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Review

Physiologically relevant organs on chips

Kyungsuk Yum[1,2], Soon Gweon Hong[1], Kevin E. Healy[1] and Luke P. Lee[1]

[1] Department of Bioengineering, University of California, Berkeley, CA, USA
[2] Department of Materials Science and Engineering, University of Texas, Arlington, TX, USA

Recent advances in integrating microengineering and tissue engineering have generated promising microengineered physiological models for experimental medicine and pharmaceutical research. Here we review the recent development of microengineered physiological systems, or also known as "ogans-on-chips", that reconstitute the physiologically critical features of specific human tissues and organs and their interactions. This technology uses microengineering approaches to construct organ-specific microenvironments, reconstituting tissue structures, tissue–tissue interactions and interfaces, and dynamic mechanical and biochemical stimuli found in specific organs, to direct cells to assemble into functional tissues. We first discuss microengineering approaches to reproduce the key elements of physiologically important, dynamic mechanical microenvironments, biochemical microenvironments, and microarchitectures of specific tissues and organs in microfluidic cell culture systems. This is followed by examples of microengineered individual organ models that incorporate the key elements of physiological microenvironments into single microfluidic cell culture systems to reproduce organ-level functions. Finally, microengineered multiple organ systems that simulate multiple organ interactions to better represent human physiology, including human responses to drugs, is covered in this review. This emerging organs-on-chips technology has the potential to become an alternative to 2D and 3D cell culture and animal models for experimental medicine, human disease modeling, drug development, and toxicology.

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1 Introduction

Human physiology and pathophysiology require understanding of cells and tissues, their interactions, and how they generate organ-level functions. Because of the limitation in directly observing and manipulating the human body and underlying cell and tissue structures in vivo, experimental studies of human physiology have relied on ex vivo biological models, such as purified biomolecules, cultured cells, and model organisms [1–4]. As the physiological relevance of such models with respect to humans increases, the experimental complexity, along with required time, cost, and resources, also increases. Medical and life science researchers have thus adopted models that are as simple, robust, and reproducible as possible but still sufficiently represent the physiological phenomena of interest [5–8].

Cell cultures are often the ex vivo models of choice. However, conventional 2D and 3D *static* cell culture models often fail to reproduce the critical aspects of human physiology, because cell culture approaches can be difficult to adapt *dynamic* 3D microenvironments and the simultaneous study of multiple tissues and their interactions [5, 8–10]. For example, 3D cell culture models, in

Correspondence: Prof. Luke P. Lee, Department of Bioengineering, 408C Stanley Hall, Berkeley, CA 94720-3220, USA.
E-mail: lplee@berkeley.edu

Abbreviations: 3D, three-dimensional; **2D,** two-dimensional; **μCCA,** microscale cell culture analog; **ADME,** absorption, metabolism, distribution, and elimination; **AVP,** arginine vasopressin; **CCA,** cell culture analog; **ECM,** extracellular matrix; **HUVEC,** human umbilical vein endothelial cell; **IdMOC,** integrated discrete multiple organ co-culture; **IMCD,** inner medullary collecting duct; **PBPK–PD,** physiologically based pharmacokinetic–pharmacodynamic

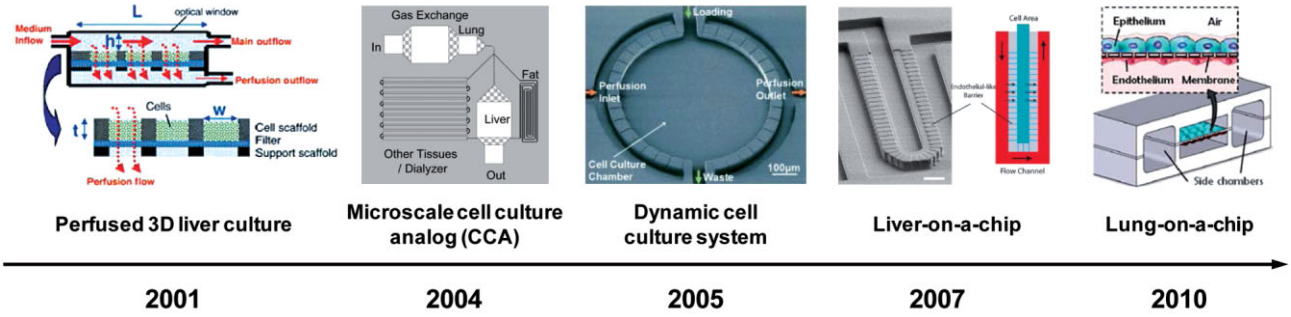

Figure 1. Development of microengineered physiological model systems: microfabricated array bioreactor for 3D liver culture with cross-flow perfusion (2001) [21], microscale cell culture analog (CCA) (2004) [3], dynamic cell culture system with continuous perfusion (2005) [24], liver-on-a-chip device with endothelium-like barriers (2007) [25], and mechanically active lung-on-a-chip device (2010) [26]. Reproduced from [3, 21, 24-26] with permission.

which cells are grown within 3D scaffolds, allow cells to interact with neighboring cells and the extracellular matrix (ECM) [11]; such cell–cell and cell–ECM interactions improve tissue-specific functions. However, 3D cell culture models do not reconstitute highly dynamic microenvironments of living organs crucial for reproducing organ-specific functions, such as dynamic mechanical microenvironments, time-varying gradients of biomolecules, and tissue–tissue interfaces. Therefore, despite their experimental complexity, lack of experimental throughput, and cost, animal models continue to be used [2, 6, 12]. However, in addition to ethical concerns, the relevance of animal models to human physiology is often questionable as data obtained from animals can prove difficult to extrapolate to humans [2, 6, 9, 10, 12].

The integration of microengineering and tissue engineering has recently introduced a new biological model that has the advantages of both in vitro cell culture and in vivo animal models, namely simplicity, high-throughput, and physiological relevance [3, 5]. For example, microfabrication techniques, such as replica molding and microcontact printing, can create microscale structures and patterns that can be designed to construct physiologically relevant mechanical, biochemical, and structural microenvironments [13, 14]. In particular, microfluidics, the science and technology that manipulate small amounts of fluids in channels with dimensions of tens to hundreds of micrometers, is inherently ideal for such applications [15]. Microfluidics offers the ability to precisely control fluid flows for transporting nutrients, generating biomolecular gradients, and applying a flow-induced shear stress and mechanical strain to cultured cells [4].

The early applications of microengineering and microfluidics to cell biology emerged from surface engineering of 2D cellular microenvironments to control the shape, location, and growth of cells, cell–cell interactions, and the expression of tissue-specific functions of cells [3, 13, 14, 16–18]. This technology has also enabled cell-seeded 3D scaffolds with microfluidic vascular networks [19]. As the technology matures, recent efforts have moved toward creating physiologically relevant microenvironments for specific tissues and organs [5, 9, 10, 12].

This emerging technology, named organs on chips, uses microfabrication techniques to construct organ-specific cell culture microenvironments that reconstitute tissue structures, tissue–tissue interactions and interfaces, and dynamic mechanical and biochemical stimuli found in specific human organs to create functional tissue and organ models. For example, organ-specific 3D microarchitectures, microfluidic vascular networks, biochemical gradients, and mechanical stimuli have been incorporated into single microfluidic cell culture systems. Because such physiological complexities are introduced by engineering the microenvironment, this approach maintains the simplicity and throughput of cell culture models [5]. Furthermore, because this approach can use human cells and culture them in microenvironments that mimic those in the human body, the organs-on-chips has the potential to better represent human physiology than animal models. Figure 1 shows representative microengineered physiological systems developed in the past decade, including a microfabricated array bioreactor for 3D liver culture with cross-flow perfusion [20, 21], a microscale cell culture analog (CCA), a microscale physical representation of a physiologically based pharmacokinetic (PBPK) model for toxicology and drug development [22, 23], a dynamic cell culture array with continuous perfusion of medium [24], a liver-on-a-chip device based on the dynamic perfusion cell culture [25], and a mechanically active lung-on-a-chip device that reconstitutes multiple physiological features of the human lung [26].

Here we review the recent development of microengineered physiological (microphysiological) systems, or organs on chips, that reproduce the physiologically relevant, critical features of specific organs and organ–organ interactions in the human body. We first review microengineering approaches to construct the key elements of physiologically important, dynamic mechanical and biochemical microenvironments and 3D microarchitectures of human organs in microfluidic devices. We then give

examples of individual microengineered organ models that incorporate such key elements of microengineered microenvironments into single microfluidic cell culture systems to reproduce organ-level functions of specific organs in vitro. We finally discuss multiple organ model systems that simulate multiple organ interactions to better reproduce human physiology, particularly for predicting human responses to drugs for drug development.

2 Key elements of microenvironments

Microengineered cell culture systems have created various dynamic microenvironments found in the human body, including blood flow, fluid-induced shear stress, and gradients of oxygen, growth factors, and other biochemical signal (Fig. 2). For example, Leclerc et al. [27–29] developed a microfluidic device with continuous fluid perfusion to supply the nutritional medium for cell culture and studied the effects of perfusion flow rate and glucose and oxygen supply on the cell culture (Fig. 2A). Compared to diffusion-based nutrient transport in traditional static cultures in dishes, the perfusion cell culture enhanced the nutrient transport via convection through the microfluidic medium channels. The perfusion device also led to significant increase of albumin production of fetal human hepatocytes compared to static culture conditions [28]. Hattori et al. [30] also developed a microfluidic perfusion cell culture array device, "microenvironment array chip," in which they created combinatorial cell culture microenvironments composed of four types of soluble factors and ECMs (total 16 different microenvironments) for screening cell culture environments (Fig. 2B). Similarly, King et al. [31] demonstrated a microfluidic parallel perfusion cell culture system that can control the dynamics of soluble cellular microenvironments by using a "flow-encoded switching" design strategy. The "flow-encoded switching" strategy uses a laminar flow microfluidic device to control the temporal aspects of cellular stimuli (state of the network) with the ratio of two flow rates (single flow control parameter).

Recent microengineered cell culture systems have been focused on reproducing physiologically relevant dynamic microenvironments of specific tissues and organs. For instance, Maidhof et al. [32] developed a bioreactor that simultaneously provides two critical factors for the development of cardiac tissues: synchronous medium perfusion and tissue contraction driven by electrical stimulation. The simultaneous application of nutrient perfusion and electrical stimulation improved the differentiation of cardiac cells and their assembly into functional cardiac tissue constructs.

In this section, we will discuss how we can use microengineering approaches to construct the key elements of physiologically relevant, dynamic mechanical microenvironments, biochemical microenvironments, and microarchitectures of specific organs and tissue in microfluidic devices.

2.1 Dynamic mechanical microenvironments

Cells and tissues in living organs experience various mechanical forces. For example, endothelial cells that line the interior surface of blood vessels are exposed to a fluid-induced shear stress from blood flow, shear stress across the vessel from interstitial plasma flow, and the interfacial mechanical force between the cells and the surrounding matrix [33, 34]. Such mechanical stimuli have been recognized as important factors for various physiological processes and critical determinants of differentiated functions of cells and tissues [33, 34].

In this section, we will discuss dynamic mechanical microenvironments: flow-induced shear stress and dynamic mechanical strain.

2.1.1 Flow-induced shear stress

Early microfluidic cell culture systems developed to generate flow-induced shear stress have mostly been used to study the effects of fluid-induced shear stress on cell adhesion, mechanics, morphology, and growth [35–39]. Recent studies have focused on reproducing physiologically relevant shear stresses to understand their effects in the context of specific tissues and organs [40]. For example, Huh et al. [41] constructed a microfluidic airway model of the human lung that consists of two microfluidic channels, which represent apical (airway lumen) and basal compartments of the airway epithelium, respectively, and are separated by a porous polyester membrane. They used this device to reproduce three physiological conditions by introducing air, single-phase liquid, and liquid plugs into the microchannel: (i) normal breathing, (ii) the motion of liquid during total liquid ventilation or fetal breathing movements in the developing lung, and (iii) lung injury during airway reopening, respectively. This lung-on-a-chip device revealed that the fluid mechanical stresses generated by the propagation and rupture of the liquid plug can induce significant injury of the small airway epithelial cells. Interestingly, the device also generated cracking sounds when plugs ruptured and caused mechanical cell damage.

Another example is a kidney-on-a-chip device that reproduces luminal fluid shear stress (0.2–20 dyn/cm^2) and transepithelial osmotic gradients produced by urinary flow in the collecting duct system of the kidney [42, 43]. Jang et al. [42, 43] used the kidney-on-a-chip device to study the role of luminal fluid shear stress in the reorganization of actin cytoskeleton and the translocation of water transport proteins (aquaporin-2) of inner medullary collecting duct (IMCD) cells of the kidney.

Microfluidic cell culture systems were also designed to study how fluid forces modulate angiogenesis. To understand the collective effects of fluid and chemical

factors on endothelial sprouting, Song and Munn [34] developed a microfluidic cell culture system that comprises two parallel microfluidic channels, lined with human umbilical vein endothelial cells (HUVECs), and a central microchannel of a 3D collagen ECM that separates the two parallel microfluidic channels, into which

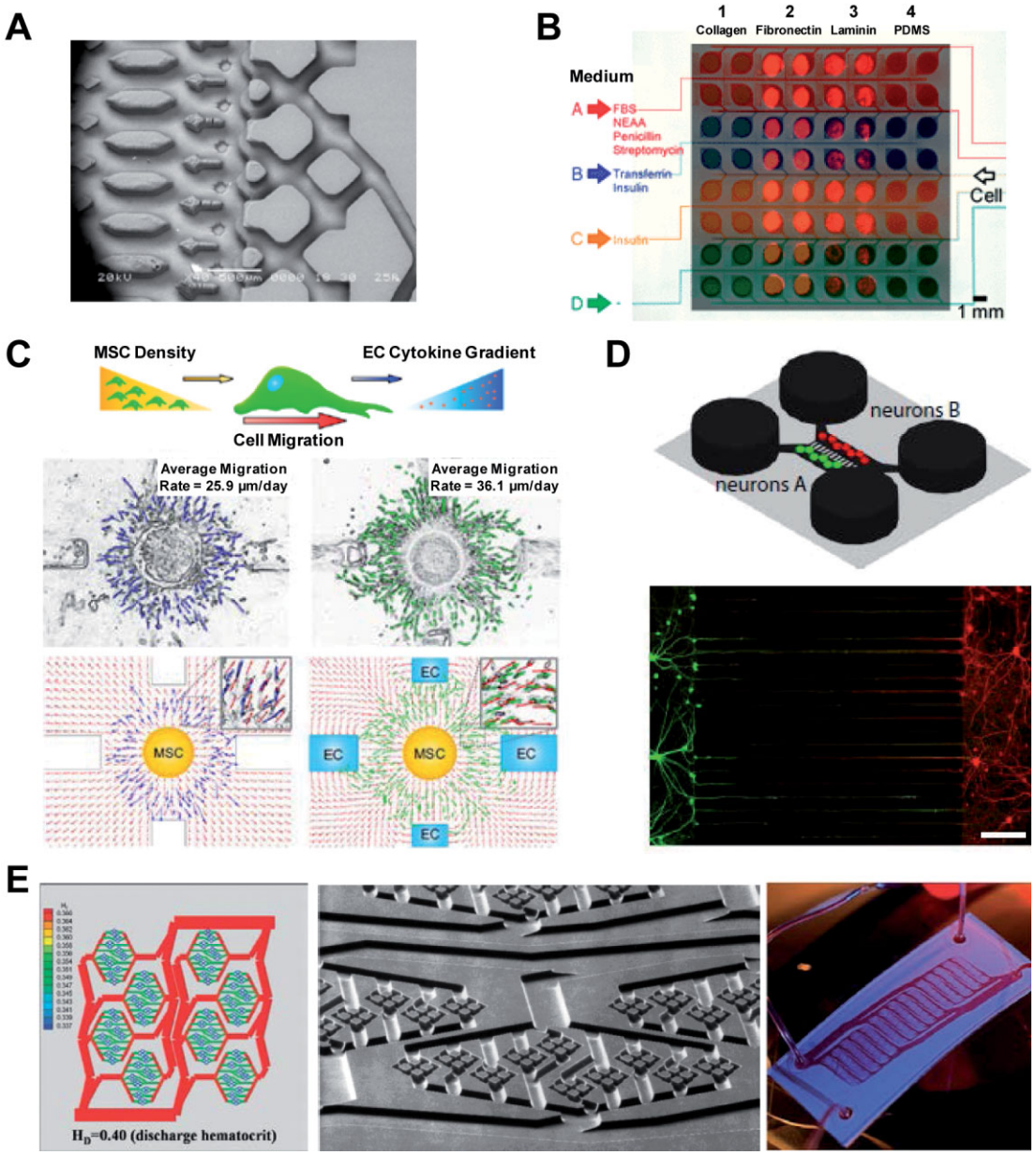

Figure 2. Key elements of physiologically relevant microenvironments. (**A**) Microfluidic device with continuous perfusion to supply the nutritional medium for cell culture [28]. (**B**) Microenvironment array chip composed of soluble factors and extracellular matrices for screening cell culture microenvironments, in which Chinese hamster ovary (CHO)-K1 cells were cultivated for demonstration [30]. (**C**) Schematic of human MSC migration in response to concentration gradient generated by endothelial cells (EC) (top), cell migration vectors generated from phase contrast cell images for MSC-only and MSC-EC coculture conditions, and overlays of the measured cell vectors and modeled vector fields for the two culture conditions (bottom). The insets show the correlation of two vector fields in the outlined regions [66]. (**D**) Brain-on-a-chip: compartmentalized microfluidic device with microgrooves that connect the two rectangular compartments containing two independent populations of neurons and guide the growth of dendrites and axons to form synapses in the microgrooves. Neurons on the left expressed GFP (green fluorescent protein) whereas neurons on the right expressed RFP (red fluorescent protein). Scale bar, 150 μm [78]. (**E**) Computational fluid dynamic model for predicting hematocrit distribution within an engineered vascular network that mimics capillary vasculature (left), microfabricated master mold for polymer casting of an engineered microvascular network (middle), and bilayer hepatocyte culture device that consists of an engineered microvascular network layer for blood flow and oxygenation and a chamber for hepatocyte culture, and an nanoporous membrane that separate the microvascular network and the hepatocyte culture (right) [84]. Reproduced from [28, 30, 66, 78, 84] with permission.

HUVECs can migrate. This microfluidic system generated multiple mechanical and chemical signals to recreate the physiological microenvironment of endothelial cell angiogenesis: tangential fluid shear stress from blood flow, transverse interstitial shear stress, and gradients of vascular endothelial cell growth factors. An interesting finding is that tangential shear stress, exerted by blood flow in vivo, attenuates endothelial cell sprouting and transverse interstitial shear stress (e.g. stress produced by extravasating plasma) enhances the rate of morphogenesis and sprout formation.

2.1.2 Dynamic mechanical strain

In addition to flow-induced shear stress, cells and tissues in the human body continuously experience organ-specific tensile and compressive forces during the normal operation of organs. To reproduce such mechanical microenvironments of the human lung in vitro, Douville et al. [44] developed a multilayered microfluidic lung-on-a-chip device that uniquely mimics the combined solid and fluid (surface-tension) mechanical stresses induced by the cyclic wall stretching and the propagation of an air–liquid meniscus in alveoli of the human lung. Previously reported in vitro models of ventilator-induced lung injury generated either cyclic stretching [45, 46] or air–liquid interface flow over the cells on nonstretching substrates [41, 47, 48]. This lung-on-a-chip device [44] created more physiologically relevant mechanical microenvironments for alveolar epithelial cells during ventilation by simulating both fluid and solid mechanical stresses. This study showed that combined solid and fluid mechanical stresses (cyclic stretch and surface tension forces, respectively) significantly increase cell death and detachment compared to solid mechanical stress alone, supporting clinical observations that cyclic stretch alone is not sufficient to induce the level of the cell injury as seen in ventilator-induced lung injury.

Another example is a human-breathing lung-on-a-chip device that reconstitutes mechanically active microenvironment of the alveolar–capillary interface of the human lung [26, 49]. Fluid-induced shear stress was generated by introducing the culture medium into the capillary channel, which creates a physiological level of fluid-induced shear stress (1 dyne/cm^2). Introduction of air into the alveolar channel created air-induced shear stress. In addition to these stresses, the lung-on-a-chip device reproduced the cyclic strain from the breathing movement in the human lung (10% at 0.2 Hz) by applying cyclic suction to the hollow side chambers, thus causing the mechanical stretching of the flexible membrane between the alveolar and capillary compartments [26, 49]. The lung-on-a-chip device was also used to create a human disease model-on-a-chip of pulmonary edema [49].

In addition to reproducing, the mechanical microenvironments of muscular organs, Grosberg et al. [50] designed a heart-on-a-chip device that uses muscular thin films (MTF), elastic biohybrid constructs that consist of 2D engineered muscle tissues on elastomeric thin films, to measure contractility of engineered cardiac tissues. They also adapted the MTF-based heart-on-a-chip to develop muscle-on-a-chip devices with both striated and smooth muscle cells [51].

2.2 Biochemical microenvironments

Elucidating the fundamental mechanism of gradient-driven biochemical signaling has offered new insights into various physiological processes, including immune responses, wound healing, cancer metastasis, and stem cell differentiation [52, 53]. Diffusive mixing in streams of laminar flow in microchannels at low Reynolds number conditions leads to generating stable, spatially and temporally controlled gradients of soluble molecules, difficult to achieve with conventional methods [53, 54]. Early studies with microfluidic gradient generators focused on creating different types of biomolecular gradients and understanding their biological effects in 2D cellular microenvironments (i.e. chemotaxis) [55, 56]. Recent work has created 3D biochemical microenvironments that mimic biological processes occurred in the human body [57–62]. For example, a microfluidic cell culture system that comprises hydrogel-incorporating chambers between surface-accessible microchannels has been used to study angiogenesis under well-controlled gradients of growth factors in 3D microenvironments [61–64].

Another approach to generate physiologically relevant biochemical gradients is to pattern chemoattractant-secreting (source) and chemoattractant-scavenging (sink) cells in defined locations in microfluidic channels. For instance, Torisawa et al. [65] developed a microfluidic cell culture system that recapitulates physiological gradients of chemokine (CXCL12) in cancer-stroma microenvironments by patterning chemokine-secreting cells (source), chemokine-scavenging cells (sink), and migrating cancer cells in spatially defined positions inside microchannels. This approach enabled efficient chemotaxis under shallower yet more physiological gradients of chemoattractants and showed that the presence and location of sink cells is critical for efficient chemotaxis. Similarly, Eng et al. [66] developed a shape-coded hydrogel-based method to create patterned 3D cellular microenvironments and used this method to control the geometrical pattern of the coculture of human mesenchymal stem cells (MSC) and endothelial cells (EC) (Fig. 2C). They used this system to study the migration of MSC in the controlled biochemical gradient generated by the patterned coculture.

Other examples include a perfusion bioreactor in a liver model with co-cultures of hepatocytes and fibroblasts, which generates physiological oxygen gradients [67]. A perfusion-based microfluidic system was also used to

study the dynamic motion of primary human hepatocytes cells in 3D cell culture environments [68]. A paper-based multi-layer cell culture system (cells-in-gels-in-paper) was also developed to study the behavior of tumor cells and microvascular endothelium in response to oxygen gradients [69, 70].

2.3 Physiologically relevant microarchitectures

Recent advances in microfabrication have brought more complex and sophisticated cell culture microenvironments that reconstitute in vivo-like 3D microarchitectures, including multiple tissue structures, tissue–tissue interfaces, and microvascular networks [5]. In this section, we will discuss three major approaches to construct the physiologically relevant microarchitectures: microstructures in single-layer microfluidic devices, 3D compartmentalization, and microfluidic vascular networks.

2.3.1 *Microarchitectures in single-layer microfluidic devices*

Fabricating microstructures and culturing different types of cells in predefined regions can reproduce the structural microenvironment in the human body, critical to generate functional tissue and organ models [34, 61–65, 71]. For example, Sudo et al. [60] developed a microfluidic coculture system that consists of an intervening 3D gel scaffold (e.g. type I collagen) between two parallel microfluidic channels, in which liver and vascular cells were cultured on each sidewall of the scaffold. They used this

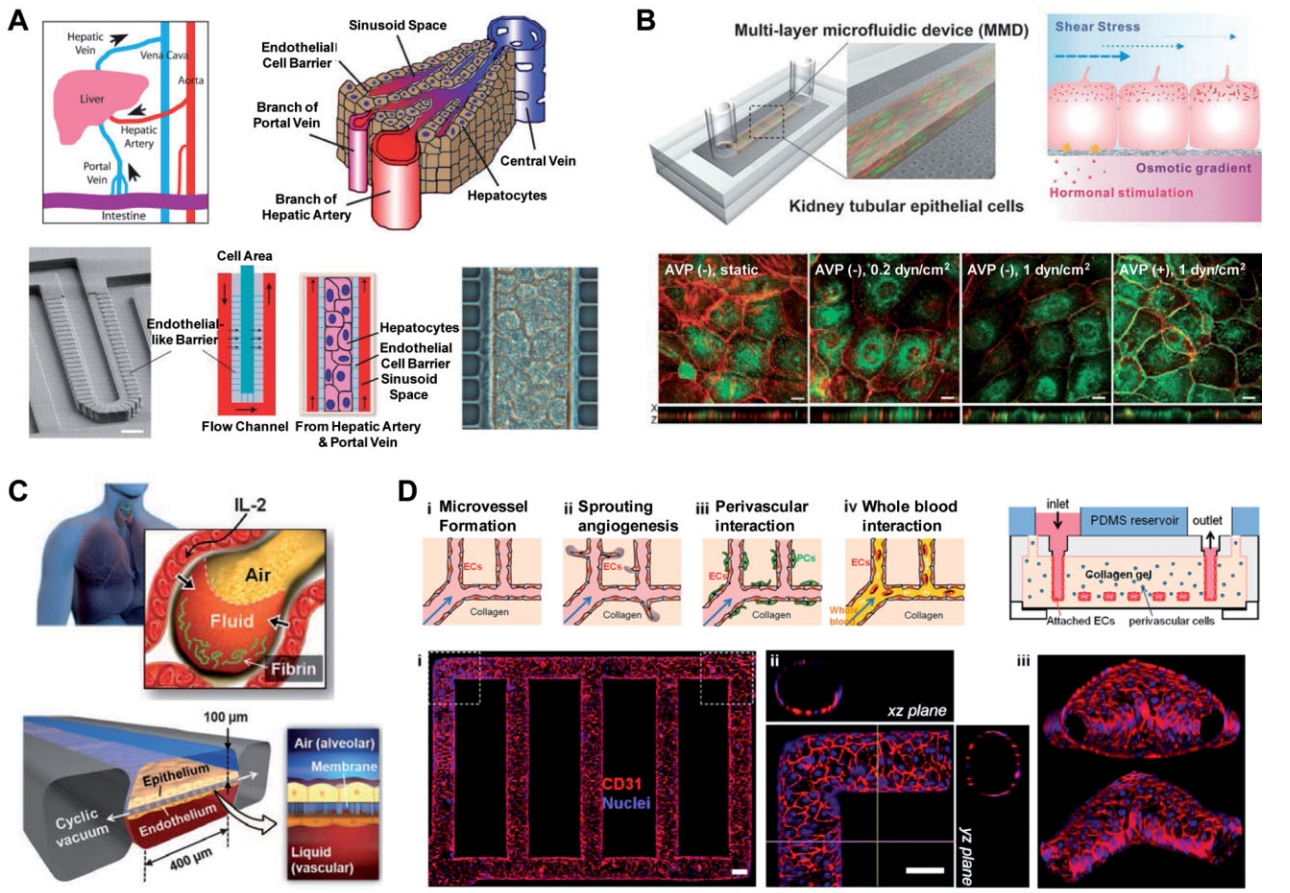

Figure 3. Microengineered individual organ models. (**A**) Liver-on-a-chip: a biologically inspired artificial liver model with endothelium-like barriers that mimic the endothelium of the sinusoid in the human liver. Scale bar, 50 µm [25]. (**B**) Multi-layer microfluidic kidney-on-a-chip. Kidney tubular epithelial cells cultured on the membrane are exposed to a flow-induced shear stress and a transepithelial osmotic gradient defined by the simulated urinary flow in the microchannel. The images below show the effects of luminal fluid shear stress and arginine vasopressin (AVP) stimulation on F-actin (red) and water transport proteins (green), and x–z reconstruction under the images. Scale bar, 10 µm [43]. (**C**) Biologically inspired human breathing lung-on-a-chip device. The lung-on-a-chip device reproduces physiological breathing movements in the living lung by mechanically stretching the membrane that mimics the alveolar-capillary interface [49]. (**D**) Endothelialized microfluidic vascular networks in engineered 3D tissues. Schematic cross-sectional view of a section of microfluidic vessel networks illustrating (i) microvessel formation, (ii) endothelial sprouting, (iii) perivascular interaction, and (iv) whole blood interaction (top left). Schematic of microfluidic collagen scaffolds with microfluidic endothelial vessel networks (top right). Horizontal confocal images of endothelialized microfluidic vessels (i) and views of corner (ii) and branching sections (iii) (below). Red, CD31; blue, nuclei. Scale bar: 100 µm [87]. Reproduced from [25, 43, 49, 87] with permission.

microfluidic coculture system to study angiogenesis in 3D cultures of hepatocytes and microvascular endothelial cells. A similar microfluidic device with three flow channels and two collagen scaffolds that separate the flow channels was also constructed to study capillary growth and endothelial cell migration under various coculture conditions [72].

Microfluidic coculture systems can create in vitro 3D models for cell–cell signaling studies during disease development and progression. For example, a simple Y-shaped microfluidic coculture system was used to study the transition of ductal carcinoma in situ to invasive ductal carcinoma in breast cancer progression, where the laminar flow-based patterning generated two side-by-side compartments to culture mammary epithelia cells and human mammary fibroblasts in each compartment [73]. Grafton et al. [74] developed a simple microfluidic breast ductal system with branched microchannels of decreasing size. They used this microfluidic system with branched microchannels as an in vitro testing platform to characterize targeting and toxicity of superparamagnetic submicron particles and their use for therapeutics.

Compartmentalized microfluidic devices have also been used to arrange neuronal cells and direct their growth to induce the physiological connections of neurons as found in vivo [75–79]. For example, Taylor et al. [75, 78] developed compartmentalized microfluidic devices to guide the growth of axons and dendrites by using parallel microgrooves, which also allowed them to visualize and manipulate synapses and presynaptic and postsynaptic cell bodies (Fig. 2D). Peyrin et al. [80] fabricated a similar microfluidic system with two compartments connected by asymmetrical microchannels, "axon diodes," to generate oriented neuronal networks.

2.3.2 3D compartmentalization

The concept of 3D compartmentalization that creates microengineered compartments with physiologically defined microenvironments and physiological interfaces between the compartments can create 3D microarchitectures in human organs (examples are also shown in Fig. 3) [81]. For example, the concept of 3D compartmentalization was applied to kidney-on-a-chip (Fig. 3B) [42, 43] and lung-on-a-chip devices (Fig. 3C) [26, 41, 44, 49] by constructing multi-layer microfluidic devices. A similar two-layer microfluidic device with a porous membrane was also designed to produce a 3D metastatic cancer model to study the interactions between circulating breast cancer cells and microvascular endothelium under physiological flow conditions [82].

2.3.3 Microfluidic vascular networks

Human organs require dense microvasculature to maintain the function of the cells in the organs. The development of artificial microvascular networks to transport nutrients and oxygen and remove wastes is critical not only for developing engineered tissues for clinical applications but also for maintaining vital functions of cells in organs-on-chips devices; vascular networks are particularly important for microengineered physiological systems of highly metabolic organs, such as heart, liver, and kidney. In addition, angiogenesis is critical for understanding various physiological processes, including wound healing and tumor growth [83]. Artificial microfluidic vascular networks have also been used to mimic physiological blood flow and meet the metabolic demands of effective transport of oxygen and nutrients and removal of wastes (Fig. 2E) [84, 85].

Miller et al. [86] constructed patterned vascular networks in engineered 3D tissues by using a 3D printing method. The vascular networks could be lined with endothelial cells and perfused with high-pressure human blood. The perfused vascular networks also sustained the metabolic function of primary hepatocytes in engineered 3D tissue constructs. Endothelialized microfluidic vascular networks constructed in 3D tissue scaffolds by using the injection molding method [19] are another example of physiologically functioning, perfusable vascular networks [87] (Fig. 3D).

3 Microengineered individual organ and tissue models

A number of microengineered physiological models have been developed to reproduce key features of specific tissues and organs or biological processes. In this section, we discuss the microengineered key elements of microenvironments (discussed in the previous section) can be incorporated into single microfluidic cell culture systems to construct microengineered models of specific tissues and organs. We particularly highlight microengineered physiological models of liver, kidney, lung, and vascular networks.

One of early examples of organs on chips is a microengineered liver model (Fig. 3A) [25]. This liver-on-a-chip device reconstitutes the physiological microarchitecture of the liver, including the hepatic cord-like structure, sinusoids, and highly permeable endothelial cell barriers that separate hepatocytes in the cord-like structure and sinusoids. The endothelium-like barriers were particularly designed to reproduce the physiologically relevant diffusive transport of nutrients and wastes between the hepatocytes and the sinusoid through the endothelium in the liver. A similar concept was applied to other types of liver-on-a-chip devices for drug screening applications [57, 88, 89].

A microengineered kidney model was also developed. Jang et al. [42, 43] reconstituted dynamic mechanical and biochemical microenvironments of the collecting duct system in the kidney, including luminal fluid shear stress (0.2 to 20 dyn/cm^2) and transepithelial osmotic gradients

exerted by urinary flow on renal tubular epithelial cells (Fig. 3B). They constructed the kidney-on-a-chip device by stacking two compartments, an upper flow channel and an underlying static well, and a porous membrane that separates the two compartments and then culturing primary rat IMCD cells on the flow channel side of the membrane. The kidney-on-a-chip device provides an insight into how dynamic microenvironments, such as fluid shear stress, hormonal stimulation, and osmotic gradients, induce depolymerization of actins and trafficking of water transport proteins (aquaporin-2) of the kidney cells.

Another example is lung-on-a-chip devices that reconstitute one or several distinct features of the human lung, including the tissue–tissue interface, physiological breathing movements, and the air–liquid interface of the lung [26, 41, 44, 49]. The lung-on-a-chip device (Fig. 3C) [26, 49] particularly reconstituted the microstructure of the alveolar–capillary interface, which consists of the epithelial cells of the alveolus facing air, the endothelial cells of the capillary facing blood, and the permeable basement membrane between the two tissue layers. This device also reproduced multiple dynamic mechanical microenvironments found in vivo, including the breathing movements of the alveolus, which induces the continuous exposure of the lung tissue to mechanical stretching, the air-induced shear stress exerted on the epithelial cells, and the blood-induced shear stress exerted on the endothelial cells. This lung-on-a-chip device uniquely reproduced the complex responses of the human lung to bacteria and inflammatory cytokines introduced into the alveolar space.

Perfusable vascular networks were also constructed in 3D engineered tissues (Fig. 3D) [86, 87]. For example, Zheng et al.[87] constructed endothelialized microfluidic vascular networks within 3D tissue scaffolds and demonstrated their biological functionality in vitro (Fig. 3D). They fabricated the microfluidic vascular networks by seeding HUVECs and perivascular cells into microfluidic vascular networks constructed in 3D tissue scaffolds (type I collagen) by using injection molding techniques [19]. Compared to previous angiogenesis models, such as lateral endothelial sprouting into a collagen gel isolated from two microchannels lined with HUVECs and 3D sprouting from microbeads in a bulk gel [34], the endothelialized microfluidic vascular networks uniquely reproduced the key features of vascular networks, including initiation of angiogenesis from native-like endothelialized vessels with luminal flow and control of mechanical and chemical microenvironment of the endothelium. The endothelialized microfluidic vascular networks showed the formation of appropriate endothelial morphology and barrier functions (Fig. 3D, i), allowing to study angiogenic remodeling (Fig. 3D, ii), interactions between endothelial cells and perivascular cells (human brain vascular pericytes and human umbilical arterial smooth muscle cells) (Fig. 3D, iii), and interactions of whole blood and endothelium under flow (Fig. 3D, iv).

Other microengineered physiological systems have also been developed to build heart [32, 50], muscle [51], brain [75, 76, 78, 79, 90–92], gut [93, 94], pancreatic islet [95], eye [77], tumor [34, 96] models.

4 Microengineered multiple organ models

An early development of microphysiological systems for multiple organ models came from the need for new model systems for human toxicology and drug screening to overcome the limitation of conventional cell culture models, particularly the lack of dynamic organ–organ interactions [1, 2]. Shuler et al. [1, 2] proposed a CCA and a microscale CCA (μCCA) of a physiologically based a pharmacokinetic–pharmacodynamic (PBPK–PD) model as an alternative to computational PBPK, cell culture, and animal models (Fig. 4A) [6, 7, 97, 98]. The microscale CCA is a physical representation of a PBPK model, in which multiple cell culture compartments, representing different organs with physiological tissue-to-tissue size ratio, are interconnected through microfluidic channels under physiologically relevant fluid flow conditions to predict the time-dependent absorption, metabolism, distribution, and elimination (ADME) of drugs in the human body and human responses to drugs. Li [99, 100] also proposed the integrated discrete multiple organ co-culture (IdMOC) system to overcome the shortcomings of in vitro biological models, such as the lack of multiple organ metabolism and interactions (Fig. 4B). Li realized the concept of multiple organ interactions by constructing multiple small inner wells with cells from specific organs within a large outer well containing the overlying medium that interconnects the physically discrete multiple organ cells in the small inner wells. However, the simple expansion of static cell culture platforms for multiple organ interactions in the IdMOC system has a limitation that it does not consider the dynamic nature of organ–organ interactions, such as dynamic exchange of metabolites between organs, and the circulation system in the human body, which changes ADME [6].

A common limitation of the μCCA and the IdMOC is that the simple representation of individual organs by using cell cultures may not properly reproduce organ-specific functions. The current interest is to integrate multiple organs-on-chips devices, each of which is designed to reproduce the key features of specific human organs (as discussed in the previous section). Such approaches would allow for assessing human responses to drugs on both individual organ and multiple organ levels, including off-target toxicity [101].

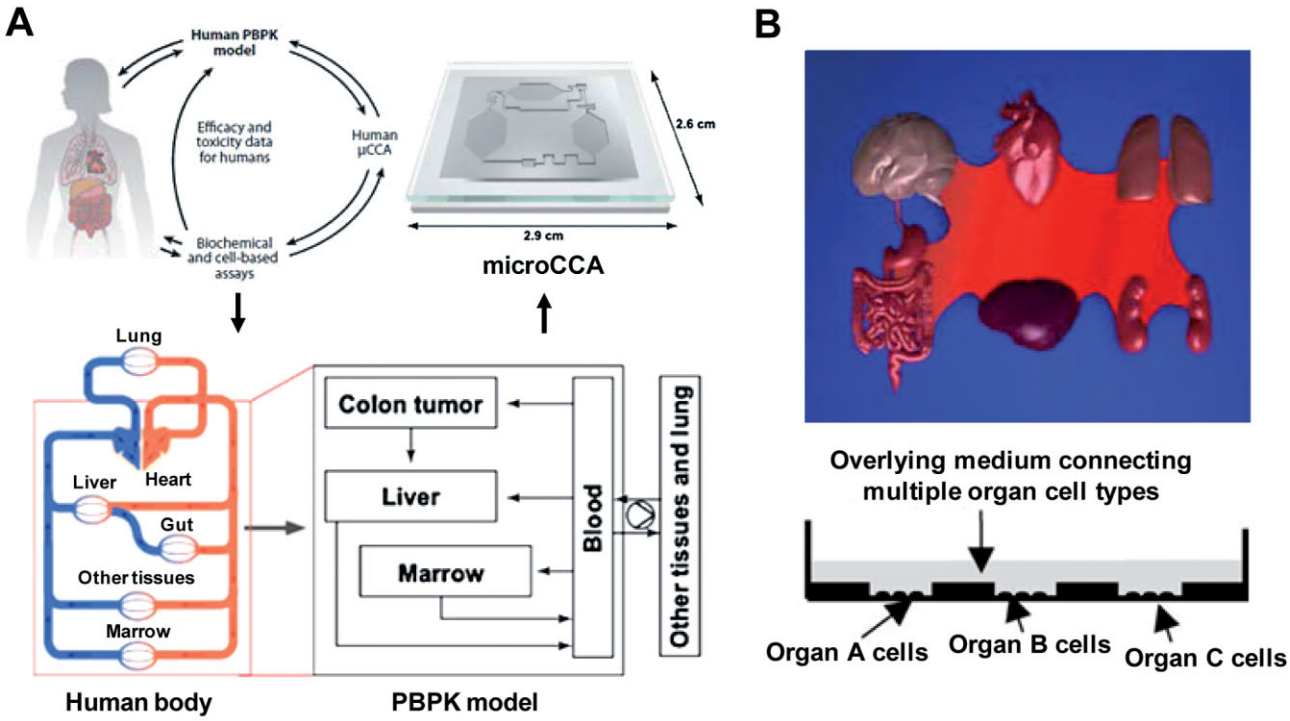

Figure 4. Microengineered multiple organ models. (**A**) Microscale cell culture analog (CCA): a microscale CCA of a physiologically based pharmacokinetic–pharmacodynamic (PBPK–PD) model of the human body to predict human responses to drugs and their metabolites [6, 7]. (**B**) Integrated discrete multiple organ co-culture (IdMOC) system, consisting of multiple inner wells with cells from specific organs, representing physically discrete organs, within a large outer well containing the overlying medium, which interconnects the physically discrete organ cells [100]. Reproduced from [6, 7, 100] with permission.

5 Concluding remarks

The microphysiological systems, or organ on chips, have been emerging as a physiological model for human physiology, drug development, and toxicology. The organs-on-chips technologies reconstitute dynamic microenvironments that reproduce the key features of specific human tissues and organs in microfluidic cell culture systems. Organs-on-chips devices have already begun to serve as an alternative to 2D and 3D cell culture models for studying biological mechanisms in the context of specific tissues and organs [26, 49]. The greater potential of human cell-based organs on chips is in creating human disease models and predicting human responses to drugs and chemicals. For example, one of the main reasons for the failure of new drugs is that animal models often do not predict the efficacy and toxicity of drugs in the human body, because of considerable difference between human and animal metabolism (e.g. liver toxicity). Therefore, the development of organs on chips that can predict the efficacy and toxicity of drugs in the human body, more accurately than animal models, could improve success rates in clinical trials and reduce the time and cost for drug development. Another benefit is that the precise experimental control possible in organs on chips will allow for mechanistic studies at high temporal and spatial resolutions, at a level difficult to be achieved with complex animal models, thus enhancing our understanding of fundamental mechanisms in human responses to drugs.

There exist scientific and technological challenges for the success of organs on chips. This emerging technology requires further scientific validation and characterization to define their capability and limitation for practical biomedical applications. For example, before their use for drug screening, characterization of organs-on-chips devices, by using drugs of which the clinical efficacy and toxicity are well characterized, is required to validate the capability and limitation in predicting human responses to drugs. Another important area for further investigation is to develop mathematical models that can correlate data from organs on chips and in vivo experiments to extrapolate data obtained from organs on chips to humans, including PK–PD models [6, 7, 12].

The wider use of organs on chips, including transferring this technology from the laboratory to clinical and industrial applications, requires the development of user-friendly, standardized organs on chips systems. The design concepts toward these goals include developing scalable, robust, and easy-to-use systems, increasing the compatibility with existing biological techniques, such as high throughput screening systems, developing the on-chip capability for real-time sensing and control of cells

and tissues and their surrounding microenvironments, and standardizing designs and interfaces to potentially develop multiple organ systems.

In addition to constructing physiologically relevant microenvironments from the engineering side, the supply of relevant human cells from the biology side and their use in organs on chips will be another important factor for the future success. Combining organs on chips and human induced pluripotent stem cells technologies in this regard could solve the issue of the availability of primary human cells and improve the relevance of organs on chips to human physiology. More importantly, this approach would also create patient-specific, human tissue and organ models for personalized medicine, drug screening, and toxicology [102–106].

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6 References

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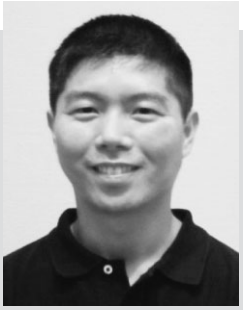

Kyungsuk Yum is Assistant Professor in the Department of Materials Science and Engineering at the University of Texas, Arlington. He received his BS in Mechanical and Aerospace Engineering from Seoul National University and PhD in Mechanical Science and Engineering from the University of Illinois at Urbana-Champaign. Before joining the University of Texas, he worked as a postdoctoral research associate in Chemical Engineering at the Massachusetts Institute of Technology and in Bioengineering at the University of California, Berkeley. His research interests are biologically inspired materials and engineering systems, nanobiotechnology, nano-biomanufacturing, and nanomaterials.

Luke P. Lee is Arnold and Barbara Silverman Distinguished Professor of Bioengineering at UC Berkeley, the Director of the Biomedical Institute of Global Healthcare Research & Technology (BIGHEART) and a Co-Director of the Berkeley Sensor & Actuator Center. He is a 2010 Ho-Am Laureate. He received his BA in Biophysics and PhD in Applied Science & Technology from UC Berkeley. He has more than 10 years of industrial experience in integrated optoelectronics, Superconducting Quantum Interference Devices (SQUIDs), and biomagnetic assays. His research interests are bionanoscience, nanomedicine for global healthcare and personalized medicine, and Bioinspired Photonics-Optofluidics-Electronics Technology and Science (BioPOETS).

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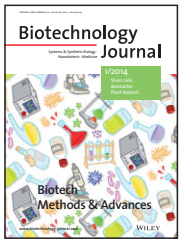

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Review
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