Chapter 13
Bioinspired Engineering of Organ-on-Chip Devices

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Abstract The human body can be viewed as an organism consisting of a variety of cellular and non-cellular materials interacting in a highly ordered manner. Its complex and hierarchical nature inspires the multi-level recapitulation of the human body in order to gain insights into the inner workings of life. While traditional cell culture models have led to new insights into the cellular microenvironment and biological control *in vivo*, deeper understanding of biological systems and human pathophysiology requires the development of novel model systems that allow for analysis of complex internal and external interactions within the cellular microenvironment in a more relevant organ context. Engineering organ-on-chip systems offers an unprecedented opportunity to unravel the complex and hierarchical nature of human organs. In this chapter, we first highlight the advances in microfluidic platforms that enable engineering of the cellular microenvironment and the transition from cells-on-chips to organs-on-chips. Then, we introduce the key features of the emerging organs-on-chips and their proof-of-concept applications in biomedical research. We also discuss the challenges and future outlooks of this state-of-the-art technology.

Keywords Bioinspired materials · Microfluidics · Organ-on-chip · Cellular microenvironment · Disease modeling · Drug testing

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Abbreviations

ADMET	Adsorption, distribution, metabolism, elimination and toxicity
BBB	Blood-brain-barrier
EBs	Embryonic body
ECM	Extracellular matrix
ECs	Endothelial cells
EMT	Epithelial-to mesenchymal
ESCs	Embryonic stem cells
FSS	Fluidic shear stress
iPSCs	Induced pluripotent stem cells
MEMS	Micro-electromechanical system
MSC	Mesenchymal stem cells
PDMS	Polydimethylsiloxane
PK/PD	Pharmacokinetics and pharmacodynamics
TEER	Trans-epithelial electrical resistance

13.1 Introduction

Appropriate model systems drive the development of biological and biomedical research. These model systems seek to recapitulate human physiology and pathology from the molecular level to the cellular, tissue and organ levels, thus providing insights into disease etiology, diagnostic therapeutics and disease prevention. *In vivo*, the body can be viewed as a variety of cellular and non-cellular materials interacting in a highly ordered manner. The complex and hierarchical nature of all living things inspires the multi-level recapitulation of the human body and development of biological model systems consisting of multiple cell types, with internal (cell-cell, cell-matrix) and/or external (e.g. cell-environment) interactions in a more relevant organ and multi-organ context.

Conventionally, animal models are often used to closely recapitulate human physiology in a variety of biomedical research areas, but they fail to faithfully mimic human responses due to the presence of confounding variables and differences between animal and human biology. It is also quite difficult to carry out real-time observation, utilization and throughput assays in animal models. While simplistic models, such as two dimensional (2D) monocultures of cells in a Petri-dish, have their merits to study the biological process of specific cell types, these formats often lack cell-cell and cell-matrix interactions that are necessary to maintain and define the specific phenotypes of cells. They also fail to mimic the cellular functions and intercellular communication that is present in tissues or organs. While three-dimensional (3D) cell aggregates and spheroid cultures can improve the cellular functions to some extent, they still lack many features that are critical for sustaining organ development and function, such as spatiotemporal biochemical cues, vascular perfusion, mechanical cues or multiple cell co-cultures. Obviously, most current

model systems are far from being able to fully reconstitute functions spanning cellular, tissue and organ levels, but it is crucial to develop such biological systems that can address specific scientific questions in biomedical research.

Considerable advances in microfabrication and microfluidics technology have expanded our ability to culture cells in a tightly controlled complex cellular microenvironment in a spatiotemporal manner, thus mimicking the tissue microenvironment *in vivo*. The microfluidic culture platform can provide living cells with continuously perfused medium in microchannels at the microscale [1–5]. Integration of microfabrication with microfluidics technologies that enable precise control of dynamic fluid flow has made it possible to create cellular microenvironments that present cells (e.g. human cell lines, primary cells or stem cells) with appropriate organ-relevant chemical gradients and dynamic mechanical cues. These new capabilities have provided an impetus for the development of alternative cell-based *in vitro* models, which better mimic the complex structures and functional complexity of living organs, termed organs-on-chips [6–9]. These organs-on-chips combine microfluidics with bioengineering and cell biology, allowing study of the diverse biological processes and physiopathology of the human body in ways that are not possible using animal models and conventional 2D or 3D cell culture systems.

In this chapter, we first introduce the microfluidic culture systems that can offer dynamic cell cultures with enhanced capabilities. Then, we introduce the design considerations and key components required for engineering the cellular microenvironment using microfluidic chips. We further summarize the emerging transition from cells-on-a-chip to organs-on-a-chip, and the proof-of–concept applications of engineered organ-on-chip devices. We also discuss the challenges and future perspectives of this state-of–the-art technology.

13.2 Microfluidic Cell Culture System

Microfluidic technology allows precise manipulation of fluid in a microscale device created with technologies developed by the semiconductor industry and micro-electromechanical systems (MEMS). Advances in microfabrication and soft lithography have enabled microfluidic culture systems to reconstitute dynamic, controlled spatio-temporal physico-chemical microenvironments to mimic *in vivo* conditions, distinguishing them from the existing cell culture platforms.

Microfluidic culture devices are usually generated by soft lithography pioneered by Whitesides et al. using polydimethylsiloxane(PDMS) [10, 11]. The classic process of PDMS chip fabrication begins from a mould manufactured using photoresistor silicon [12, 13]. A mixture of silicone rubber and cross-linking agent is poured into the mould and is then easily peeled off the substrate after cross-linking. The PDMS block can be reversibly sealed to other substrates, such as glass and PDMS simply by direct contact. It is also easy to irreversibly bond the PDMS chip to a PDMS substrate, silicon or glass by plasma oxidizing the PDMS surface to form Si-O-Si bonds or by using PDMS as glue [14]. These features make it possible to fabricate multilayer microfluidic chips with flexible microstructure

configurations and channels for compartmentalizing cell culture by simply stacking PDMS pieces to connect different layers [15, 16]. The high elasticity of PDMS enables the integration of pneumatic microvalves into chips to realize complicated cell manipulations on-chip [17–22]. The major superiority of PDMS is its permeability to oxygen and its biocompatibility, which are necessary for long-term cell culture in sealed microchambers or microchannels. The property of PDMS of being optically transparent is favorable for observing cellular behavior and detecting molecules expressed in cells using a brightfield or fluorescence microscope. Besides PDMS, other polymeric materials have been utilized for fabricating configurable microstructures (e.g. microwell, micropillars and microchannels) for microfluidic cell culture [23–28]. In addition, natural polymers, such as agarose, fibrin and collagen, can also be used for generating cell-laden microfluidic chips with the creation of an *in vivo*-like extra-cellular matrix (ECM). More recently, paper-based substrates, which have a fibrous structure analogous to native ECM, have been developed to fabricate chips with 3D microstructures to support the culture of cancer cells, stem cells and cardiomyocytes [29–34]. The diverse properties of these materials have broadened the applications of microfluidics in cell-based biomedical research.

Microfluidic systems were initially applied to cell culture by simply lining the cells in the microchannels or microchambers in a 2D manner with perfused medium in a controlled way. They are often used to study cell growth, proliferation, differentiation and in drug testing. Lately, microfluidic technology has gradually moved to create 3D cell culture models *in vitro* due to its capability to produce and manipulate micron-sized 3D spheroids in a high-throughput manner with great reproducibility [35–37]. Cell spheroid formation is mostly based on the self-assembly features of cells. Although some conventional methods can produce cellular spheroids, such as non-adhesive culture substrates and spinner systems, these methods fail to create uniform cellular spheroids rapidly with controlled size. Recently, microfluidic devices have been developed to produce uniformly sized cellular aggregates [38–40]. These microfluidic based 3D aggregates can be used for generation of multiple types of spheroids from cancer cells, liver cells, and adult stem cells, as well as embryonic bodies (EBs) from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) [41–46]. The cellular spheroids formed by single or multiple cell types can promote cell-cell interactions via gap junctions between cells and thus reduce the distance between cells and the chemical signals secreted from adjacent cells via paracrine pathways. Thus, these 3D cellular spheroids are effective in creating a variety of functional microtissues *in vitro*. A dynamic culture model in a microdevice can also accelerate the proliferation and maturation of tissues.

Microfluidic cell-laden culture systems are an ideal platform for studying cell-ECM interactions as present in the tissue microenvironment. This approach can realize the spatial arrangements of different cellular spheroids via specific microchannel designs, facilitating the study of spheroid-spheroid, and spheroid-ECM interactions. Compared to existing 3D methods, microfluidic-based 3D cell-laden culture has many advantages, including controllable size, arbitrary shape,

macro-tissue reproduction, and easy manipulation of different hydrogels [47–51]. Cell-laden gels as a building block have potential for construction of functional tissues or organs. Zhang et al. proposed a novel and straightforward strategy to produce shape-controlled collagen building blocks via a membrane-templated microdevice [52]. This strategy enables the collagen blocks to self-assemble into 3D tissue-like microstructures with spatial distributions of cell types. These studies open new opportunities to investigate the mechanisms of tissue or organ development and tissue engineering applications.

13.3 Microengineering the Cellular Microenvironment

In order to conduct reliable microfluidics-based cell cultures, it is critical to mimic the cellular microenvironment encountered in vivo (Fig. 13.1). To construct a biomimetic cellular microenvironment in vitro, complex and multi-purpose designs are required to integrate micro-fabricated substrates with microfluidics technologies and cell biology. The in vivo cellular microenvironment is composed of both biochemical and mechanical signals produced by cells and the ECM. These stimuli may

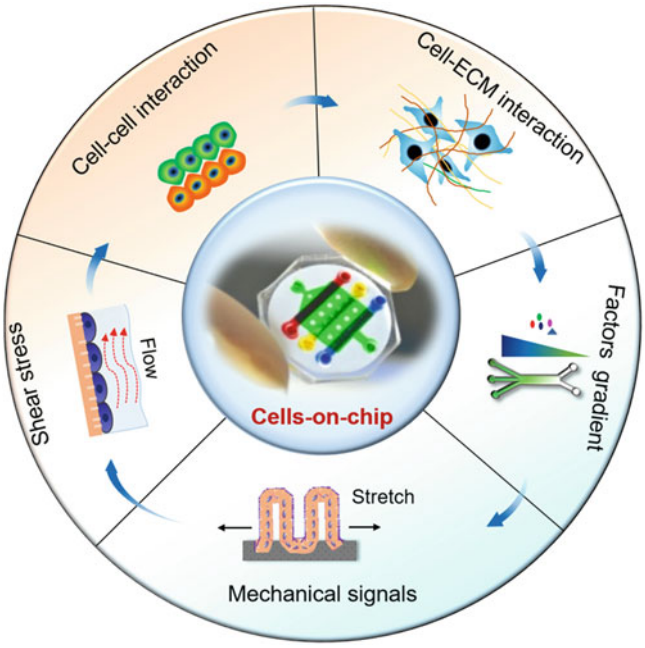

Fig. 13.1 Engineering the cellular microenvironment on chip. The microfluidic device provides cells with a controllable microenvironment, including biochemical and biophysical cues involved in maintaining cellular microenvironments

guide tissue organization and growth via orienting cell polarization and migration, balancing the growth and apoptosis, and regulating functional protein expression and cellular behavior to construct a functional and coordinated tissue. Cell-cell communications within cellular microenvironments share several common features, such as short communication distance between cells and other stimuli, continuous nutrient supply and waste removal, and synergistic actions of total cells to external stimuli. Microfluidic devices offer a powerful tool to reconstruct the cellular microenvironment via providing precise control of intercellular communication, as well as biochemical and biophysical cues that are necessary for the formation and development of tissues or organs.

13.3.1 Cell-Matrix Interaction

Extracellular matrix proteins secreted by different cell types provide important physical support for cells in the processes of tissue/organ formation [53, 54]. These matrix proteins direct cell fate and behavior via promoting cell-cell and cell-matrix interactions. The receptor proteins on cellular membranes can recognize the specific ligands on cells or extracellular matrix proteins and trigger intracellular signaling pathways. Microfluidic platforms exhibit the ability to integrate the ECM within microsystems to form gradients of nutrients, oxygen and soluble factors [55–57] via spatio-temporal control of the cellular microenvironment. For example, a microfluidic-based turning-assay device was designed to realistically mimic the microenvironment of neuronal growth cones in vivo by combining precise gradients of soluble guidance cues with surface-bound guidance signals. The surface-bound laminin gradient enabled to tune the polarity of the neuronal growth cone in response to gradients of neurotrophic factors [58]. Lanfer et al. fabricated aligned collagen matrices using a microfluidic set-up in order to study the effects of collagen on the growth and differentiation of mesenchymal stem cells (MSC) [59, 60]. Using microfabrication techniques, Chin et al. created an array of 10,000 microwells (coated by polyornithine and laminin) with 20–500 μm in diameter on a glass coverslip to study the combinatorial effects of growth factors and laminin protein on the proliferation and phenotypes of rat neural progenitor cells [61]. With these microdevices, the cell-matrix interactions could be studied *in vitro* in a biomimetic manner, which improves our understanding of the formation mechanism and development process of tissues/organs with anisotropic architecture.

13.3.2 Cell-Cell Interactions

Cell-cell interactions guide development and morphogenesis, as well as promote wound healing of tissues or organs because the body is composed of a variety of cell types working synergistically in organized structures. Commonly, cell-cell

interactions under physiological conditions may happen either by direct contact relying on a tight cell-cell junction or by indirect contact via local diffusion of soluble factors or the system of endocrine regulation. It is feasible, with microfluidic technology, to manipulate and culture multiple cell types within a compartmentalized microdevice, which can facilitate the study of cell-cell interactions. Qin et al. developed a series of functional microfluidic chips to realise cellular co-cultures and investigate the interactions between different cell types. Two cell types, MSC and salivary gland cancer cells, were co-cultured in a compartmentalized PDMS microdevice fabricated with two separate chambers and PDMS pillar structures. This study demonstrated that MSCs could be recruited by cancer cells and this effect could be mediated by TGF-β, secreted by cancer cells [62]. It is well known that the first step of tumor cell metastasis is the transfer of circulating tumor cells across the vascular side in a tumor microenvironment. This work designed a 3D microfluidic cell co-culture model to investigate the interaction between cancer cells and endothelial cells, in which the device consists of a vessel-like cavity, endothelium and perivascular matrix containing chemokines [63]. This device enabled the modeling of tumor-cell metastasis in a dynamic manner and visualized observation of transendothelial invasion of cancer cells in real-time, something that is not possible by conventional methods.

The study of neurobiology requires the creation of cellular microenvironments containing multiple types of brain cells and biochemical cues in a precisely controlled manner. A microfluidic system with large open cell culture reservoirs was designed to generate neuronal microenvironments that enable to mimic axon transport and synapse formation via dynamic analysis. In this work, the motor neurons were co-cultured with C2C12-derived myotubes for substantial time periods on the devices in order to mimic the neuro-muscular junction [64]. This device provides a new platform to study the interaction between different cells, such as effector cells and target cells within the cellular microenvironment. Obviously, microfluidic culture systems offer advantages beyond existing methods by allowing to manipulate different cells in a flexible and organ relevant context.

13.3.3 Control of Biochemical Microenvironments

13.3.3.1 Gradients of Soluble Factors

In addition to cellular components, biochemical factors in the local tissue microenvironment may function as regulatory signals to guide various types of cellular behavior, such as cell growth and differentiation, migration and angiogenesis, by forming gradients of soluble molecules. It is quite difficult to generate physiologically relevant biochemical gradients on traditional 2D and 3D culture models.

The ability of microfluidic techniques for producing chemical gradients holds great promise for mimicking and investigating the role of spatially defined soluble microenvironments in guiding cellular behavior and adversity of biological responses [65–69]. Classical microfluidic gradients were generated by manipulating diffused mass transport across the interface between adjacently flowing liquid streams in microchannels under laminar flow at low Reynolds numbers [70, 71]. To validate the chemo-attraction of leukocytes under inflammatory stimuli, Han et al. created a 3D microfluidic cell culture device that generated spatially controllable and stable gradients of two chemo-attractants [72]. A microfluidic gradient chip containing hydrogel-incorporating chambers between surface-accessible microchannels has been utilized to investigate angiogenesis under growth factor gradients in 3D microenvironments [73]. Aside from these methods of gradient formation, relying on laminar flow, Torisawa et al. developed a novel microfluidic device to generate physiologically relevant biochemical gradients by patterning chemo-attractant-secreting (source) and chemo-attractant-scavenging (sink) cells in spatially defined locations inside microchannels [74]. This culture system with more physiological chemoattractant gradients can be broadly used for engineering tissue microenvironments for the study of complex intercellular communications.

13.3.3.2 Control of Oxygen Concentration

Oxygen gradients play a crucial role in maintaining homeostasis in specific tissues, promoting angiogenesis and inducing acute cellular response under inflammatory conditions. Cells in the human body can respond to a wide range of relative oxygen concentrations; for example, normal arterial oxygen content in human brain ranges from 5 to 10 ml/dl. However, the cells in conventional culture systems are usually maintained in atmospheric condition of approximately 20% O_2, which is higher than that in the body [75–77]. Microfluidic devices enable to control parameters of the cellular microenvironment and provide a unique opportunity to generate gaseous gradients with high spatio-temporal resolution [78–80]. A new type of microfluidic device capable of generating oxygen gradients for cell culture was developed based on spatially confined chemical reactions. This device requires a minimal amount of reagents and efficiently controls the oxygen gradients in cell cultures [81]. Derda et al. developed a paper-based cell culture system composed of stacked layers of paper that were impregnated with suspensions of cancer cells in an ECM hydrogel. This system enabled control of oxygen and nutrient gradients in a 3D microenvironment and allowed the analysis of molecular and genetic responses. The paper-supported gels provide a uniquely flexible platform to investigate cellular responses to 3D molecular gradients and to simulate tissue or organ level functions [32, 82].

13.3.4 Control of Biophysical Microenvironments

13.3.4.1 Fluid Flow-Induced Stress

Fluid flow exists ubiquitously in the human body, including in blood vessels, lymphatic vessels, and its role is mass transport and the distribution of soluble factors. The fluid flows in the body span a wide range of fluid velocities from 0.1 μm/s to 0.3 m/s [83, 84]. Different flow velocities may induce various responses of different cell types. It is advantageous for biomimetic microfluidic cell culture devices to enable the simulation and generation of fluidic shear stress (FSS) within microchannels in order to investigate the effects of FSS on cellular adhesion, growth, protein expression and morphology. It is beneficial to be able to reproduce physiologically relevant shear stresses and to study their roles in regulating the specific tissues or organs at levels relevant for organs [85–87]. One significant feature of microfluidic devices lies in their enabling of integrated bioassays. An integrated microfluidic perfusion chip was developed to simultaneously produce multiple-parameter FSS in order to study the effects of fluid flow stimuli on the fate of MSC, chondrocytes and Yes-associated protein (YAP) expression associated with the regulations of cell proliferation, survival, differentiation and organ size [88]. Wang et al. constructed a microfluidic-based vascular-like chip to mimic blood vessels *in vivo* with the aim to study the role of fluid flow in the arrangement of human iPSC-derived endothelial cells (ECs) [89]. In this study, the FSS promoted the arrangement of ECs comparing with static cultures. In addition, this work also mimicked vascular inflammatory reactions under flow conditions by analyzing the interactions of ECs and inflammatory monocytes and the response of ECs to the inflammatory factor TNF-α. The cells showed a physiologically relevant feature under fluidic conditions not possible with conventional cell culture systems. FSS can also modulate cellular behavior via a reorganization of the cytoskeleton (F-actin stress fibers) in cells [90]. Jang et al. developed a microsystem to investigate the effects of luminal FSS on the reorganization of the actin cytoskeleton of inner medullary collecting duct cells of the kidney as well as the translocation of water transport proteins anchoring on the cellular membrane [91, 92]. A biomimetic microfluidic chip was designed to mimic the flow of urine within the proximal tubule and to study the effects of complement C3a on the epithelial-to mesenchymal (EMT) of proximal tubular epithelial cells after being exposed to serum proteins [93].

Due to the microstructure design of the microfluidic devices, they can also be utilized for recapitulating the effects of fluid forces on modulating angiogenesis associated with tumor biology. To investigate the collective roles of fluid and soluble factors on endothelial sprouting, Song and Munn designed a microfluidic culture device that consists of two parallel microchannels lined with HUVEC and a central microchannel filled with collagen gel which separates the two parallel mcirochannels [94]. Multiple mechanical and chemical cues were generated in this microfluidic cell culture system to mimic the physiological microenvironment of ECs angiogenesis.

13.3.4.2 Tissue Mechanics

In addition to fluid shear stresses, cells also experience organ-specific mechanical cues, such as tensile and compressive forces under normal physiological and pathological conditions. A multilayer microfluidic device was designed to study the combinatorial effects of solid mechanical and surface tension stress induced by cyclic wall stretching and the propagation of the air-liquid interface in the alveoli of the lung [95]. This platform generated more physiologically relevant mechanical cues and provided more detailed information than previous models of ventilator-induced pulmonary injury which were based on cyclic stretching [96, 97] or air-liquid interface flow over the cells respectively [98, 99].

Previous studies of gut absorption and metabolism, using human intestinal epithelial cells (e.g. Caco-2), failed to reproduce most of the differentiated organ-specific features of living intestine by culturing these cells on plastic flasks or Transwell inserts as well as microfluidic chips [100–102]. The main reason is that epithelial cells in culture are not able to experience the natural mechanical stimuli including fluid flow and cyclic peristaltic motions in the normal intestine *in vivo*. Recently, Ingberet al. developed a multilayer microfluidic gut-on-a-chip device with a flexible, porous ECM-coated membrane between two PDMS chips containing microchannels. The intestinal epithelial cells were cultured on the membrane substrate in the microchannels and experienced physiological mechanical strains including trickling flow and cyclic mechanical distortion [103, 104]. These mechanical stimuli induced the cells to spontaneously form robust intestinal villi structures that hold the features of tight junctions between cells, coating with brush borders and mucus. The mechanical cues also promoted differentiation of the cells into four different cell lineages of the small intestine, including absorptive, mucus-secretory, enteroendocrine and Paneth, and produced a higher resistance epithelial barrier.

In addition, mechanical strains also influence the biological behaviors of stem cells. Gao et al. created a simple membrane-based microfluidic device to study the effects of cyclic tensile stress on the proliferation and differentiation of MSC [105]. This tensile stress was generated by deforming the elastic PDMS membrane sandwiched between the two layer PDMS chips via negative pressure. The MSCs cultured on the membrane were subjected to different magnitudes of tensile force. The results recapitulated the results that higher tensile stress could promote proliferation, osteogenesis and inhibit adipogenesis of MSCs, providing a new method using tensile stress to regulate the osteogenesis/adipogenesis balance in the development and damage repair of organs.

13.4 From Cells-on-Chip to Organs-on-Chips

In vitro cell culture systems can reflect some biological behavior and functions of cells. However, how cells as the basic building blocks of all living organisms can be made to assemble into functional tissues and organs *in vitro* has been, and

remains, a major challenge of recent decades. Organ-on-a-chip technology rapidly advanced on the basis of integration of microfabrication and microfluidics technology that enable to recapitulate the dynamic and complex tissue microenvironment from the cellular to the tissue and organ level [106, 107]. Organs-on-chips have progressed to the point where it has become feasible to engineer the structural arrangements and functional complexity of living organs by culturing cells in microfluidic channels with multicellular microarchitecture, tissue-tissue interfaces, and biochemical/physical cues in an organ-relevant context (Fig. 13.2). It should benoted that this technology is not intended to engineer a whole living organ but rather to rebuild minimal functional units that represent some specific functions of tissues or organs [108]. In earlier work, the concept of organ-on-a-chip focused on a simple design with perfused microchannels or chambers containing one cell type (e.g., ECs, hepatocytes or intestinal epithelial cells) that represents the functions of one tissue type. Recently, more complex organ-on-chip devices were developed with multi-layer structures connected by porous membranes. Different cells were cultured on opposite sides of membranes to establish interfaces between different tissues (e.g. the blood-brain barrier (BBB) or the lung alveolar-capillary interface). These devices can combine simultaneously chemical signals (e.g. soluble factors) with physical cues (e.g. fluidic shear stress, mechanical pressure and cyclic mechanical strains). They can also be used for real-time analysis of organ-specific responses to drugs, toxins or other environmental perturbations and circulating immune cells. Nowadays, multiple types of organs-on-chips have been created for studying the various biological processes at physiological or pathological levels in ways that are not possible by traditional cell culture and animal models [109–112] (Table 13.1).

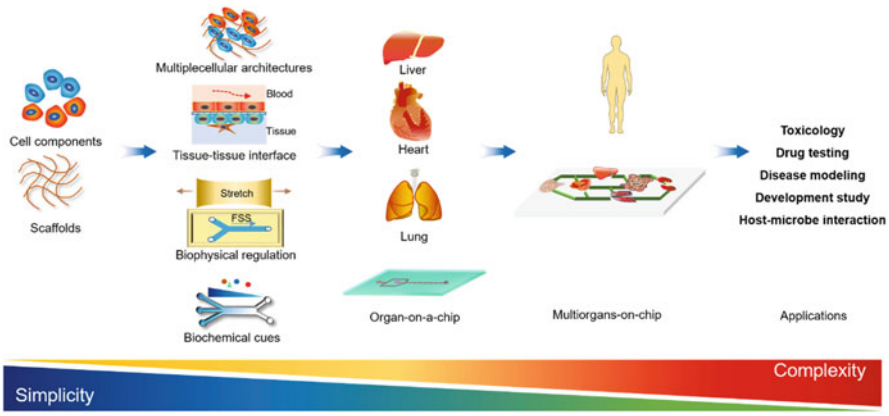

Fig. 13.2 Schematic illustrations of the biomimetic diagram for engineering organs-on-chips

Table 13.1 Summary of the presented organs-on-chip devices categorized by organ types, cell resources, chip features and applications

Organ	Cell source	Preparation process	Applications	References
Brain-BBB unit	Brain ECs (bEnd3) and astrocytic cells (C8D1A) or rat glial cells (C6) (M, R)	Porous polycarbonate sandwiched by PDMS chips with microchannels integrating TEER sensors	Physiological BBB models and toxicity of brain-targeting drugs	[159, 199]
	Brain ECs (R) and astrocytic cells (R)	Two independent vascular channel surrounding tissue compartment in center Two channels for 3D gels, Two channels for media 9 trapezoidal structures in gel channels	Functional analysis	[200, 215]
Neurovascular unit	Neurons and astrocytes (R) HUVEC and cerebral microvascular ECs (H)		Functional analysis	[160]
Heart	iPSC-derived Cardiomyocytes(H);	Microcontact printing using PDMS stamps PDMS thin film	Disease model	[201]
	Cardiomyocytes (R)		Functional heart tissue; Drug testing	[150, 152, 202]
	Cardiomyocytes (M)	Tri-layer cell-laden hydrogel in a perfused PMDSchip	Functional heart tissue; drug testing	[145]
Lung Alveolar-capillary interface	Alveolar epithelial cells, pulmonary microvascular EC (H)	Two PDMS layers with microchannels sandwich a microporous PDMS membrane. Fluid flow, mechanical strain.	Pulmonary inflammation and infection using TNF-a and coli bacteria,toxico logy of nanoparticulat	[115, 116]
	Bronchial epithelial cells(Beas-2B)(H)	A porous silicon membrane is sandwiched between two PDMSmicrochannels	Pulmonary edema and drug toxicity drug testing Lung inflammation model Disease modeling (chronic obstructive pulmonary disease, COPD)	[203]
Lung small airway	Airway epithelial cells and lung microvascular ECs.	PDMS layers with channels separated by semiporous polyester membrane Fluid flow, mechanical strain.		[171]

Intestine	Intestinal epithelial cells (H)	Two PDMS layers with microchannels sandwich a microporous PDMS membrane. Fluid flow, mechanical strain.	Co-culture with microflora Intestinal villus differentiation absorption function host-microbe interaction	[102, 103, 104]
Kidney	Primary kidney inner medullary collecting duct(IMCD)cells (R)	Sandwiched assemble of PDMSmicrochannels, polyester porous membrane, PDMS well Fluid flow shear(1 dyn/cm2)	Physiological renal tubule model; drug screening	[91, 92]
	Primary renal proximal tubular epithelial cells(H) Renal proximal tubular cell line (HK-2)(H)	Fluid flow shear(0.2dyn/cm2) Flow rate (0.6 μl/min)	Drug transport and nephrotoxicity	[204] [93]
	Immortalized glomerular EC (GEnCs) and podocytes (MPC-5)(M)	Flow rate 0.001, 0.002 and 0.003 dyn/cm2	Kidney disease (proteinuric nephropathy) Kidney disease (hypertensive nephropathy)	[205]
Liver	Primary hepatocytes(R); hepatic stellate cell line(LX2)(H)	Multiple chambers reconfigurable coculture device with biosensors	Disease modeling	[206]
	Primary hepatocytes(R)	PDMS device containing multiplexed microchannels	Function analysis; Drug toxicity	[27]
	Primary hepatocytes(H&R)	PDMS chip containing central cell culture region and the outer flow channels with endothelial-like barrier consisted of a set of parallel channels	Function analysis	[126]
	Primary hepatocytes (R);primary adrenal medullary ECs (R); Bovine aortic ECs.	Two PDMS with channels sandwich a porous membrane. Flow perfusion.	Function analysis; Disease model(hepatitis)	[207]
Vasculature	Primary dermal microvascular EC (HDMECs)(H)	PDMS chip containing two compartments and three pump membrane.	Physiological vascular tissues	[208]
	Cordblood ECs (H); lung fibroblasts (H)	Long microchannel connects multiple microchambers	Structure and function analysis	[179, 209, 210]

(continued)

Table 13.1 (continued)

Organ	Cell source	Preparation process	Applications	References
Multiorgans Liver, lung, kidney, adipose	C3A; A549;HK-2;HPA	Multi-channel 3D microfluidic cell culture system (3D-μFCCS)under perfusing medium	Co-culture model	[169]
Neurospheres/liver	Undifferentiated NT2 (H); liver HepaRG cells (H)	PDMS chip containing two compartments and three pump membrane.	Toxicology	[165]
Heart, muscle, nerve, liver	HiPSC-derived cardiomyocytes(H); Skeletal Myofibers(H); Motoneurons(H);hiPSC- derived neurons (H); hepatocellular carcinoma	Two holders separate culture devices with pumpless. Continuous gravitational flow.	Organ-organ communication; Drug toxicity;	[211]
Intestine, liver, skin,kidney	HepG2/C3A(H); intestinal epithelial cells (H); HepaRG and primary hepatic stellate cells(HHSteC) (H); prepuce (H); proximal tubule cell line (RPTEC/TERT-1)(H)	Two PDMS layer with two fluid flow circuits with separate peristaltic on-chip micropump	ADME model	[168]
Intestine, liver	colorectal carcinoma cells(HCT-116 eGFP) (H);	Hang drop technique for fabrication of multi-cell microfluidic chip	Function analysis	[212]
Liver, tumor, Marrow	primary liver cells (R) HepG2/C3A (H); colon cancer cells (HCT-116)(H); Myeloblast cell line (Kasumi-1)(H)	Microscale cell culture (μCAA)	PK-PD model	[166]
Multiple heart chips	iPSC-derived cardiomyocytes (H)	Lego-like plug & play system(μOrgano)	Function analysis	[213]
Spleen	Red blood cells (H)	Device containing fast-flow and slow-flow channel and pillar matrix in slow-flow channel	Spleen filtering function	[214]

H human, *R* rat, *M* mouse

13.4.1 Bioengineering Organs on Chip

13.4.1.1 Lung on a Chip

The lung is the primary respiratory organ in humans. Much effort is devoted to save lives by improving lung health and preventing lung diseases in biomedical research. The alveolar-capillary interface is the fundamental functional unit of the living lung. Some methods developed to-date reproduce the geometry of the lung epithelial-endothelial interface via culturing epithelial and endothelial cells on opposite sides of a thin porous membrane. However, these models failed to mimic the mechanical microenvironment of a living breathing lung [113]. Tavana et al. developed a similar design to monitor the effect of pulmonary pressure on the cell culture system [114]. This model focused on the role that surfactants play in the damage of alveolar epithelial cells. The results demonstrated that the surfactant, Survanta, reduced the cell injuries induced by the liquid plug flow. This lung-on-a-chip system is helpful for the investigation of cellular and sub-cellular effects in airway reopening.

Huh et al. developed a microfluidic lung-on-a-chip device comprising two PDMS layers with microchannels separated by a nanoporous membrane with the purpose to analyze the effects of liquid plug flow on human epithelial cells [99]. Using a similar microdevice, this biomimetic microsystem was used to mimic the structural alveolar-capillary interface of the lung, and the physiological pulmonary respiratory movement via compression and expansion of the system [115]. In this system, human alveolar epithelial cells and pulmonary capillary endothelial cells were cultured on the opposite side of the flexible PDMS membrane that was used to reproduce physiological breathing. Two microchannels on both sides of the PDMS membrane acted as vacuum chambers to mimic breathing movements. The authors found that uptake of nanoparticles by the epithelial cells and endothelial cells increased and that the transport of nanoparticles into the underlying vascular channels was stimulated by the mechanicals constrains of air being pulled in and out of the device. The authors also introduced bacteria and inflammatory cytokines into the air and human bloodborne immune cells into the vascular channels in order to mimic the innate cellular response to pulmonary infection of bacterial origin. Another lung disease model was devised in order to mimic the development and progression of human pulmonary edema induced by the cancer drug interleukin-2 (IL-2) using the same lung-on-a-chip device [116]. Similar to previous chips, air was introduced into the upper alveolar channels and liquid was introduced into the lower vascular channels. The results demonstrated that IL-2 leaked into the alveolar channels increasingly under the cyclic mechanical strains and drug DSK2193874 could inhibit the leakage induced by IL-2. This human disease model-on-a-chip could bring research one step closer to predicting the efficacy of a new drug for pulmonary edema.

13.4.1.2 Gastrointestines on a Chip

The gut is one of the essential components for the maintainance of immune responses and for the natural development of the human body. It is necessary to construct *in vitro* cell-based gut models that aid in the study of the structural, mechanical, absorption functional, drug transportation and pathophysiological properties of the living gut [117]. Several models were developed using human intestinal tumor-derived Caco-2 cells to simulate the intestinal structure and function including Transwell filter inserts and miniaturized microfluidic devices [102, 118–120]. In other work, the researchers developed a new approach to rebuild the normal 3D microarchitecture of the intestinal lining *in vitro* via culturing intestinal epithelial cells on hydrogel substrates that were microfabricated to simulate the size, shape and density of human intestinal villi [121]. However, *these in vitro* models are not able to recapitulate the mechanical microenvironment of the gut, including peristaltic movements and intraluminal fluid flow of human living intestine that is crucial for normal organ physiology and pathology.

To overcome these limitations, Kim et al. demonstrated a functional human gut-on-a-chip microfluidic device composed of two microchannels separated by a porous flexible, clear PDMS membrane pre-coated with ECM and lined with Caco-2 cells [103] (Fig. 13.3). This model adopted fluid flow conditions at a low shear

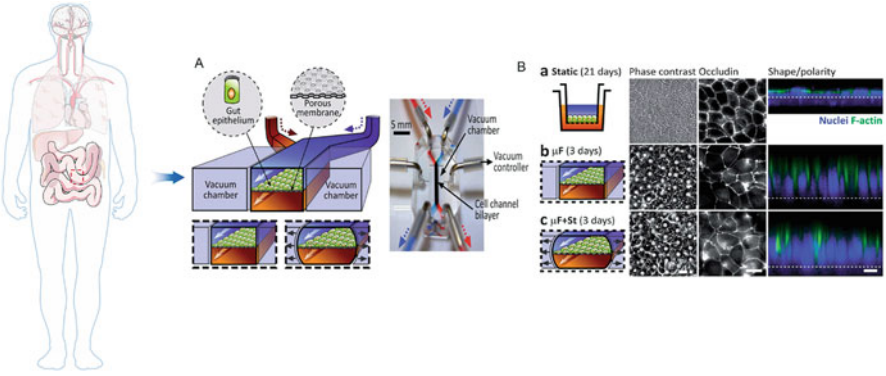

Fig. 13.3 Human gut-on-a-chip. (**A**) Scheme of a gut-on-a-chip device showing the flexible porous ECM-coated membrane lined by gut epithelial cells cross horizontally through the middle of the central microchannel, and full height vacuum chambers on both sides. (**B**) Morphology of the Caco-2 epithelial cells cultured in the static Transwell system for 21 days (*a*) versus in the gut-on-a-chip with microfluidic flow (30 μL h^{-1}; μF) without (*b*) or with (*c*) application of cyclic mechanical strain (10%; 0.15 Hz; μF + St) for 3 days. A scheme (left) shows the system layout; fluorescence views (center) show the distribution of the tight junction protein, occludin, in the epithelial monolayers; and the confocal fluorescence views (right) show a vertical cross section of the epithelium highlighting cell shape and polarity (nuclei in blue and F-actin in green). The regular array of small white circles in (*b*) and (*c*) are pores visible beneath the epithelial monolayer; the dashed white line indicates the top of the anchoring substrate (bar, 20 μm). Reproduced from [103] with permission

stress (0.002 $N \cdot m^{-2}$) and cyclic strain (10%; 0.15 Hz) simultaneously to mimic the intraluminal fluid flow and physiological peristaltic motions in living intestine. Caco-2 cells formed confluent polygonal epithelial monolayers with tight junction protein expression and exhibited differentiated intestinal barrier functions in the microfluidic device after only 3 days culture, which was less than that required on Transwell culture system (>21 days). The flow velocity also influenced the differentiation and polarization of epithelial cells. More interestingly, this study demonstrated for the first time that the planar columnar epithelium spontaneously deformed to form undulations and folds when Caco-2 cells were maintained in the device with the flow and cyclic strain for extended periods of time. These folds exhibited the morphology of normal intestinal villi formed by polarized columnar epithelium with basal nuclei and separated by crypts. In addition, the authors tried to culture the normal intestinal microbial flora, Lactobacillus rhamnosus GG, on the apical surface of differentiated intestinal epithelial monolayers under continuous flow and cyclic strain context. The results showed that normal intestinal microbes can be co-cultured for over 1 week on the apical surface of the cultured epithelium. Based on this gut-on-a-chip device, the same group further explored the structure and function of epithelium using the tumor-derived Caco-2 cells in detail [104]. The cells formed undulating structures containing basal proliferative crypts and the four types of differentiated epithelial cells (absorptive, mucus-secretory, enteroendocrine and Paneth) of normal intestinal villi. Furthermore, the intestinal models on chips behaved similarly to normal intestinal physiology, such as more efficient glucose reuptake, higher cytochrome P450-3A4 isoform activity and mucus production than previous static culture systems. Thus, this human gut-on-a-chip may play a critical role in studying the mechanical regulation of intestinal function and host-microbe symbiosis and evolution.

The stomach is another important organ in the digestive system with specific chemical and physical microenvironments. Gastric mucus serves in protecting the epithelial cells of the stomach wall from injures by the acidic digestive juices in the gastric lumen. Li et al. reported an *in vitro* microfluidic device that replicated a dynamic stomach acid barrier [122]. This study used native mucins obtained from pig gastric mucus and perfused continuously mucus liquid into the epithelial lumen. Several models of the mass transport were constructed to investigate the effects of H^+-mucin interaction on the diffusion of H^+ and acid penetration was monitored using the pH sensitive dye Oregon Green by live fluorescence microscopy. This work indicated that a continuously secreted mucin layer can hinder acid diffusion. This stomach-on-a-chip holds promise for the study of the barrier functions provided by the mucus layer and the interaction of the mucus layer and drugs.

13.4.1.3 Liver on a Chip

The liver is a major organ with multiple functions for protein synthesis, detoxification of various substrates, digestion and metabolic activities in the human body. Multiple liver-on-a-chip devices were fabricated to construct liver microsystem due to their ability to mimic the complex *in vivo* microenvironment [123–125]. To mimic the functional unit of the liver, Lee et al. developed a biologically inspired artificial liver sinusoid microdevice [126]. In this microfluidic chip, an endothelial-like barrier formed by microstructure served a mass transport function similar to the liver acinus. Primary rat and human hepatocytes were co-cultured in the configuration for 7 days without ECM coating. This model has also been applied to test the hepatotoxicity of diclofenac. With the advances of microfabrication technology, well-organized liver microtissues were created that simulated both the structural and physiological functions of the living liver in contrast to the conventional approaches using random co-culture methods. Micropatterned substrates can offer a suitable microenvironment to explore the cell-interactions in co-culture systems [127–130]. Although 2D patterning is convenient to control the spatial position of hepatocytes and other supporting cells, fresh primary hepatocytes cultured on 2D substrates rapidly, lose their capacity to proliferate, to form differentiated structures and their liver-specific functions compared to 3D culture microenvironments [131]. 3D culture systems can promote the hepatocytes' functions and maintain their differentiated properties for extended times *in vitro*, as well as mimic *in vivo* structural features including lobule and tubular architectures as closely as possible [132, 133]. Currently, several methods have been developed to construct 3D liver microtissues, including a spinner culture system, non-adhesive surfaces, pellet culture models, cell sheets and a hanging drop [134], but the microfluidic-based procedures may be more effective as they offer a convenient and straightforward platform in order to form uniformly sized and shaped 3D structures [135, 136].

Continuous perfusion is of great importance in 3D cell culture systems in order to maintain the long term function and viability of hepatocytes. Microfluidic technology enables the precise control mechanism for perfusing medium with nutrients and controlling its chemical composition not possible using traditional culture system. The fluid flow promoted and maintained the 3D tissue-like structure and cell-specific function of primary human hepatocytes and stem cell differentiation into hepatocytes [137–139]. Dash et al. reported that hepatocytes exhibited an enhanced capacity of metabolism of specific cytochrome P450 enzymes in a flow system compared with non-flow conditions [140]. Trietsch et al. developed a stratified 3D cell culture model incorporated in a microtiter plate format. The hepatocytes and fibroblasts were co-cultured in the way of mixed or side-by-side format to evaluate the toxicity of rifampicin. Furthermore, this device was used to study the invasion and aggregation of breast cancer cells [141]. To mimic the functional unit of the liver, a microfluidic culture device was created to rebuild the microscopic structure of the hepatic cords [142]. The asymmetric tip of the device with two separate compartments can house two cells side-by-side and the aligned hepatocytes can self-organize and form bile canaliculi along the cord-like microscale structure. Recently,

Esch et al. fabricated a low-cost cell culture device to culture 3D liver microtissues containing multiple liver cell types including primary hepatocytes, stellate cells, fibroblasts and Kupffer cells under fluid flow condition [143]. These 3D liver microtissues had good enzymatic activity and responded to bacterial lipoprotein. All these 3D liver microsystems represent an important step towards adoption of organ-on-a-chip technology for disease modeling and drug development.

13.4.1.4 Heart on a Chip

Microengineering cardiac tissue can potentially drive the development of efficacy and toxicity of drugs via reproducing crucial cardiac physiological features. Currently, several heart-on-chip systems have been explored to mimic physical, mechanical and biological functions of living heart which are not realized using conventional 2D culture systems [144–149]. Parker et al. designed a microfluidic device to culture rat cardiomyocytes using fibronectin-coated flexible elastomeric cantilevers cultured to form anisotropic muscular tissue [150]. The average systolic stress and diastolic stress were within the stress range published previously using isolated muscle strips [151]. These muscular thin films exhibited a chronotropic effect as the concentration of epinephrine increasing by dose-response curve experiments. This platform was further improved by combining a fluid flow control system and the platinum electrodes to collect accurate functional contractile response to a cardiac drug isoproterenol (β-adrenergic agonist) [152]. This high throughput heart-on-a-chip device is very useful for collecting large quantities of high quality functional data, testing cardiac drug and integration with other organs. Serena et al. reported a microfluidic device based on micropatterning techniques which can selectively treat areas of the cell array and perform multi-parametric assays [153].

Microfluidic technology can be integrated with stem cells to generate human derived cardiac tissues. The cardiac bodies derived from iPSCs were captured in niches along a perfusion microchannel in a microfluidic device. The cardiomyocyte clusters exhibited well-developed sarcomeres and cardiac protein markers (Nkx2.5 and Cx43) as well as *in vivo*-like cardiac function [154, 155]. In addition, a non-invasive recording method was introduced in this study to calculate the beating frequency of cardiac bodies using an ordinary microscope. These 3D cardiac bodies responded to different drugs rapidly in a similar manner to living heart tissue in a body. Other researchers explored different microfluidic approaches to form cell-laden hydrogels in order to evaluate the functionality of the cardiac tissue constructs. Aung et al. produced 3D perfused cardiomyoctes-laden hydrogels within a microfluidic chip and monitored the contractile stress of cardiac tissues in real-time and *in situ* measurements [156]. A cardiac microphysiological system was developed to recapitulate a minimal human cardiac microtissue in a central microchannel of the microfluidic device. This biomimetic system can drive the self-organization of human iPSC-derived cardiomyocytes into aligned 3D heart organoids which presented spontaneous beating at physiological level [157, 158]. The heart microtissues were also treated with multiple drugs at different concentra-

tions. The results demonstrated that half maximal inhibitory concentration (IC50) and half maximal effective concentration (EC50) values were more consistent with the data on tissue scale references compared to studies at cellular level.

13.4.1.5 Blood-Brain-Barrier on Chip

The blood-brain-barrier (BBB) is a selective yet dynamic barrier between the central nervous tissues and the circulatory system, which is formed by microvascular ECs, pericytes and the perivascular end-feet of astrocytes. The dysfunction of this barrier is associated with brain tumors, Alzheimer's and neurodegenerative diseases. Reproducing the physiological characteristics and functional responses of the BBB in a reliable model will greatly promote the development of novel therapeutics for central nervous system diseases [199, 200]. To mimic the *in vivo* BBB, a microfluidic BBB (μBBB) was created by lining a fibronectin-coated polycarbonate membrane with brain microvascular endothelial cells and astrocytes on the opposite side of the membrane [159]. This BBB exhibited a well-developed biological function, including higher trans-epithelial electrical resistance (TEER) across the barrier, and being more impermeable to large molecules. A more complex microfluidic device of the neurovascular unit was created by co-culturing rat brain microvascular ECs and a mixture of three different cell types derived from rat brain (astrocytes, neurons and microglia) on opposite sides of a porous membrane [160]. The endothelial cells formed a good barrier and the neuronal cells fired inhibitory as well as excitatory potentials after having been cultured for 10 days. Moreover, a tissue-mimetic neuroinflammation model was also established on this chip by stimulating the ECs with inflammatory factor TNF-α for 6 h, leading to the significant activation of resident microglia and astrocytes on the neural side.

13.4.1.6 Multiple Organs on a Chip

The advances of single organ-on-a-chip technology highlight the importance of interaction between different cell types within the same functional unit of the organ. More complex microfluidic chips integrating tissue-tissue interfaces and compartmentalized microchambers connected with microchannels (e.g., blood-brain-barrier, lung alveolar-capillary barrier) make it possible to create multiple organs on a chip. These multi-organs on a chip are designed to maintain multiple cell types in different culture compartments in one device for long-term culture and integrate them into one system in order to mimic organ-organ interaction. In the past decade, several multiorgans-on-chip devices have been developed for potential use in disease modeling and drug toxicity screening applications. To mimic physiologically relevant organs realistically *in vivo*, some key aspects need to be considered to design the chip, such as the size of the organs, interactions between different organs and the organ volume-medium ratio (scaling of organ sizes and vascular flow *in vivo*). Wikswo et al. discussed scaling arguments about

microengineering multi-organs on a chip to guide the design of a universal cell-culture medium without red blood cells [161]. It is mentioned that the prototype drug metabolized by the liver component could have therapeutic effects. Thus, the liver should be placed upstream of the drug target organs in designing the multi-organs on a chip.

The field of multiorgans-on-chips is explored and investigated for different purposes [162]. To study tissue cross-effects, liver and intestinal slices isolated from the same rat were cultured in one microdevice [163]. The liver and intestinal slices remained functional under flow conditions, indicating the potential of this chip for the study of inter-organ interactions. Wagner et al. cultured human primary hepatocytes and skin biopsies simultaneously in one microfluidic device for up to 28 days [164]. These studies demonstrated that the primary tissues from living body can maintain their functions *in vitro* under dynamic culture condition. Moreover, a 3D two-organs-on-chip device was created to support the survival of differentiated neurospheres and liver spheroids in a combined media circuit over 14 days [165]. In the study it was suggested that the two combined tissues were more sensitive to the 2, 5-hexanedione compared to respective single-tissue cultures, maybe due to the tissue-tissue interactions. As is well established, most orally adminstred drugs are absorbed and metabolised in the small intestine or the liver and excreted by the kidney. However, it is impossible to mimic these processes *in vitro* using conventional cell-based models.

A typical example of a three organs-on-chip device was designed with a 3D hydrogel culture in which three cell lines were cultured to assess metabolism-dependent cytotoxicity of anti-cancer drugs [166] (Fig. 13.4). In this microsystem, cancer cells, liver cells and myeloblasts were cultured in separate chambers representing cancerous tissue, liver and bone marrow. Tegafur, an oral prodrug of 5-fluorouracil, was introduced into this system to test the cytotoxicity to the three cell lines. Compared with conventional 96-well microtiter plates, the micro cell culture system enabled to reproduce the metabolism of Tegafur to 5-fluorouracil in the liver and the resulting death of cells caused by 5-fluorouracil, while cells in the 96-well microtiter plate did not display the same results. Esch et al. designed a microscale body-on-a-chip system containing gastrointestinal tract, liver and other tissues to mimic the oral uptake of nanoparticles. The nanoparticles were transported across the GI epithelium and interacted with liver cells resulting in the damage of the latter [167].

To improve the functions of multiorgans-on-chip, a four-organ-chip microphysiological system was proposed to maintain the functionality of four organs containing human intestine, liver, skin and kidney over 28 days [168]. Considering the size ratio of tissues on chip compared to human living organs, the intestine and skin tissues on chip are at a size 100,000-fold smaller than their counterpart organs. 3D liver microtissues, equivalent to ten liver lobules, were found to mimick liver function. Human proximal tubule cell lineage RPTEC/TERT-1 was used to form the proximal tubule barrier on a polymeric membrane to mimic the metabolite excretion. A peristaltic micropump integrated in this chip enables pulsed medium flow interconnecting the four organs through microchannels. This device considered

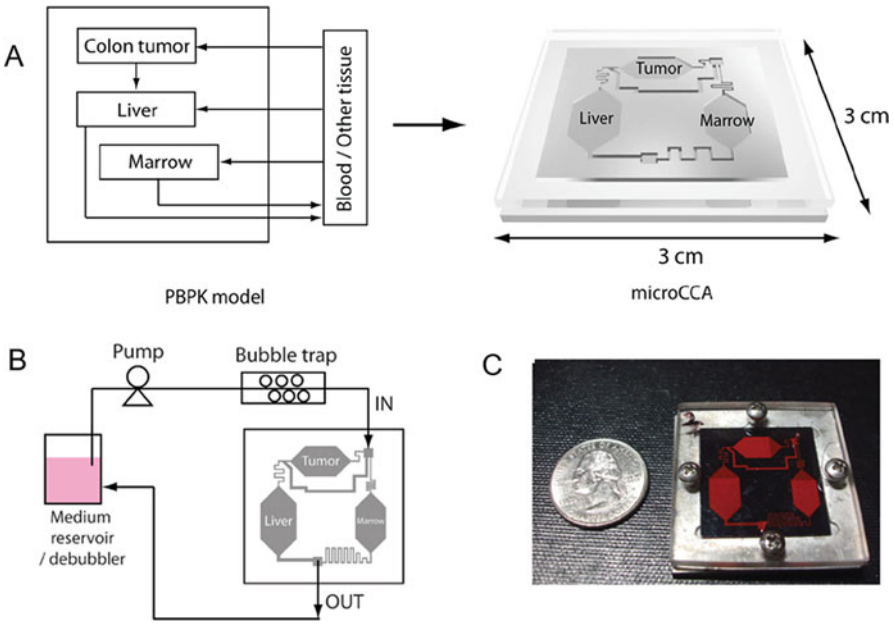

Fig. 13.4 Design of multi-ogans on a chip. (**a**) Liver-marrow-cancer on a chip. Hepatoma cells (HepG2/C3A), Myeblasts (Kasumi-1) and colon cancer cells (HCT-116) are embedded in a 3D hydrogel and cultured in separate chambers representing the liver, marrow and tumor respectively. Medium flows through the cell culture chambers via connecting channels with a pump mimicking blood flow. (**b**) Schematic diagram of operation setup of a single chip with medium recirculation. Medium is withdrawn from a reservoir, after circulating through the channels and chambers in a chip, medium goes back to the reservoir for recirculation. (**c**) A picture of an assembled Liver-marrow-cancer on a chip with red dye for visualization of channels and chambers. Reproduced from [166] with permission

the physiological fluid-to-tissue ratio which is important to accurately simulate the drug metabolism. This microsystem exhibited remarkably robust homeostasis and functionality of the four organ equivalents, presenting itself as a good platform for *in vitro* adsorption, distribution, metabolism, elimination (ADME) modeling. A new type of four organs-on-chip device was fabricated to culture four human cell types representing liver, lung, kidney and the adipose tissue, respectively [169]. A common culture medium is the key factor to be considered in the development of multiple cell type culture systems that should satisfy all types of cells. This is also the critical question for designing multi-organs on a chip in the future.

13.4.2 Integrated Analysis System

One of the most powerful opportunities for microfluidic organs-on-chip for biomedical applications is the capacity of integrated analysis system to monitor and control

organs in real-time. Existing automated techniques enable us to monitor cellular proliferation, morphology and molecular changes in multiwell plates. However, it is difficult to monitor the complex multiple parameters on organ microsystems including gradients of chemical factors and of oxygen, fluid flow shear, mechanical cues, matrix composition and metabolites. Currently, several types of microsensors for real-time monitoring of organ conditions have provided measurements of basic cell culture parameters including oxygen, pH, temperature, and molecular composition (e.g., glucose and lactate). More specialized sensor measurements are widely used to monitor the trans-epithelial electrical resistance (TEER) which usually exists in biological barriers, such as the blood-brain barrier, intestinal epithelial-capillary barrier and the lung alveolar-capillary barrier. Recent efforts indicate that TEER measurements in organs-on-chip systems present specific challenges and are highly sensitive to slight changes in the cell barrier membrane [170]. Hence, the automated and precise measurements of TEER in a complex organ microsystem would increase reliability and offer key information on the organ condition and responses to different drugs.

13.5 Proof-of-Concept Applications of Organs-on-Chip

13.5.1 Disease Modeling

Disease modeling is one of the main applications of organs-on-chip technology, ranging from genetic diseases to infectious, cancerous and degenerative diseases, and is helpful for understanding the disease etiology, diagnostic strategies and effective therapies. Several proof-of-concept disease models have been reported to date.

13.5.1.1 Inflammatory-Related Diseases

Inflammation reactions are closely related to many diseases and can lead to serious pathological symptoms. Pneumonia is one of most common lung diseases, characterized by its complexity, acute onset and difficulty to control it. Establishing an *in vitro* model to study the disease mechanism and to be able to screen for efficient drugs is an urgent requirement in order to address this challenging disease. The breathing lung-on-a-chip, as mentioned above, recapitulated the epithelium-endothelium interface under fluid flow and cyclic mechanical strain conditions to mimic the functional unit of the lung. Inflammatory models were developed in this system by administrating immune activator TNF-α or bacteria which increased expression of surface ICAM-1 of endothelial cells and recruitment of human neutrophils [114]. Based on the lung-on-a-chip system, the same research group further mimicked a pulmonary edema model by the cancer drug interleukin-2 (IL-2) which can cause pulmonary vascular permeability and lung edema [115]. This lung

disease model also recapitulated that the mechanical strain caused by the breathing motions promote increased vascular leakage and pulmonary edema induced by IL-2 but not the circulating immune cells. An organ-level lung small airway-on-a-chip model was created to study human chronic obstructive pulmonary disease (COPD) and drug response using patient and healthy lung microvascular endothelial cells and airway epithelial cells [171]. This model effectively rebuilt many properties of the structures and functions of human lung bronchioles and maintained them for weeks *in vitro* which is crucial for studying chronic disease *in vitro* including cell types and cilia structure. More importantly, interleukin-13 (IL-13) stimulated the epithelium leading to goblet cell hyperplasia, cytokine hypersecretion and ciliary functional decline of asthmatics. Using the robust in vitro lung-on-a-chip model, it is possible to screen for synergistic effects of lung epithelium and endothelium on cytokine secretion, discover new biomarkers of disease exacerbation and evaluate responses to anti-inflammatory drugs.

In addition, neuroinflammation was studied by a more complex microfluidic chip supporting the neurovascular unit containing endothelial barrier on one side of a porous membrane and three various brain cell types (astrocytes, neurons and microglia) on the other. Inflammatory factor, TNF-α, stimulated the vascular endothelium which activated adjacent microglia and astrocytes. This process is similar to what happens *in vivo* in situations such as neuroinfectious diseases [160].

13.5.1.2 Brain diseases on Chip

Neurodegenerative diseases such as Alzheimer's disease (AD) seriously threaten human health. In order to better understand disease etiology and to develop efficient treatment strategies, it is indispensable to seek a suitable *in vitro* model for exploring the mechanisms of brain diseases. Lee et al. presented a microfluidic device to create 3D neurospheroids which more closely simulate the *in vivo* brain microenvironment (Fig. 13.5) [172]. The uniform neurospheroids formed in concave microwell arrays are exposed to all directions and are able to interact. By introducing an interstitial level of flow using an osmotic micropump system, this model system demonstrated that the flow could promote neurospheroid growth, improved neural network formation compared with cultures under static conditions, as well as enabled long-term cultures without the need for peripheral devices. To test the potential of this microfluidic microsystem as an *in vitro* brain disease model, the authors added the amyloid-β peptide, which is generally viewed as the major contributor in AD, on 3D neurospheroids under interstitial flow conditions. The study indicated that the neurotoxic effects of amyloid-β led to a decline in neural cell viability and destroyed neural networks via inducing synaptic dysfunction. Taken together, this biomimetic brain-on-a-chip device recreates a 3D cytoarchitecture and interstitial flow of brain *in vivo*, as well as mimics the pathological changes of AD *in vitro*. This platform has great potential for investigating neurodegenerative disease pathology and treatment strategies as well as for drug testing applications.

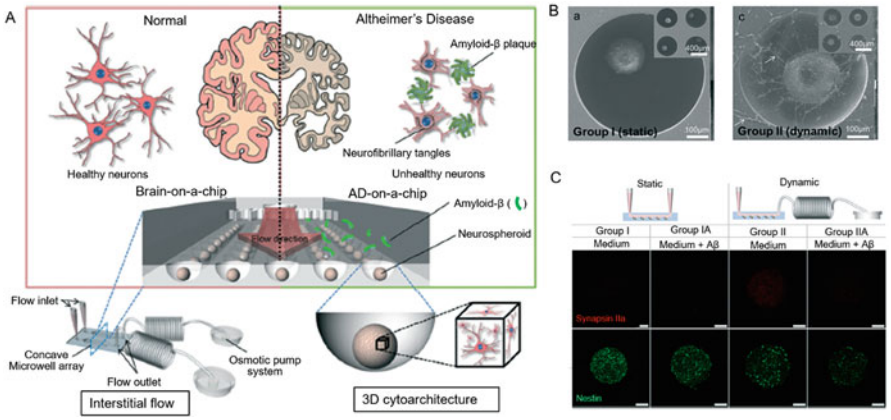

Fig. 13.5 Creating Alzheimer's disease modeling on chip. (**a**) Schematic diagram of a brain-on-a-chip device. A concave microwell array is utilized for homogeneous neurospheroid formation and a continuous flow mimicking interstitial flow is applied via an osmotic pump system. (**b**) Dynamic culture significantly promotes the growth and function of neurosopheroids. (**c**) Amyloid-β treatment via an osmotic micropump significantly reduced the viability of neurospheroids and caused a significantly more pronounced destruction of neural networks, compared to the amyloid-β treatment under static conditions. Reproduced from [172] with permission

13.5.1.3 Cancers on Chip

Cancers are a large family of diseases that seriously threaten human health and life. The development of cancers involves abnormal cell growth and invasion of other important organs. It is very helpful to establish *in vitro* models to study the biological behavior of cancer cells and anticancer drug effects. Currently, numerous groups have developed cancer-on-a-chip models by mimicking tumor microenvironments consisting of complex cell-cell and cell-matrix interactions, chemokine/cytokine gradients and biophysical cues [173–175]. Xu et al. fabricated a bilayer PDMS microfluidic chip to mimic the glioblastoma invasion into ECM under different concentrations of oxygen [176]. This work investigated the role of hypoxia and EMT in glioblastoma and hypoxia promoted the proliferation of the cancer cells and EMT-associated protein expression, and enhanced cell migration. The mechanism linking EMT and cancer cell behavior could be related to the Hypoxia-Inducible Factor 1α or 2α (HIF1α or HIF2α), indicating that developing inhibitors of HIFs may be a novel therapeutic drug. Li et al. demonstrated a high throughput microdevice containing tunable cell micro-niches, which performs flow-based analysis of large cell populations to evaluate various responses of lung adenocarcinoma cells to different ECM proteins and soluble factors [177]. This study indicated that tumor-cell growth is related to TGF-β and TGF-βR2 inhibitor drugs in a 3D matrix but not in a 2D culture. Angiogenesis of tumors is a key step of tumor metastasis towards distant target organs. Several works were performed to study the intravasation and extravasation of tumor cells using microfluidic devices [178, 179]. Other

studies have utilized similar microfluidic technology to investigate the molecular mechanisms of cancer cell-immune cell crosstalk [180].

13.5.2 Drug Testing

13.5.2.1 Efficacy and Toxicity Testing

One challenge of drug development is the poor efficacy and unexpected toxicity in clinical trials caused by an absence of predicted therapeutic effects. The main reason for undesired outcomes is that existing approaches fail to accurately predict *in vivo* drug efficacy before clinical application. Human organ-on-a-chip systems that model human physiological and pathological functional units of living organs provide a promising tool to address the limitations of existing methods. They allow to reconstruct and pharmacologically modulate key aetiologies and clinical relevant responses at various levels of biological complexity and to test unanticipated off-target toxicity. As described above, a heart-on-a-chip composed of 20 rat cardiomyocyte thin films was utilized to evaluate the inotropic effects of the β-adrenergic agonist isoproterenol which are similar to those previously determined in rats [152]. A recent study reported a micro-engineered 3D model of EMT during cancer progression for testing drug efficacy [181]. This model demonstrated EMT-induced tumor dispersion and phenotypic changes by culturing lung tumor spheroids in a matrix gel close to microchannels inhabited with endothelial cells. In addition, 12 drugs, including approved drugs, as well as prospective drugs that are in the early discovery pipeline, were perfused into the vascular microchannels to evaluate the potential of this model as a drug screening platform. The ability of these drugs to inhibit EMT was tested by direct visualization of the cancer spheroids. The results showed that efficacious concentrations derived from the cancer-on-a-chip were higher than that from the 2D system, but are also closer to the range of effective drug concentrations determined in clinical trials. Several micro-engineered models of breast cancer and multiple organ models of uterine cancer, bone marrow and liver have also demonstrated a similar difference between effective drug concentrations determined by organs-on-chip platforms and those by conventional 2D culture systems [182, 183]. To investigate the communication between different brain regions, organotypic brain slices from rat hippocampus and entorhinal cortex were cultured in compartments interconnected by microchannels [184]. A glutamate receptor antagonist (kynurenic acid) was introduced to one microchannel in order to selectively inhibit the spontaneous electrical excitation of the treated brain tissue but not on the other brain slice. This design allows for the selective pharmacological administration of only one tissue and evaluation of its effects across the synaptic connection.

Recently, Qin et al. developed a novel *in vivo*-like 3D blood-brain barrier model that replicates the complex multicellular architecture, functions and mechanical properties of the BBB *in vivo* [185] (Fig. 13.6). The BBB model encompassed essential components, including primary brain microvascular endothelial cells,

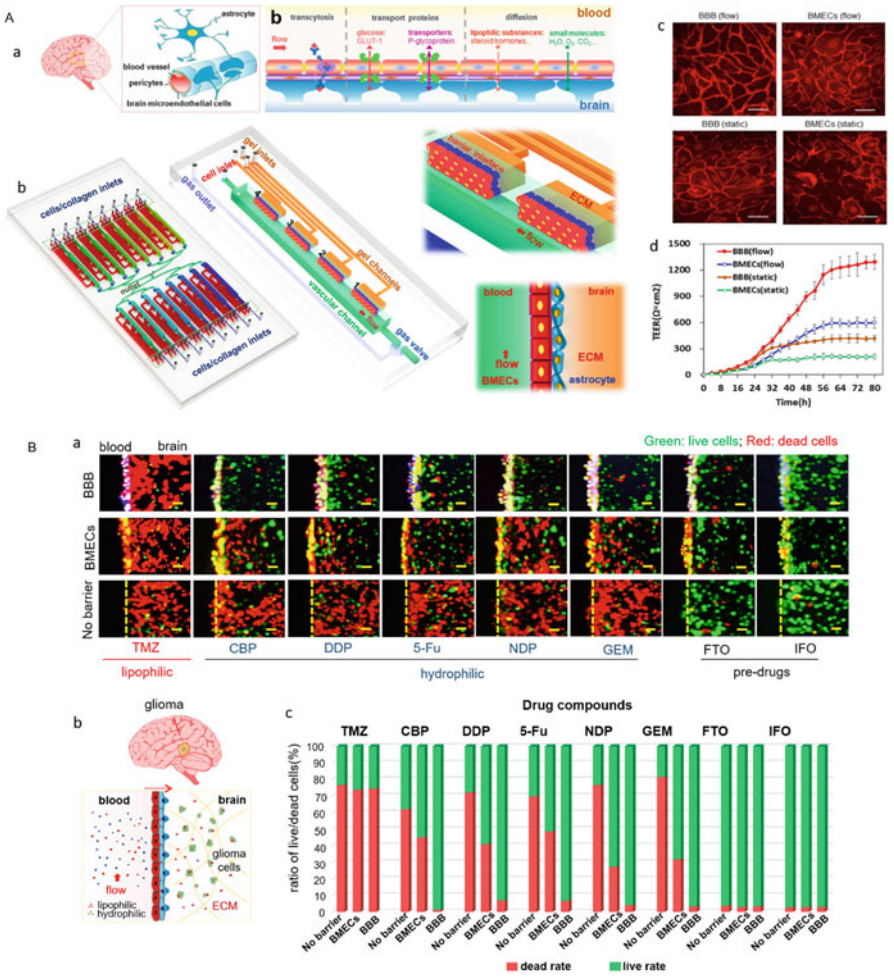

Fig. 13.6 Engineering 3D dynamic blood-brain-barrier (BBB) in brain tumor microenvironment on chip. (**A**-*a*) Cellular constituents and structural features of the BBB *in vivo*. (**A**-*b*) The details of the BBB microdevice are represented. It is composed of 16 independent units with fluidic channels, ECM gel channels, gas valve. The BMECs, astrocytes and 3D ECM gel formed the functional BBB under fluidic flow conditions. (**A**-*c*) Expression of adhesive protein, VE-cadherin, in integral BBB and BMECs under fluidic flow or static conditions. (**A**-*d*) TEER values in BBB and BMECs under fluidic flow and static conditions. (**B**-*a*) Cytotoxicity of eight chemotherapeutic agents to glioma cells (U87 cell line) in various barrier groups. (**B**-*b*) Schematic illustration of diffusion of lipophilic and hydrophilic drug compounds across the BBB *in vivo*. (**B**-*c*) Quantitative data of live/dead ratio of U87 cells induced by the different drugs introduced into the vascular channels of the BBB. Reproduced from [185] with permission

astrocytes and ECM which orchestrate to form a more stringent structure of a BBB (Fig. 13.6). In this microsystem, the TEER value in the BBB interface under dynamic culture condition is around 1300 $\Omega \times cm^2$, a value that far exceeds that reported in Transwell-based BBB models in a static context [186, 187]. Moreover, this BBB model was used to understand the interplay of the BBB and exogenous cancer cells in the course of brain metastasis in real-time. As is well-known, efficiently delivering a drug to the brain is still a challenge at present because of the selective permeability of the BBB to different exotic molecules. In this work, the *in vitro* BBB model exhibited a robust and realistic experimental result with regard to drug response of brain tumors including primary and secondary cancers and the high throughput design is very useful to screen multiple drugs simultaneously, which is not possible in other BBB models *in vitro* and in animal experiments. This physiological BBB microsystem provides a versatile and valuable platform for neuroscientific research, drug testing and pharmaceutical development.

Unanticipated adverse drug effects are another very common cause of the costly withdrawal of marketed drugs and clinical trial failures. Animal experiments often fail to reveal important toxic effects in humans and can lead to unnecessary rejection of drug candidates due to the genetic background and biological discrepancy and animal-specific pathways of toxicity. Organ-on-a-chip devices used in liver toxicity have been reported recently to analyze the metabolism of hepatotoxicity. For example, a microfluidic device was developed to culture human hepatocytes in order to monitor metabolic responses of hepatocytes to flutamide (a type of anticancer drug) and the hepatotoxic properties of flutamide and its active metabolite hydroxyflutamide. This study showed the metabolic signatures of toxic responses and described metabolic pathways induced by the drug and its metabolites in hepatotoxicity. The human liver-on-a-chip model leveraged in this work was influential in reducing biological noise inherent to *in vivo* metabolomics models, and indicating a potential source of hepatotoxicity-specific biomarkers. The secondary toxic effects of drug metabolites generated in the liver on other organs were also demonstrated by two organ liver-kidney organ-on-a-chip devices [188]. This study mimicked the systemic interplay between the two organs and recapitulated nephrotoxic responses to the ifosfamide metabolites produced by hepatocytes. More recently, drug-induced cardiotoxicity was also tested using an organ-on-a-chip platform by rebuilding and monitoring the contractile functions of heart muscle by means of quantitative analysis [189, 190].

13.5.2.2 Pharmacokinetic and Pharmacodynamic Studies

Pharmacokinetic and pharmacodynamic (PK/PD) models have been broadly used to analyze and predict the whole body response to drugs in a time-dependent manner by featuring the mechanistic basis of multi-organ interactions. Multiorgans-on-chips were developed to analyze the bioaccumulation, distribution, and toxicity of selected chemical compounds [191]. The toxicant naphthalene was converted by hepatocytes into its reactive metabolites which depleted the intracellular glutathione of lung cells as soon as circulating to the lung tissue chamber. Adipocytes differentiated from

3 T3-L1 in the fat tissue chamber moderated the glutathione depletion induced by naphthalene, but preferentially accumulated hydrophobic compounds. This study is the first model of adsorption, distribution, metabolism, elimination and toxicity (ADMET) that performed all of these functions on the same device. Over the past decade, many researchers have explored the utilization of organs-on-chips to investigate drug ADMET features, to support PK/PD modeling, and to evaluate drug efficacy [192–194]. Li et al. developed a new and multilayer organs-on-chip device to assess drug metabolism and its active metabolite drug efficacy and cytotoxicity in four organ-specific cells simultaneously representing the liver, breast cancer, lung tumor, and normal gastric cells [195]. In this study, the prodrug capecitabine (CAP) was first metabolized in a top liver tissue chamber with hepatocytes (HepG2) and its intermediate metabolites, 5′-deoxy-5-fluorocytidine (DFUR), was further metabolized into 5-fluorouracil (5-FU) by targeting cancer tissue and normal tissue cells (Fig. 13.7). This work recapitulated that the CAP exhibited strong cytotoxicity on breast and lung cancer cells, but not in normal gastric cells.

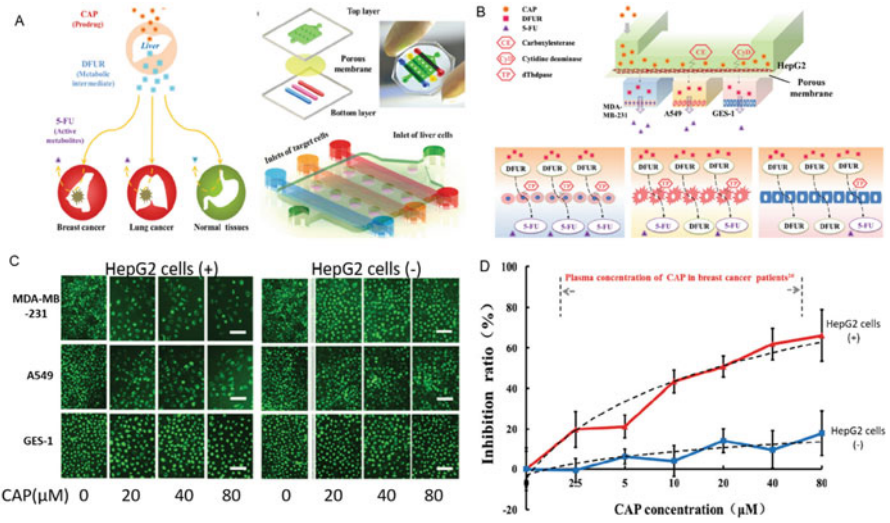

Fig. 13.7 Liver dependent drug metabolism on a multiorgan-on-chip. (**a**) Schematic diagram of organs-on-a-chip. The microfluidic chip consists of two layers separated by a porous membrane. The top layer was seeded with HepG2 cells representing the liver and the three channels on the bottom layer were cultured with breast cancer cells (MDA-MB231), lung cancer cells (A549) and normal tissue cells (GES-1). (**b**) Schematic illustration of drug metabolism on chip. CAP was introduced from the top layer and presented to HepG2 cells. CAP was metabolized by carboxylesterase and cytidine deaminase within HepG2 and transformed into DFUR. The DFUR were subsequently presented to the target cells on the bottom layer and transformed into cytoxic 5-FU by dThdpase expressed by target cells. Since the target cell lines express different levels of dThdpase, the cytotoxic effects vary. (**c**) Cytoxic effects of CAP on each cell type with or without HepG2. (**d**) Dose-dependent effects of CAP on the inhibition of breast cancer on chip. Reproduced from [195] with permission

All these studies demonstrate an opportunity for organ-on-a-chip devices in the field of drug development to simulate and predict the key physiological responses involved in drug metabolism, bioactivity, efficacy and toxicity, and increase likelihood of success in clinical trials.

13.5.3 Host-Microbe Interaction

The gut microbiome can deeply impact many aspects of human bio-behavior, such as mental activities, stress responses via brain-gut communication [196]. The balance of intestinal microbial diversity is crucial to maintain human health status and alteration of intestinal microbiota is related to many acute and chronic diseases including inflammatory bowel disease, diabetes, obesity and cardiovascular diseases [197].The gut microbial community is a dynamic ecosystem that can be influenced by many factors, such as food type, living condition and host genetics. Therefore, establishment of a stable host-microbe ecosystem *in vitro* is crucial for understanding the human intestinal diseases, regulating nutrient and drug absorption. However, existing 2D, static culture methods fail to rebuild functional intestinal structure and establish a stable symbiosis between host intestinal epithelium and a certain population of bacterial cells for extended time periods because bacterial overgrowth occurs rapidly within 1 day.

Alternatively, microfluidic devices can provide a desirable culture platform to co-culture the host cells and bacteria under dynamic conditions. The human gut-on-a-chip was developed using intestinal epithelial cells that grew into 3D villi stably on optically clear, microporous PDMS membranes sandwiched by two PDMS layers with parallel hollow microchannels. In this microsystem, multiple commensal microbes were directly co-cultured with epithelial cells for more than 1 week. This study recapitulated the individual contributors containing the peristalsis-associated mechanical deformations, gut microbiome and inflammatory cells to intestinal bacterial overgrowth and inflammation [198]. Lack of epithelial deformation generated by peristalsis-like motion led to bacterial overgrowth similar to that observed in inflammatory intestinal diseases. Most importantly, this *in vitro* intestinal model replicated results from past human and animal studies which demonstrated that antibiotic and probiotic treatment can suppress villus injury caused by pathogenic bacteria. The intestinal epithelial cells were also stimulated by immune cells and lipopolysaccharide endotoxin to produce four pro-inflammatory cytokines (IL-6, -8, -1β,and TNF-α) that can trigger villus injury and compromise intestinal barrier function. In future studies, gut-on-a-chip devices can be used to investigate the host-microbe interaction in a physiological or pathological context.

13.6 Conclusion and Outlooks

Novel bioengineered organs-on-chips platforms can provide tight environmental control of tissues and organs along with the physiological transport and signaling as present in the human body. These approaches offer unique advantages in studying the intercellular communications, tissue microenvironment, disease modeling and drug testing beyond the existing methods. These novel strategies complement traditional cell culture and animal model systems by providing the biomimetic niche of native tissues/organs, including multicellular architectures, tissue-tissue interfaces and spatio-temporal control of biochemical and biophysical cues. In addition, the compatibility of organs-on-chips with existing analysis methods and integrated biosensors has enabled real-time readout of crucial physiological and pathological information, thus extending their utility for biomedical applications.

While the emerging organs-on-chip technology has advanced our understanding of the cellular behaviors within the context of organ-relevant functions, much work remains to be done to fully mimic organ functions and to move toward a human-on-a-chip model with greater relevance for disease studies and drug discovery. Among these challenges, the cell source is one of the key issues for building organs-on-chips analogous with organs in the human body. Human primary cells are ideal resources to model human tissues/organs. To meet both ends, human iPSCs have been explored to rebuild organs-on-chips. Patient-specific iPSCs provide a promising cell source to engineer the disease-on-a-chip for personalized medicine. In order to study organ-organ interactions in multiorgans-on-chips, the identification of an appropriate common medium is also of great concern because the different cell types need various nutrient components when these cell lineages are assembled. To address this challenge, human plasma or serum might be a desirable substitute as supplements of culture medium for multiple organ-specific cell lineages.

In addition, the choice of material is another major concern in fabricating and designing the organs-on-chips. At present, PDMS is the most widely used material due to its ease of use, high transparency, air permeability and biocompatible features. However, PDMS can absorb small hydrophobic drugs, which might cause underestimation of drug toxicity and efficacy, thus limiting its practical utility in drug discovery. Chemical modifications of PDMS surfaces and other alternative materials are required to solve this type of problem. Furthermore, more powerful analysis and monitoring systems specifically designed or modified for microfluidic chips are required to provide comprehensive information of cell behavior, metabolism and drug responses from the organs-on-chips. The available methods, such as PCR analysis, *in vivo* imaging, two-photon confocal microscopy, and mass spectrometry can be further integrated with organs-on-chips for real-time monitoring, thus boosting the development of organ-on-a-chip technology for obtaining biological information. It will be necessary to develop new biomimetic microsystem, novel materials and appropriate cell sources that support the scale-up of organs-on-chips.

Although the development of engineered organs-on-chips is still in its infancy, its potential to model diseases and predict human response under physiological and pathological conditions is tremendous. The ongoing progress of this technology to simulate various cues in organ relevant microenvironments and to combine with stem cell biology and traditional biological assays will accelerate the pace toward creating a more realistic human-on-a-chip model system that is accessible, practical and robust for end users. Collectively, future success will require the collaborative efforts from academic investigators, bioengineers, industry and regulatory agencies to advance this technology to market.

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