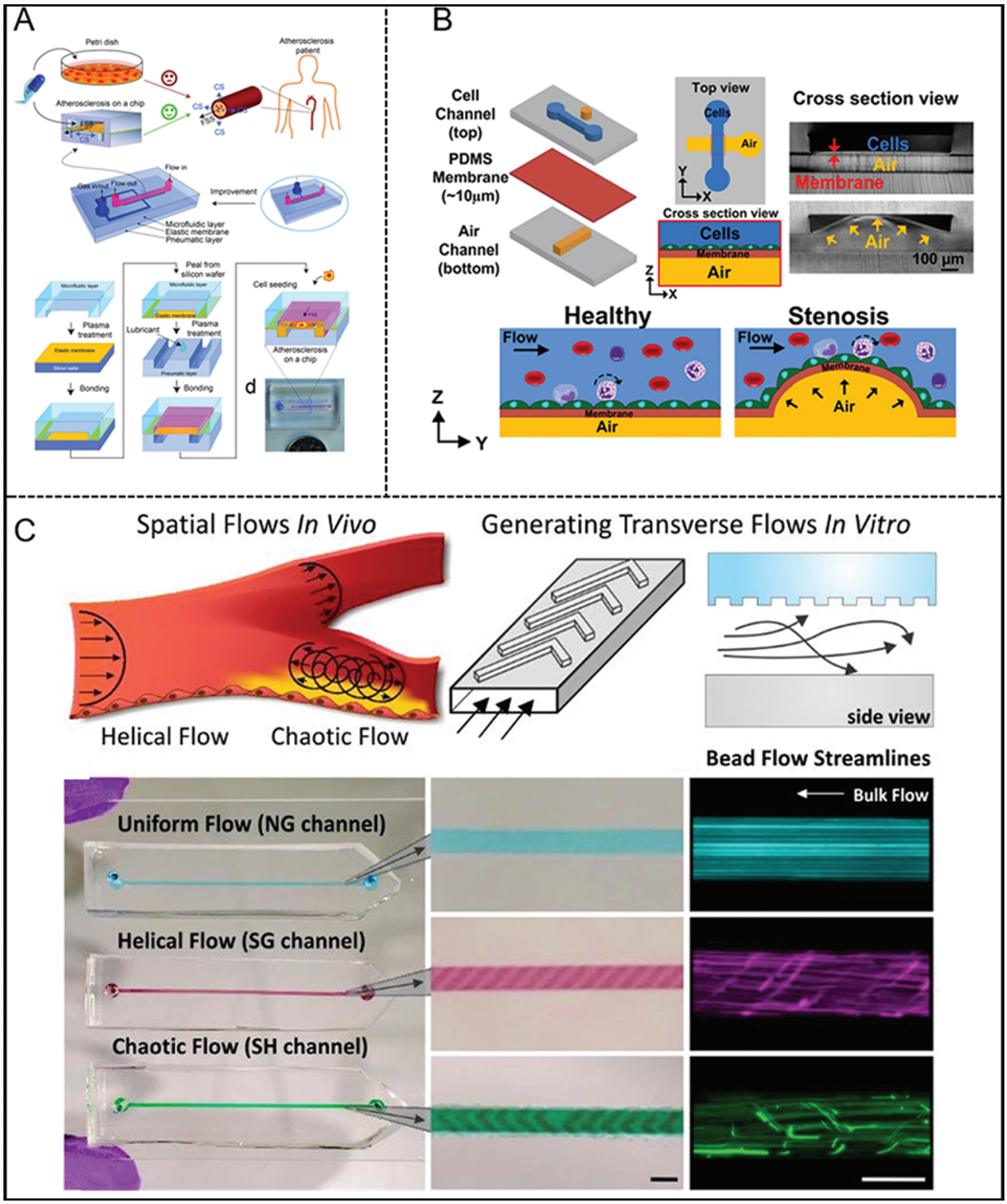

FIGURE 6. Examples of microfluidic vascular system for atherosclerosis developed by (A) Zheng *et al.* (2016), (B) Venugopal *et al.* (2018), (C) Varma *et al.* (2020).

leukocytes and endothelium (Fig. 6B). The multilayered microfluidic chip is composed of two orthogonal channels with a cell culture channel (top) and an air channel (bottom), separated by a thin PDMS membrane. It creates an adjustable 3D contraction by pumping air into the bottom channel to simulate narrow plaques of different severity. As mentioned above, helical flows may prevent the occurrence of vascular diseases, such as AS. Therefore, it is of great importance to design experimental models to specifically study helical flows. Jia *et al.* (2019) developed a microfluidics-based model to fabricate biomimetic helical microfibrous with various complex helical structures. The micro-channels had good perfusability and were able to simulate the swirling blood flow of helical blood vessels. Consequently, the model could reproduce blood function *in vitro* and be applied for blood-vessel-on-a-chip applications (Jia *et al.*, 2019). Another platform developed by Varma *et al.* (2020) was able to provide helical flow as well (Fig. 6C). In this platform, grooved surfaces on the channel ceilings created uniform, helical, and chaotic flow. Specific coupling between the spatial profile of flow and human endothelial cell phenotype was characterized in this work (Varma *et al.*, 2020).

Conclusions and Future Challenges

We firstly elaborated the contribution of physical forces, such as shear stress, blood pressure, and stretch strain, to ECs in molecular and cellular aspects, including mechanical responsive molecules or proteins involved in cell cytoskeleton and cell-cell communication. Then, we summarized the advanced progress of vascularized micro-tissues using microfluidic technology. These *in vitro* microfluidic vascularization chips reproduced the mechanical microenvironment of blood vessels of the human body. Overall, the development of organ-on-chip has taken a big step forward, and future technological advances may further accelerate the development of reproducing cell heterogeneity and complex three-dimensional structure models of *in vivo* tissues on *in vitro* microfluidic platforms.

However, there are also several challenges in vascular microfluidic systems. Current microfluidic platforms are usually composed of PDMS. The material has its disadvantages in that the absorption of small molecules and cytokines may greatly affect the accuracy of the bioassays. Therefore, more applicable biomaterials are required to develop to modify or replace PDMS. However, the current disease-specific microfluidic chip for drug screen may not fully reproduce the journey of a drug through the human body. It is difficult for vascular chips to reproduce the dynamic structure, the environment, and function changes in the process of organogenesis because they are designed and constructed in a predetermined manner. Furthermore, although the function and applications of the physiological organ-on-a-chip platforms have been accepted, there is still in deficiency of more personalized clinical applications based on microfluidic chips.

Availability of Data and Materials: Data supporting this article are detailed in this manuscript.

Author Contribution: The authors confirm contribution to the paper as follows: Study conception and design: Xiao Liu, Xiaoyan Deng, Yubo Fan. The modification of article: Jing Du, Li Wang. Figure integration: Kexin Li. Draft manuscript preparation: Haoran Su. All authors reviewed the results and approved the final version of the manuscript.

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