Recent Progress in the Development of Microfluidic Vascular Models

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The blood vessel is part of the circulatory system, and systemic circulation provides the blood supply to all tissues. Arteries are pathways through which the blood is carried, and the capillaries have a key role in material exchange to maintain the tissue environment. Blood vessels have structures appropriate for their functions, and their sizes and cell types are different. In this review, we introduced recent studies of the microfluidic vascular models. The model structures are classified mainly as poly(dimethylsiloxane) and hydrogel microchannels and self-assembled networks. Basic phenomena and functions were realized in vascular models, including fluid shear stress, cell strain, interstitial flow, endothelial permeation, angiogenesis, and thrombosis. In some models, endothelial cells were co-cultured with smooth muscle cells, pericytes, and fibroblasts in an extracellular matrix. Examples of vascular models involving the brain, lung, liver, kidney, placenta, and cancer were also introduced.

Keywords Microfluidic device, microchip, organ-on-a-chip, bioassay, cell-based assay, cell culture, blood vessel, endothelial cell, extracellular matrix

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1 Introduction

Development of a microfluidic organ model called organ-on-a-chip, which is used for bioassays and especially for drug development, is a major topic in bioanalytical chemistry. In addition to the main organs, including the lungs and liver, blood vessels are important targets of bioassays. Blood vessels are...
Blood vessels have structures that are appropriate for their functions, and their sizes and cell types are different. The aorta originating from the heart has an inner diameter of 12 mm, with a thick three-layered vascular wall to withstand the blood flow. The tunica intima, which is the innermost layer, is composed of endothelium; it is in direct contact with the blood flow. Tunica media is made from smooth muscle cells (SMCs) and elastic tissues. Smooth muscle assumes vascular constriction and vasodilation. The tunica externa is composed of fibrous tissues.

The artery branches into thinner vessels called the arterioles, which have an inner diameter of 15 μm, with a two-layered structure composed of the endothelium and smooth muscle. The arterioles branch into capillaries, which have an inner diameter of 3 μm. Capillaries consist of a layer of endothelium branching into tissue to create a blood vessel network, and they have three types of tubular structures. Continuous capillaries are located in the muscle, brain, bone, lung, and skin, in which endothelial cells (ECs) are continuously connected by adherens and tight junctions and sometimes surrounded by pericytes. Capillaries in the brain have especially significantly tight junctions to prevent permeation. Fenestrated capillaries surround the epithelium in the kidney, intestines, and endocrine glands, which have 50- to 80-nm pores and fenestrae for material exchange. Discontinuous capillaries are found in the liver, spleen, and endocrine glands, and there is a gap between the ECs. Therefore, blood cells easily pass through the capillary wall.

Functional abnormalities of the blood vessels are closely related to angiogenesis, cancer metastasis, and blood vessel diseases. Conventionally, studies of the blood vessels and related diseases are performed with experimental animals or cells cultured two-dimensionally under static conditions. However, the results obtained from the animal tests are not always applicable to humans, and in vitro cultured cells are not good models of vascular diseases due to dimensional differences and the absence of blood flow.

During recent organ-on-a-chip research, three-dimensional (3D) primary cultures of human cells were created to mimic parts of the human body. Using these in vitro models, it is possible to culture cells in the extracellular matrix (ECM) gel with mechanical stimulation to mimic the microenvironment of organs. In the past 5 years, many models with vascular structures have been reported, including organ-specific vessels (Fig. 1).

Various ECMs and human primary cells from the aorta, pulmonary artery, umbilical vessel, and microvessels of the skin, bladder, and uterus are commercially available as experimental materials. Various human vascular models from the aorta to capillaries in various organs have been developed by embedding cells and ECMs in microfluidic devices to culture them with fluidic stimulation, thus simulating the blood flow and strain to mimic the environment of human blood vessels.

In this review, we introduced recent studies of microfluidic vascular models. In Chap. 2, structures of the devices are classified. Basic phenomena and functions of the vascular models, including permeability, angiogenesis, and thrombosis, are explained in Chap. 3. In Chap. 4, the blood vessels in organ and cancer models are reviewed.

2 Device Structures

In vitro vascular networks are mainly constructed using two distinct approaches: ECs cultured on a microchannel surface and self-assembled vascular networks. Recently, it was reported that an intact vascular network with surrounding tissue was introduced in a microdevice.

2-1 Poly(dimethylsiloxane) microchannel

Most microfluidic devices for vascular models are made of a silicone elastomer called poly(dimethylsiloxane) (PDMS). PDMS devices are produced by a soft lithography replica-molding method during which the devices are replicated from a reusable mold. The PDMS replica is bound to another PDMS sheet or a glass slide. Tubes are connected with a pump to infuse fluid into microchannels via access holes. With this method, channels with various patterns are easily created.

To fabricate a microdevice with cell culture microchannels to mimic complex tissue, a porous membrane or ECM was incorporated in the PDMS device. In the membrane-based devices, ECs were cultured horizontally on a porous membrane that separated the upper channel from the lower channel (Fig. 2a). In the ECM-containing microfluidic devices (Fig. 2b), narrowly spaced PDMS micropillars were used to confine an ECM gel between the two adjoining channels. With this device, ECs were cultured on the sidewall of the ECM gel.

2-2 Hydrogel microchannel

In some devices, microfluidic channels are fabricated using an ECM gel (Fig. 2c). Using this approach, a simple linear channel is fabricated in many cases. A needle or rod has been used as a template that was physically removed by pulling it out of the surrounding ECM gel, typically collagen type I or fibrin.

Fig. 1 Illustration of human blood vessels. The organs shown are described in Chap. 4.
ECs were seeded on the channel surface to form a tube structure with a monolayer of ECs. Zheng et al. fabricated a hydrogel device having a more complex structure by using a PDMS mold. In comparison to PDMS devices, the hydrogel devices closely mimicked a natural extracellular environment.

Self-assembled network

Vascular ECs are suspended in an ECM hydrogel and cultured in a device in which the cells reconstitute three-dimensional (3D) vascular networks spontaneously. This approach does not require an extra channel structure to guide vasculatures, and the device design is similar to ECM-containing devices (Figs. 2b and 3). ECs or normal human lung fibroblasts (NHLFs) were suspended in a mixture of fibrinogen and thrombin, and each suspension was introduced separately in discrete gel channels and allowed to form. Kim et al. found that vasculogenic morphogenesis of human umbilical vein endothelial cells (HUVECs) was dependent on discrete co-culturing with NHLFs; HUVECs without co-cultured NHLFs failed to form interconnected networks. Although a mixed co-culture of HUVECs and NHLFs in the central channel also formed well-interconnected vascular networks after 5 days of culture, these networks were not connected to the adjoining medium channels and not perfusable. This result was in sharp contrast to that of the open networks when NHLFs were cultured in separate channels. The capillary networks that formed spontaneously consisted of perfusable vessels with diameters ranging between 1 and 100 μm.

Analyses of Phenomena in Vascular Models

3-1 Blood flow

Mechanical stimulation plays a crucial role in the vascular system. ECs and SMCs are affected by complex mechanical stimuli such as shear and tensile stresses, compressive strain, and interstitial flow.

3-1-1 Fluid shear stress

Experiments in which fluid shear stress (FSS) has been applied to ECs cultured in a microchannel have been reported. FSS ranges from 10 to 70 dyn/cm² (1 dyn/cm² = 0.1 Pa) in the artery and 1 to 6 dyn/cm² in the vein, which can be reproduced in the microchannel with a syringe pump. Among the earliest attempts, Frame et al. designed branching microchannels (20 – 50 μm in diameter) etched on a borosilicate glass slide to reproduce the geometry of the arteriolar microcirculation. Cells cultured with FSS showed a different response than those without FSS. For example, FSS made the EC-EC junction stronger. It was also reported that FSS induced proliferation of SMCs.

Cells cultured with FSS showed a different response than those without FSS. For example, FSS made the EC-EC junction stronger. It was also reported that FSS induced proliferation of SMCs. Sato et al. developed a microfluidic cell culture device for pulmonary hypertension studies that withheld high shear stress. Pulmonary artery SMCs were collected from the device to analyze mRNA, and they found that shear stress caused a...
microenvironment of blood vessels. By imposing FSS-only, separately to vascular cells to mimic the hemodynamic FSS and CS. The chip delivered FSS and CS simultaneously or integrated two major mechanical stimulations in blood vessels.31 (b) Illustration of a microfluidic device used to form a quasi-circumferential strain mimicking the mechanical strains in blood vessels (Fig. 4a).31 This device consisted of an array of platforms for a cyclic circumferential strain stimulation study of flow. Zhou et al. confirmed that blood vessel closure in pulmonary hypertension protein involved in regulating cell cycle progression. This result 7.5-fold increase in the transcription levels of cyclin D1, a protein involved in regulating cell cycle progression. This result confirmed that blood vessel closure in pulmonary hypertension disease is caused by the abnormal proliferation of SMCs.

3-1-2 Cell strain

Cell strain is another mechanical stimulus induced by blood flow. Zhou et al. reported on the development of a microchip platform for a cyclic circumferential strain stimulation study of blood vessels (Fig. 4a).31 This device consisted of an array of microfluidic channels with widths ranging from 20 to 500 μm. The channels were covered with suspended deformable membranes on which cells were cultured and stimulated by cyclic circumferential strain of up to 20% via hydrodynamic actuation of the fluid in the microchannel to mimic the biomechanical conditions of small blood vessels. They showed that human mesenchymal stem cells (MSCs), which can be differentiated into osteoblasts, chondrocytes, adipocytes, and vascular SMCs, were cultured with continuous stimulation of cyclic stretch (CS) over a period of 7 days. Localization and alignment of MSCs were observed when mechanical stretch was larger than 10%, and proteins in multiple signaling pathways, including SMAD1/SMAD2 and canonical Wnt/β-catenin, were detected.

Zheng et al. reported a flow and CS microdevice.32 The chip integrated two major mechanical stimulations in blood vessels, FSS and CS. The chip delivered FSS and CS simultaneously or separately to vascular cells to mimic the hemodynamic microenvironment of blood vessels. By imposing FSS-only, CS-only, and FSS plus CS stimulation on rat MSCs and HUVECs, the alignment of the cellular stress fibers varied with the type of cells and stimulation.

3-1-3 Interstitial flow

Flow of interstitial fluid, which is a solution that surrounds cells and originates from blood plasma extravasating from capillaries through pores and intercellular clefts in the vascular wall, plays an important role in vascular development. Moya et al. reported development of an in vitro perfusable capillary network in a microdevice by applying the interstitial flow (IF) to mimic the human microvascular environment.33 A continuous capillary network was spontaneously generated from cells, ECM, and angiogenic stimuli. The capillaries anastomosed with adjoining microchannels, allowing perfusion in the capillary network.

Kim et al. developed a microfluidic 3D vascular model to investigate the role of IF during vasculogenic formation and angiogenic remodeling of microvascular networks.23 In this model, HUVECs co-cultured with stromal fibroblasts spontaneously organized into an interconnected microvascular network that expanded to adjoining avascular regions by a manner of neovessel sprouting. Angiogenic sprouting was promoted only when the directions of IF and sprouting were opposite. The vasculatures switched between active angiogenic remodeling and non-sprouting states according to IF conditions.

3-2 Angiogenesis

Angiogenesis, which is the formation of new blood vessels, is a major research topic in biology, and especially in development, cancer, tissue engineering, and regenerative medicine. Kim et al. fabricated a microdevice for angiogenesis with five parallel channels: a central EC channel and two stromal cell channels separated by two culture medium channels (Fig. 4b).21 To induce angiogenic sprouting, HUVECs were seeded on the sidewall of the fibrin gel filled in the central channel, and NHLFs were cultured in the stromal channel on the opposite side to expose HUVECs to a gradient of NHLF-secreted factors. Within 24 h of co-culturing, formation of tip cells and angiogenic sprouting were observed. The tip cells guided growth of the sprouts across the fibrin gel until they reached the opposite end of the channel. By day 2, ECs formed tubular structures. Defined lumina around the stalk region appeared by day 3. After day 4, the lumenized vessels connected with the medium channels. The microdevice was applied to angiogenesis experiments with cancer cell-secreted factors. U87MG cells, which are highly malignant human glioblastoma multiforme cells, were seeded in the stromal channel. Within 24 h of coculturing, HUVECs invaded the fibrin gel in response to the U87MG-derived factors. Compared to the NHLF-induced sprouts, the U87MG-induced sprouts exhibited inefficient formation of perfusable vascular networks encompassing immature and poorly lumenized vessels on day 4.

Although HUVECs were used in most angiogenesis studies, Seo et al. compared the functional angiogenic ability of human aortic endothelial cells (HAEcs) and HUVECs using a 3D microfluidic cell culture system.34 With this system, ECs induced new sprouts that invaded a scaffold depending on the gradient of the vascular endothelial growth factor (VEGF)-A, which is known as an important regulator of angiogenesis.35 HAEcs showed stronger angiogenic potential than HUVECs. Details of angiogenesis systems have been reviewed by Akbari et al.36

3-3 Permeability test

There are two kinds of microfluidic devices for conducting an...
endothelial permeability test during which a permeable porous membrane (Fig. 2a) or hydrogel (Fig. 2b) is used as culture support for ECs. Although the diameter of the microvessel performing material exchange in peripheral tissues is approximately 3 μm, those of microchannels to test the process are tens of micrometers. Kim et al. reported a permeable membrane-based microfluidic device for permeability testing of nanodrugs. EC monolayers showed a decrease in transendothelial electrical resistance (TEER), i.e., an increase in permeability, when ECs were exposed to shear stresses of 1 and 10 dyn/cm² for 24 h. A suspension of lipid-polymer hybrid nanoparticles was infused into a microchannel, and the nanoparticles that appeared in the other channel were monitored every 10 min. A significant increase in nanoparticle permeation across the EC layer was observed when cells were treated with tumor necrosis factor (TNF)-α. To compare endothelial permeability of the endothelialized microdevice with an in vivo atherosclerosis model, New Zealand White rabbits were used. A 3D dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) technique was used to analyze in vivo permeability of atherosclerotic plaque microvessels with a 3-T clinical MRI scanner. High correlation was observed in nanoparticle permeability, as determined by in vivo DCE-MRI and ex vivo near-infrared fluorescence imaging.

A porous membrane was also used to evaluate the endothelial permeability of nanoparticles instead of cultured cells. It is difficult to precisely evaluate the effects of physical parameters, including pore and nanoparticle sizes, on permeation by using conventional animal experiments and cell-based microfluidic assays because the sizes and shapes of the vascular pores are essentially not uniform. A porous membrane with uniform straight pores was integrated in a microfluidic device to study permeation of nanoparticles. Furthermore, the effects of pore sizes and pressure differences across the membrane on nanoparticle permeation were examined. The experimentally determined permeability coefficient of the 1.0-μm pore membrane against the 100-nm-diameter nanoparticles agreed well with the theoretical value.

A microfluidic microvascular interstitium model used to study the leakage of drugs from blood vessels under in vivo-like fluidic conditions was reported. MRI was used to demonstrate the compatibility of the model with experimental animals and humans. The transport of two types of contrast agents with different molecular weights in the interstitium model was observed. The obtained transport rate ratio agreed with the ratio calculated using diffusion coefficients of the agents.

3-4 Thrombosis

On vascular injury, proteins and cellular materials congregate at the injury site to create a stable blood clot, which prevents excessive blood loss. When this occurs, normal blood vessels do not clog to keep normal circulation because of the high antithrombotic activity of the vascular endothelium. In diabetic patients, however, blood clots are easily formed because of oxidative stress on the endothelium. It is well known that platelet aggregation, a process essential for thrombus formation, is tightly regulated by shear stress, although the mechanisms of shear activation of platelets, particularly in diabetes, are poorly understood. Microfluidic vascular devices are good for observing thrombus formation because ECs are cultured in transparent tubular structures to apply various FSS, and composition of a medium containing the testing agent is freely adjustable.

Jain et al. developed a microfluidic device to predict whether a blood sample had the tendency to form clots. The device mimicked a stenosed artery network to evaluate blood clotting with a small volume of samples under pathophysiological flow. By using a clotting time obtained from a phenomenological calculation model of thrombus formation, coagulation and platelet formation were accurately measured in vitro using patient blood samples. The device was integrated in an extracorporeal circuit in pig endotoxemia or its heparin therapy model for real-time monitoring of ex vivo coagulation, which was more reliable than standard clotting assays.

It is challenging to use live ECs for assays in clinical settings. However, a microfluidic device lined by ECs fixed chemically retained its ability to modulate hemostasis under continuous flow conditions similar to a living arterial vessel even after a few days of storage.

3-5 Co-culture

Mural cells of the vascular wall, namely pericytes and vascular SMCs, are essential for vascular integrity. While an EC monolayer encloses the vessel lumen, pericytes are associated with the abluminal surface of capillaries. Vascular SMCs, which cover larger arteries and veins, are thought to be closely related to pericytes. These cells stabilize vessels through physical and chemical interactions with adjacent ECs, and the absence of these cells leads to vascular leakage and hemorrhaging.

3-5-1 Pericytes

Van der Meer et al. developed 3D vascular tissue in a PDMS microfluidic device by injecting a mixture of HUVECs, human embryonic stem cell-derived pericytes, and rat tail type I collagen. The cells organized themselves into a single long tube resembling a blood vessel. Observation by confocal microscopy revealed a mature endothelial monolayer with complete platelet-endothelial cell adhesion molecule-1 existing at the cell-cell junction and pericytes incorporated in the tubular structures. Tube formation was disrupted in the presence of a neutralizing antibody against transforming growth factor-beta (TGF-β), which is essential for normal vascular development. In the engineered microvessels, inhibition of TGF-β signaling resulted in tubes with smaller diameters and higher tortuosity, which were highly comparable with the abnormal vessels observed in patients with a vascular disease known as hereditary hemorrhagic telangiectasia.

Kim et al. developed a microfluidic device to investigate interactions between ECs and pericytes during the sprouting, growth, and maturation steps of neovessel formation. A mixture of HUVECs and human placental pericytes was attached to the sidewall of a pre-patterned 3D fibrin gel and allowed to sprout across the gel. The effects of EC maturation by pericytes on the perfusable EC network were confirmed with a confocal microscope. Compared with EC monolayer conditions, EC-pericyte co-cultured vessels showed a significant reduction in diameter, increased numbers of junctions and branches, and decreased permeability. In response to biochemical factors, ECs with pericytes showed features similar to those of previous in vivo experiments.

3-5-2 Lung microvasculature with pericytes

To study vasculogenesis and vascular remodeling in the lung, Bischel et al. developed an in vitro 3D microvascular model that closely mimicked the human lung microvasculature in terms of accessibility, functionality, and cell types. To analyze their role in the generation of normal microvessels, lung pericytes obtained from the human distal airway were mixed in fibrin gel and seeded into microcompartments together with HUVECs. Soluble signals from the lung pericytes were necessary to establish vascular perfusability, and pericytes migrated toward
endothelial microvessels. Cell-cell interaction to form tight junctions as well as secretion of the basement membrane were confirmed using transmission electron microscopy and immunocytochemistry. Direct co-culture of ECs and pericytes decreased the microvascular permeability from $17.8 \times 10^{-6}$ to $2.0 \times 10^{-6}$ cm/s and resulted in vessels with significantly smaller diameters. With phenylephrine administration, vasoconstriction was observed in microvessels lined with pericytes, but not in endothelial microvessels. Per fusable microvessels were also generated with human lung microvascular ECs and lung pericytes. Lung pericytes were shown to have a notable influence on microvascular morphology, permeability, vasoconstriction, and long-term stability in the in vitro microvascular system.

3·5·3 SMCs
Tan et al. developed 3D artificial vessels mimicking the basic architecture of arteries: a collagen-rich ECM (tunica externa), SMCs (tunica media), and ECs (tunica intima). A needle-architecture of arteries: a collagen-rich ECM (tunica externa), ç 3·5·3 SMCs microvascular system. In vitro vasoconstriction, and long-term stability in the influence on microvascular morphology, permeability, was observed in microvessels lined with pericytes, but not in endothelial microvessels. Per fusable microvessels were also generated with human lung microvascular ECs and lung pericytes. Lung pericytes were shown to have a notable influence on microvascular morphology, permeability, vasoconstriction, and long-term stability in the in vitro microvascular system.

3·5·4 Lymphatic ECs
Intersitial fluid leaked from blood capillaries is absorbed by lymphatic vessels. To analyze the mass transfer in the peripheral tissue, the recovery ability of lymphatic vessels is important, as is the vascular permeability of blood. Sato et al. developed a membrane-based microfluidic microcirculation model containing both blood and lymphatic ECs to examine vascular permeability. The device had upper and lower channels that were partly aligned and separated with a porous membrane on which blood vascular and lymphatic ECs were co-cultured back-to-back. The flow culture in the device promoted the formation of EC-EC junctions, and treatment with histamine, which is an inflammation-promoting substance, induced changes in the localization of tight and adherens junction-associated proteins and an increase in vascular permeability.

4 Blood Vessels in Organ and Tissue Models
4·1 Organ models
Blood vessels in organs have an important role in material exchange, and the cells in blood vessels and organs communicate with each other to express functions. In some microfluidic models, vascular cells and organ-specific cells are co-cultured to mimic the organ-specific microenvironment.

4·1·1 Blood-brain barrier
The blood-brain barrier (BBB) is a physiological barrier of the central nervous system that regulates transport between the brain and blood. The BBB protects the brain from potentially toxic substances and regulates the transport of circulating molecules based on their physical and chemical characteristics; specific transporters maintain brain homeostasis. In vitro BBB models are essential for developing drugs that permeate the BBB and affect brain tumors, Alzheimer’s disease, and other conditions. However, the conventional transwell-based BBB models have limitations. It is difficult to culture more than two kinds of cells in a model and to apply mechanical stimulation including FSS to the cells.

Some microfluidic BBB models were developed to solve these problems. Adriani et al. developed a microfluidic BBB model with four parallel channels (Fig. 5a). In the neurovascular model, astrocytes and neurons were used to mimic brain tissues with ECs to form a blood vessel. The in vitro model had four channels, a vascular channel paved with ECs (HUVEC or hCMEC/D3) and astrocyte, neuron, and medium channels, which were separated with micropillars. Astrocytes and neurons were cultured in a collagen hydrogel. Cells communicated directly with those in the adjacent channel, allowing cell contact and signaling. As a model experiment, a neurotransmitter, glutamate, was used to determine the functionality of the EC barrier by assessing neuronal activity measured by calcium imaging. Glutamate injection in the EC channel resulted in significantly increased calcium concentrations in the neurons without the endothelial barrier compared to the experiment with ECs. This suggested that the endothelial barrier restricted the passage of glutamate and the successive increase in the calcium level in neurons.

Wang et al. developed a BBB model composed of layered microfluidic channels separated by a porous membrane. Ag/AgCl electrodes were embedded in the upper and lower microfluidic PDMS channels to allow real-time measurements of TEER across the cultured ECs. Mouse pericytes and bEnd3 ECs were co-cultured on a porous membrane back-to-back, and mouse astrocytes were cultured on the bottom of the lower channel. The live/dead assay indicated high viability of all cultured cells up to 21 days. The model showed high TEER values and low permeability of [$^{14}$C]-mannitol and [$^{14}$C]-urea, and it exhibited functional expression of an efflux pump,
P-glycoprotein, which increased with the increased culture period. Herland et al. developed a BBB model with cylindrical collagen gel in a microfluidic channel. A viscous fingering method was applied to create a cylindrical collagen gel in a microchannel, and hydrostatic pressure-driven flow was used to control the dimensions of the lumen. Multiple co-culture modes were established by either embedding astrocytes inside the gel or sequential seeding of pericytes and ECs inside the lumen. The all-human 3D BBB model formed a permeability barrier similar to that of non-human or immortalized cells, and the integrity of the endothelium was strongly dependent on the presence of astrocytes and pericytes. The BBB model exhibited responses to an inflammatory stimulus, TNF-α, that more closely mimicked BBB in the living brain than the same cells co-cultured in a static transfwell. Details of microfluidic BBB systems have been reviewed by van der Helm.

4-1-2 Lung

One of the most famous examples of a microfluidic organ-on-a-chip system is the lung-on-a-chip, which mimics pulmonary alveolus (Fig. 5b). Briefly, an upper air channel was separated from a lower liquid channel with a porous PDMS membrane on which epithelium and endothelium were separately co-cultured back-to-back to form an alveolar-capillary interface with the human lung. Two side channels parallel to the central culture channels were connected to a vacuum pump to mimic the cyclic breathing motion of the lung. In a 2010 report, the device was used for nanotoxicology studies. The lung-on-a-chip revealed that the cyclic mechanical strain accentuated toxic and inflammatory responses to silica nanoparticles. The mechanical strain also enhanced epithelial and endothelial uptake of nanoparticles for transport into the vascular microchannel. Similar effects were also observed in the mouse lung. The device was used to reproduce drug toxicity-induced pulmonary edema observed in cancer patients treated with interleukin-2. This on-chip disease model revealed that mechanical forces associated with breathing motions have a crucial role in increasing vascular leakage, which leads to pulmonary edema. These studies also led to the identification of potential new therapeutics, including angiotensin-1 and a potential new transient receptor vanilloid 4 ion channel inhibitor, GSK2193874.

4-1-3 Liver

Primary hepatocytes are often used as physiologically relevant model cells for drug toxicity screening, and the development of microfluidic liver models has been conducted. Lee et al. reported a microfluidic liver model with microvascular-like structures without ECs. The artificial liver sinusoid with a mechanically tunable matrix surrounding a biodegradable elastomer, poly(octamethylene maleate [anhydride] citrate). The scaffold supported the assembly of hepatocytes on a mechanically tunable matrix surrounding a perfusable 3D microchannel network covered with ECs. Primary rat hepatocytes mixed with 10% primary rat fibroblasts were seeded in the parenchymal space of the endothelialized AngloChip scaffold. Hepatocytes were distributed throughout the space and around the vessel network, whereas the inner lumen of the network was paved with ECs. A cardiotoxic antihistamine drug, terfenadine, was assayed with the AngloChip. Terfenadine is generally metabolized in the liver with non-cardiotoxic fexofenadine and the P450 CYP3A4 isoform. Fexofenadine was detected by liquid chromatography–mass spectrometry measurements in a medium obtained from the bioreactor outlet well 24 h after infusion of terfenadine in the device. These results indicated that the drug was transported from the vascular channel to hepatocytes, and that the device was useful as a bioreactor. Human liver AngloChips were also developed using human embryonic stem cell-derived hepatocytes and human MSCs with HUVECs to cover the inner lumen. High-density culture resulted in the formation of junctions between hepatocytes and positive staining for albumin. Secretion of urea per cell from the endothelialized human AngloChip was higher than that observed from the collagen sandwich culture.

4-1-4 Kidney

The glomerulus, which is the former half of the nephron in the kidney, is a network of capillaries composed of ECs and podocytes. Musah et al. generated functional podocytes derived from human-induced pluripotent stem cells (hiPSCs) and reproduced molecular filtration properties of the glomerulus. The hiPSC-derived podocytes were seeded on one side of a porous elastomer membrane coated with laminin with human kidney glomerular ECs on the other side of the membrane in the microdevice. The membrane was cyclically stretched and relaxed with the heart rate. Damage induced by drugs was analyzed by monitoring breakdown of the filtration barrier.

Zhou et al. also developed glomerulus-on-a-chip to create a disease model that mimicked hypertensive nephropathy. Two parallel channels were separated by a porous membrane on which glomerular ECs and podocytes were cultured back-to-back. The cells experienced fluid flow under physiological conditions to mimic the glomerular microenvironment. The results revealed that mechanical forces have a crucial role in cellular cytoskeletal rearrangement and damage the cells and their junctions that cause glomerular leakage observed in hypertensive nephropathy.

4-1-5 Placenta

During human pregnancy, the fetal circulation is separated from the maternal blood in the placenta by two cell layers: the fetal capillary endothelium and placental trophoblast. The placental barrier plays an essential role in fetal development and health by regulating the exchange of materials between the mother and fetus. To study the biology of the human placenta, Lee et al. and Blundell et al. created placenta-on-a-chip. Lee et al. created a microsystem consisting of two PDMS microfluidic channels separated by a vitrified collagen membrane. To reproduce the placental barrier, the JEG-3 human trophoblast cell line and HUVECs were seeded on opposite sides of the ECM membrane and cultured under dynamic flow conditions to form confluent epithelial and endothelial layers. Blundell et al. used a permeable polycarbonate membrane with 1-μm pores as a substrate of the cell culture instead of the vitrified collagen membrane. The BeWo b30 human trophoblast cell line was cultured on the upper side of the porous membrane, and human primary placental villous ECs were cultured on the lower side of the membrane under dynamic flow conditions. BeWo cells in the microchannel formed microvilli and reconstituted expression and localization of transport proteins, such as glucose transporters, which are critical to the barrier function of the placenta. In both reports, the physiological function of the microfluidic placental barrier was tested by measuring glucose transport across the trophoblast-endothelial interface.
Permeability was compared to that obtained by acellular devices and devices containing an epithelial or endothelial layer alone.

### 4·2 Cancer models

#### 4·2·1 Tumor angiogenesis

In tumor tissue, angiogenesis is induced by angiogenic factors that are released by tumor cells because of the effects of some growth factors and low oxygen levels. Then, the tumor develops due to nutrients and oxygen supplied from neovessels. Tumor cells invade the neovessels in the tumor to circulate through the body, which induces metastasis. Therefore, it is very important to investigate tumor angiogenesis and the development of microdevices to analyze the processes is required.

Liu *et al.* developed a microfluidic model to mimic tumor-induced angiogenesis. In this model, ECs, which were cultured on the sidewall of an ECM gel containing tumor cells, sprouted into the gel to create a 3D microvascular network inside the gel by tumor angiogenesis. The angiogenic abilities of salivary gland adenoid cystic carcinoma (ACC) and oral squamous cell carcinoma (SCC) cells were analyzed using this model. In addition, the angiogenic capabilities of ACC and SCC cells obtained with the micromodel were consistent with the results of a conventional nude mouse model. Anti-VEGF effectively inhibited tumor angiogenesis in both the microfluidic and nude mouse models.

#### 4·2·2 Tumor metastasis

Although escape of tumor cells from blood vessels, named extravasation is a key event in tumor metastasis, its mechanism remains unclear. Little is known about how hemodynamic forces influence viability, proliferation, motion, deformation of tumor cells, and their interaction with vascular ECs. Therefore, investigations of the processes are required to clarify metastasis. Huang *et al.* reported a microfluidic tumor extravasation model used to provide both mechanical simulation (FSS and CS) and biochemical microenvironments of vascular systems simultaneously. In this model, the mechanical stimulation influenced the viability and attachment on an EC monolayer of HeLa cells. The cells showed the lowest apoptosis rate and the highest ability to attach to the EC monolayer in the model. A typical biomechanical factor, TNF-α, destroyed the EC monolayer and facilitated the attachment of HeLa cells.

*In vitro* models for analyzing tumor metastasis must include microvascular networks with dimensions and barrier functions similar to the human microvascular networks, the ability to perfuse tumor cells into a network with vascular flow, and high-resolution imaging of extravasation events.

Chen *et al.* reported *in vitro* microfluidic microvascular networks that mimic the human microcirculation for real-time observation and quantitation of tumor cell extravasation. Self-assembled microvascular networks were constructed in a microdevice by introducing a fibrin gel containing ECs and fibroblasts. In the device, the microvessels formed open lumens to connect with medium channels. MDA-MB-231 breast cancer cells were introduced from the medium channel into the microvascular network to observe their extravasation (Fig. 6). Human microvascular ECs formed significantly less perfusable and less interconnected vascular networks compared to those with HUVECs. The tumor cells initiated extravasation within 2 to 6 h after cell injection and transmigrated between 4 to 48 h.

The microfluidic system is applicable to organ-specific metastasis assays when relevant cells are added to the matrix gel, thus allowing either direct physical contact or paracrine communication. Bersini *et al.* developed a bone metastasis micromodel to observe breast cancer cells migrating from the
vascular network to bone marrow. HUVECs were cultured on the sidewall of a collagen type I gel containing osteo cells. MDA-MB-231 breast cancer cells invaded the gel through the endothelial monolayer at a higher rate when the gel contained osteo cells. Micromodel experiments showed that the breast cancer cell surface receptor CXCR2 and bone-secreted chemokine CXCL5 had crucial roles in the extravasation process of breast cancer cells, and extravasated cancer cells proliferated and generated metastasis in the ECM gel containing osteo cells.

4-2-3 Blood-tumor barrier

Microvessels in a brain tumor have different characteristics than normal brain microvessels constituting the BBB. Brain tumor microvessels form the blood-tumor barrier (BTB), which is different from the BBB because astrocytes, pericytes, and neurons are not in close proximity to the microvessels and because it shows greater permeability than the BBB.

Terrell-Hall et al. developed microfluidic BBB and BTB models. In the BBB model, a vascular channel with HUVECs was connected with a chamber in which astrocytes were cultured, whereas tumor cells were cultured in a chamber of the BTB model. The HUVEC monolayer in the BTB model showed higher permeability than that in the normal BBB model. Details of tumor micromodels have been reviewed by Tsai et al. and Ahn et al.

5 Conclusions

We reviewed recent studies of microfluidic vascular models. These vascular models are low-cost and time-saving bioassay alternatives to animal models. The formation of blood vessels is a complex tissue-specific process that has a crucial role in developmental processes, wound healing, cancer progression, fibrosis, and other pathologies. Therefore, integration of blood vessels is indispensable for the development of a complex tissue or organ model to understand diseases. Although HUVECs have been used in most recent vascular models, ECs from the target organ should be used to develop an organ-specific vascular model. These microdevices will be useful tools for studying organ-specific microenvironments. Current micromodels mimic only some of the various functions of blood vessels. Therefore, micromodels mimicking various functions and phenomena involved in blood vessels will be developed in the near future.

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7 References

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