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REVIEW ARTICLE

Design and engineering of organ-on-a-chip

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Abstract
Organ-on-a-chip (OOC) is an emerging interdisciplinary technology that reconstitutes the structure, function, and physiology of human tissues as an alternative to conventional preclinical models for drug screening. Over the last decade, substantial progress has been made in mimicking tissue- and organ-level functions on chips through technical advances in biomaterials, stem cell engineering, microengineering, and microfluidic technologies. Structural and engineering constituents, as well as biological components, are critical factors to be considered to reconstitute the tissue function and microenvironment on chips. In this review, we highlight critical engineering technologies for reconstructing the tissue microarchitecture and dynamic spatiotemporal microenvironment in OOCs. We review the technological advances in the field of OOCs for a range of applications, including systemic analysis tools that can be integrated with OOCs, multiorgan-on-chips, and large-scale manufacturing. We then discuss the challenges and future directions for the development of advanced end-user-friendly OOC systems for a wide range of applications.

Keywords Organ-on-a-chip · Tissue engineering · Microengineering

1 Introduction

The high failure rates and long process of drug development are mainly attributed to the lack of human-relevant preclinical models with predictive power for clinical trials [1]. Advances in cell biology, microengineering, and tissue engineering have led to the emergence of an organ-on-a-chip (OOC) system that is designed to recapitulate key functional characteristics of living human organs [2] (Fig. 1). An OOC, which is also known as a microphysiological system, is a microengineered device that encompasses human-derived cells and physiologically relevant physicochemical microenvironments to reconstitute the microarchitecture and function of tissues [3]. OOC technology has offered the ability to accelerate clinical translational research by bridging the gap between animal studies and clinical trials.

In the last decade, significant progress has been made in the development of OOC models to mimic a variety of human tissues (Table 1). In the context of tissue engineering, substantial efforts have been made to culture appropriate human-derived cell types in appropriate biomaterials for a stable and precise recreation of tissue-specific models. Advances in stem cell engineering, particularly in induced pluripotent stem cell (iPSC) technology, have further enabled the establishment of patient-specific OOC models for patient-specific preclinical studies [4]. Moreover, many types of biomaterials, including natural and synthetic biomaterials, have been investigated to reproduce the in vivo extracellular matrix (ECM) with tissue-specific properties [5].

However, not only the biological elements but also the design and engineering aspects, such as the microarchitecture of the device, physicochemical cues, and integration of biosensors, need to be considered to faithfully reconstruct human tissues in vitro. For example, microfluidic channels within a device should be suitably designed to create tissue-specific structures and microenvironments. Fabrication techniques should also be considered as fabrication methods affect the design and performance of OOCs [6]. In microfluidic OOC devices, environmental factors including mechanical, electrical, and chemical cues need to be controlled to mimic the physiological and pathological microenvironments of tissues that regulate tissue morphogenesis and function [7]. Moreover, the incorporation of sensors into OOCs allows for real-time on-chip analysis as well as

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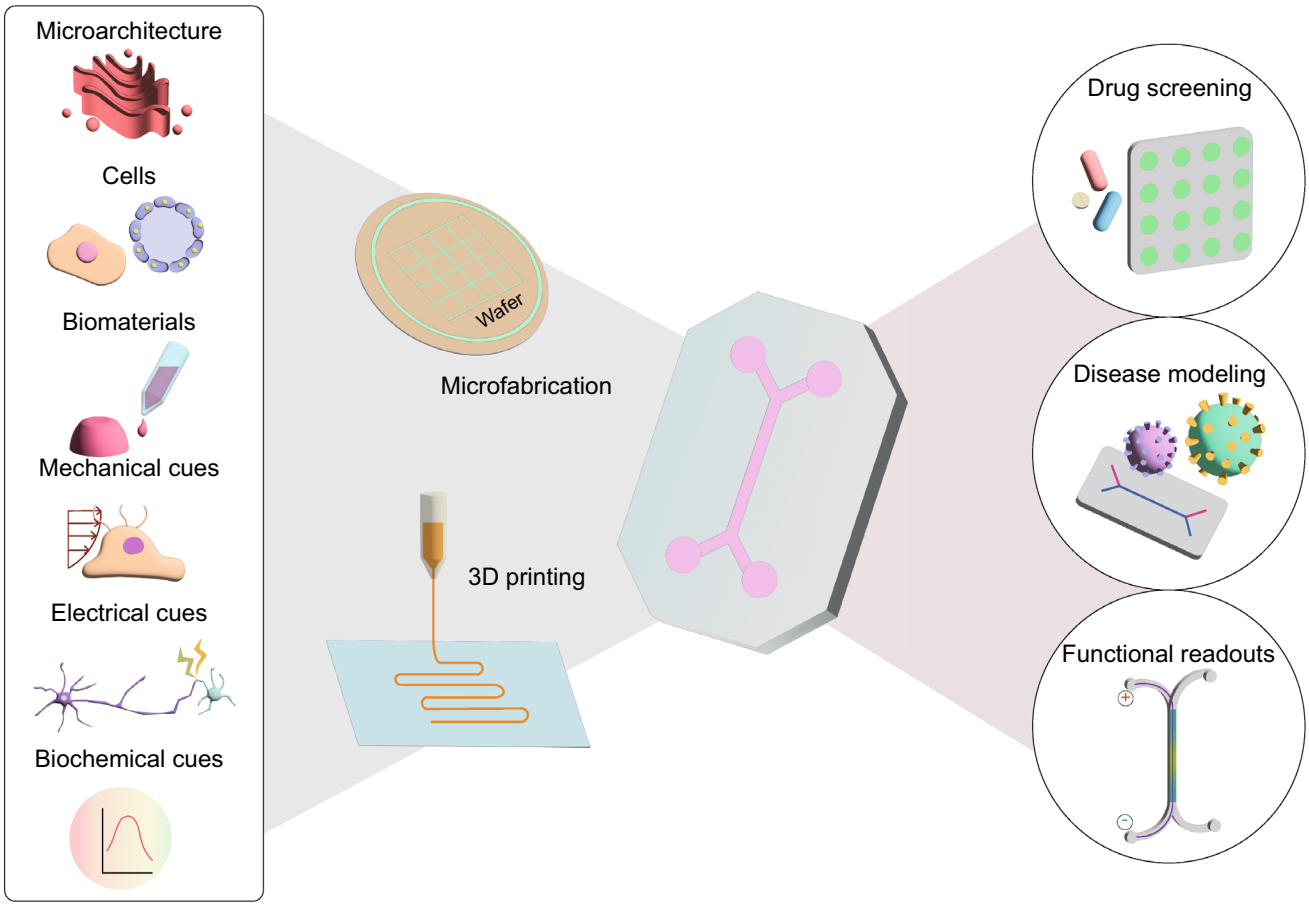

Fig. 1 From engineering considerations to applications of OOC. OOC integrates biological, biochemical, and engineering features of human organs in a microfluidic platform that is fabricated using cleanroom-based microfabrication or 3D printing technology. These OOCs can be used in a wide range of biomedical applications including drug screening, disease modeling, and monitoring tissue function

monitoring of tissue functionality and microenvironments [8].

In this review, we describe the technical advances used to engineer OOCs, highlighting engineering technologies to reconstitute the microarchitecture and microenvironment of tissues. We then cover recent advanced technologies in the OOC field for their extension to integrative systemic analysis, systemic automation, and commercialization. Lastly, we briefly discuss the direction of the future work to expand the field of OOC for a broader range of applications.

2 Engineering three-dimensional (3D) microarchitectures of tissue microenvironment

Microengineering technologies have enabled the precise in vitro reconstruction of tissue-specific microarchitectures. In particular, OOC devices are designed to recreate specific aspects of tissues for particular applications, such as studying drug transportation, monitoring barrier function, and studying cellular interactions in 3D. For drug screening, tissue barriers that control material transport between tissues were recreated to explore drug permeability and drug transport mechanisms. Double-layered microfluidic devices that have two parallel microfluidic layers compartmentalized by a porous membrane were used to recreate the barrier structures, including the blood–brain barrier (BBB) [9], intestinal mucosal barrier [10], and airway epithelial barrier [11] (Fig. 2a). The physically layered architecture of the device allows monitoring of the barrier integrity with transendothelial/epithelial electrical resistance (TEER) in real-time [9–11] and allows independent access to each layer for quantitative analysis of molecular distribution [9]. Moreover, fluid or air flow can be independently controlled in vertically separated microfluidic channels. A microengineered 3D vascular network-on-chip, which consists of multiple parallel channels laterally connected with arrays of micropillars, has been developed to recapitulate cell–cell interactions [12], vasculogenesis [13], and angiogenesis [14]

Table 1 Current progress in OOC research

Tissue	Research objectives	Details of design	Future directions	Year	References
BBB	Measuring permeability and TEER with different concentrations of various molecules	A double layered microfluidic device with a sandwiched porous membrane	- 3D culture of cells in a human brain-specific ECM - Co-culture of multiple human neurovascular unit (NVU) cells such as brain endothelial cells, pericytes, astrocytes, neurons, and microglia	2012	[125]
	Quantification of 3D nanoparticle distribution in the vascular and perivascular regions at the BBB	A hybrid design combining two vertical microfluidic layers with three parallel microchannels aligned with microposts		2020	[9]
	Developing an infectious human brain disease model to examine neurotropism and BBB penetration of pathogens	- A vertical microfluidic chip with a gravity-driven unidirectional flow - Three parallel microchannels with small posts		2021	[126]
Heart	Recapitulating a physiological environment of native myocardium	- Two compartmentalized microchambers separated by a PDMS membrane - Top compartment: subdivided by two rows of hanging posts for hosting cell construct - Bottom compartment: connected to a pneumatic actuation system to mimic beating motion	- 3D culture of human stem cell-derived cardiac myocytes - Co-culture of myocytes with other cell types (fibroblasts and endothelial cells etc.) to recapitulate the multicellular heart	2016	[67]
	Mimicking ischemia–reperfusion injury to evaluate cardioprotective effects of endothelial extracellular vesicles	3D Printed multilayered PDMS films with an embedded flexible strain gauge		2020	[127]
Lung	Reconstituting the functional human alveolar-capillary interface	- Two microchannels separated by a porous flexible membrane - Two lateral microchambers to apply vacuum that causes pressure-driven stretching of a membrane	- Mimicking surfactant lining layer at the air–liquid interface - Using a biodegradable membrane that has tunable biochemical and mechanical properties	2010	[90]
	Recapitulating the microstructure, ECM properties, air-cell interface, and the breathing motion the human pulmonary alveoli	A compartmentalized microchannels bonded with a 3D porous hydrogel showing an inverse opal structure		2021	[128]
	Reproducing the biochemical and physical properties of the alveolar basal membrane with in vivo-like dimensions	- An array of alveoli with in vivo-like dimensions - A stretchable biological membrane composed of proteins of the lung ECM		2021	[129]
Gut	Recapitulating the intestinal epithelium with the villi-like structure and the intestinal microbes	- Two microchannels separated by a porous flexible membrane - Two lateral microchambers to apply vacuum that causes pressure-driven stretching of a membrane	Adding other tissue-specific cell types such as mesenchymal cells or vascular cells	2012	[65]
	Studying the mechanisms of the villi-like epithelial morphogenesis			2019	[10]
Liver	Constructing the heterogeneous lobule-mimetic pattern with liver cells	A concentric-stellate-tip electrode array with enhanced field-induced dielectrophoresis electrodes	Co-culture of key non-parenchymal cells	2006	[130]
	Recapitulation of the structure and biological hierarchy of the liver tissue in a long-term cultured device	Hexagonal culture chamber that mimics the radial flow from the portal vein to the central vein		2017	[21]
	Studying the pathogenesis of steatosis and monitoring hepatocyte functionality	An array of interconnected hexagonal microwells		2019	[98]

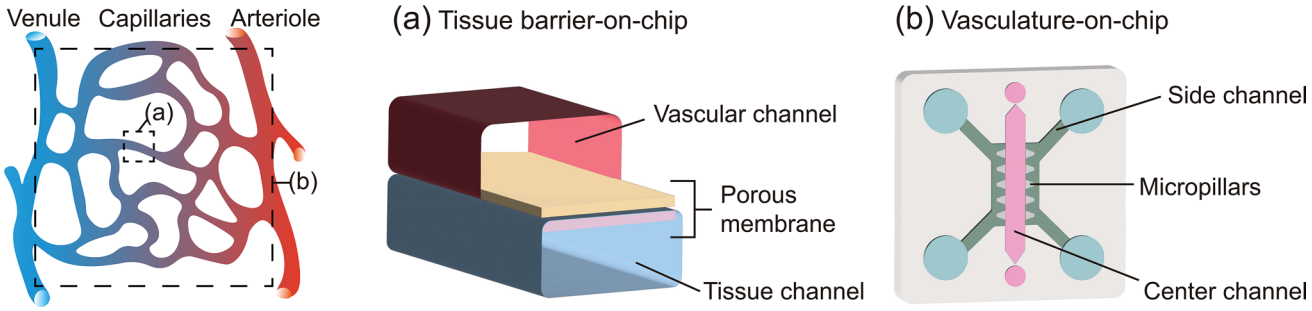

Fig. 2 Two different types of OOCs mimicking vascular microenvironments. **a** Tissue barrier-on-a-chip consisting of two microfluidic layers with a porous membrane between the layers. **b** Vasculature-on-a-chip mimicking 3D vascular networks with parallel microchannels semi-partitioned by arrays of micropillars

in 3D (Fig. 2b). The array of micropillars between two parallel channels provides a semi-partitioned structure in which a liquid–solid interface can be formed by surface tension [15]. Taking advantage of the 3D microenvironment, this type of device has been used to study direct cell–cell and cell–material interactions under 3D co-culture conditions [16], particularly to investigate the tumor vascular niche [17, 18].

Different types of OOCs have been designed to reproduce tissue-specific microarchitectures. For example, human ocular surfaces including the cornea and conjunctiva were replicated in a multi-layered elastomeric device, where eye blinking was mimicked using a computer-controlled electromechanical actuator [19]. A field-induced dielectrophoresis-based cell patterning technique [20] and the hexagonal shape of a culture chamber with the concept of lattice growth [21] were used to mimic the structure of the liver lobules (Fig. 3a). Moreover, the highly organized anisotropic layers of the musculature of the heart were replicated by patterning microgrooves on soft multilayer cantilevers [22] and the neuromuscular junction platform was designed by co-culturing 3D skeletal muscle fiber bundles formed around the pillar structures and motor neuron spheroids embedded in collagen gel [23] (Fig. 3b).

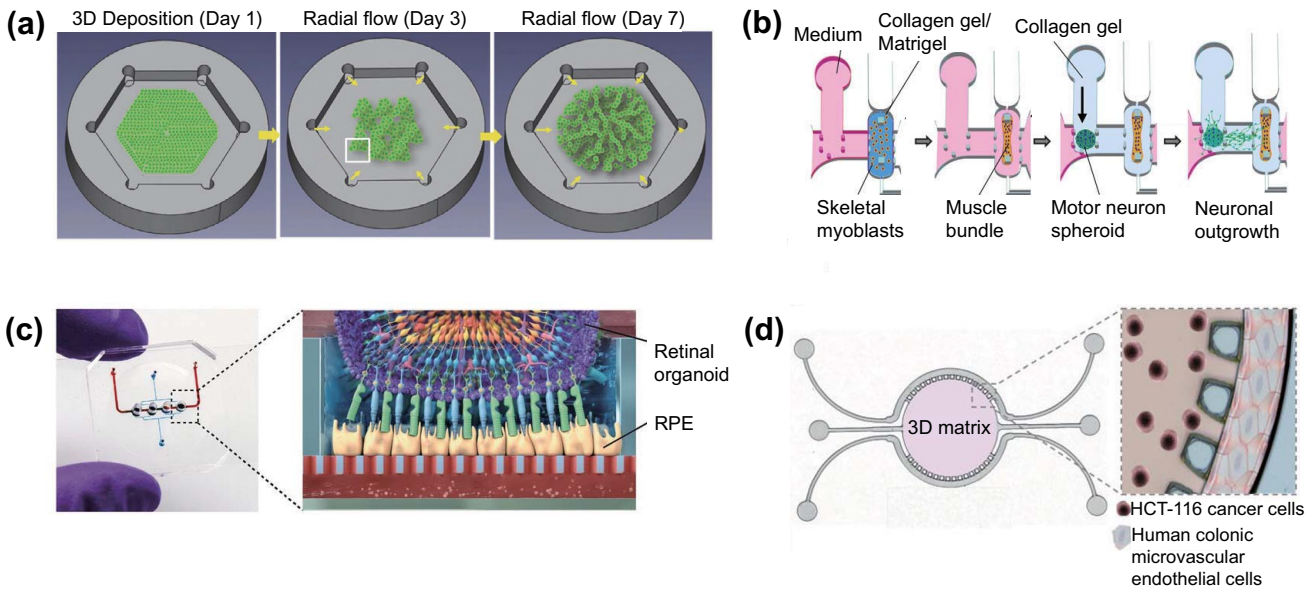

Fig. 3 Tissue-specific microarchitectures and biomaterials on chips. **a** Schematic illustration of liver-on-a-chip which mimics the structure of lattice of the liver lobules. Reproduced with permission from [21]. Copyright (2017) Wiley. **b** Schematic diagram of the 3D neuromuscular junction platform designed to co-culture 3D skeletal muscle fiber bundles and motor neuron spheroids. Reproduced with permission from [23]. Copyright (2018) AAAS. **c** Photo of the retina-on-a-chip (left) and schematic diagram of the retinal organoid and RPE in HA-based hydrogel on a chip (right). Reproduced with permission from [35]. Copyright (2019) eLife. **d** Schematic depiction and zoom-in image of the concept of colorectal tumor-on-a-chip. Reproduced with permission from [36]. Copyright (2019) AAAS

To fabricate the microarchitecture of OOC devices, cleanroom-based fabrication and 3D printing technology have been the most widely used among the various fabrication techniques [24–26]. Conventional cleanroom-based microfabrication, followed by soft lithography, allows the fabrication of high-resolution microstructures, whereas 3D printing technology enables the direct deposition of biological and functional materials, leading to a single-step fabrication of OOC devices [27–29]. Moreover, the integration of 3D bioprinting with microfluidics has enabled the creation of complex dynamic 3D microscale architectures in a high-throughput manner [30]. The evolution of 3D bioprinting technology has led to the development of printable bioink formulation [31]. Biomaterials presenting the required levels of rheological properties for 3D printing have been developed as 3D printable bioinks [32–34].

The selection of biomaterials with the desired physicochemical properties is important for mimicking the tissue-specific ECM [5, 37]. Physicochemical properties, such as surface chemistry, porosity, and stiffness profoundly affect the biochemical responses of cells that further regulate tissue homeostasis and pathogenesis [5]. Thus, various types of materials have been used to recreate tissue-specific ECMs to provide a physical and biological scaffold for cells [38]. Natural biomaterials, such as collagen, hyaluronic acid (HA), and Matrigel, are derived from living organisms and thus possess good biocompatibility. Collagen is the most prevalent protein in human tissues, particularly in connective tissues, including the bone, cartilage, skin, and cornea [39–41]. Owing to its insufficient mechanical strength, the extracted collagen is often combined with modified crosslinking methods or other high-strength materials to improve the physicochemical properties [39]. Collagen gel has been widely used to mimic the physiological environment of tumors in OOC devices, including a colorectal model [16] and a platform for studying breast ductal carcinoma [42]. HA is a glycosaminoglycan (GAG), which is an immunoneutral polysaccharide found throughout the human body [43]. HA hydrogels can be easily manipulated to possess biochemical functionality, dynamic microenvironments, and biological structures [44]. A human retina-on-a-chip model has incorporated HA-based hydrogels with retinal organoids and retinal pigment epithelia (RPE) to construct a retinal layer on a chip [35] (Fig. 3c). Matrigel is a solubilized basement membrane extracted from sarcomas and contains laminin, collagen, and heparan sulfate proteoglycan. Because of its components, Matrigel has been widely used to mimic the ECM of tumor [36] (Fig. 3d), stem cells [45], and neural tissues [9]. However, the undefined chemical composition and batch-to-batch variations of Matrigel make it difficult to reproducibly construct a tissue microenvironment [46]. Synthetic biomaterials are easily tunable to possess tissue-specific physicochemical properties [47, 48]. Moreover, synthetic biomaterials exhibit good reproducibility because they have less batch-to-batch variation than natural biomaterials [49]. Their lack of cell adhesion ligands can be supplemented by chemical modifications. Recently, hybrid natural–synthetic biomaterials that possess the advantages of both natural and synthetic biomaterials have been used as tissue scaffolds in OOCs [50].

More recently, decellularized ECM (dECM), a cell-removed remnant ECM of animal tissues or organs, has been used in OOCs to provide tissue-specific properties [51, 52]. In particular, dECM can restore the tissue-specialized spatial distribution of structural and functional ECM components. With the continuous development of decellularization protocols for various tissues, an increasing number of studies have introduced dECM into OOCs. For example, 3D liver dECM scaffolds were 3D printed to model hepatocellular carcinoma [53] and dECM from tumor-bearing and obese mammary glands were utilized for tumor cell studies [54].

In addition to physicochemical properties, electrical conductivity is an important feature of some tissues to be recapitulated in OOC. For electrically active tissue models, conductive biomaterials such as conductive hydrogels, conductive polymers, and carbon-based materials are used as scaffold materials [55]. Conductive hydrogels are representative materials for bioelectronic interfaces owing to their high stretchability, tissue-like softness, and high conductivity [56]. One study used a catechol-functionalized HA hydrogel with two electroconductive materials, single-walled carbon nanotubes (CNTs) and polypyrrole, as a conductive hydrogel for neuronal regeneration modeling [57]. Moreover, 3D printed CNTs based conductive scaffolds have been used for cardiac tissue engineering [58] and nerve regeneration modeling [59].

3 Dynamic control of tissue microenvironments

Cells in vivo are exposed to various physical stimuli, such as mechanical, electrical, and geometric actuations, as well as biochemical stimuli. In response to these stimuli, cells regulate their physiological and pathological functions. Hence, the need to recapitulate physiologically relevant extracellular stimuli has been underscored in developing in vitro tissue models [60]. In a microfluidics-based OOC model, physiological stimulation, such as fluidic shear stress, interstitial flow, cyclic strain of repetitive movements, and compression, can be precisely controlled and monitored in real-time [61] (Table 2).

Flows in biological systems are replicated in a chip by flowing culture medium through microchannels with an auxiliary device such as a syringe pump and pneumatic pump [62]. The microscale channels of the OOC devices generate flows at low Reynolds numbers, resulting in

Table 2 Engineering technology to mimic specific features of tissue microenvironments

Stimuli	Engineering technology	Mimicking tissue	Specific features	References
Shear stress	Integration of a microgap, self-contained flow loop, pneumatic pumps, and valves on a chip	Endothelial barrier	Pulsatile and oscillatory shear flow in pathogenic situations	[62]
	Connection of the vascular channel to an external syringe pump	Endothelial barrier	Oscillatory shear stress caused by disturbed flow conditions in cardiovascular disease progression	[63]
Cyclic strain	Stretching a porous membrane by applying vacuum to side channels	Lung	Cyclic breathing movement	[64]
		Intestine	Intestinal peristaltic motions	[65]
	Actuation of a micro-diaphragm using electro-pneumatic set-up	Lung	Cyclic breathing movements	[66]
	Pressurization of an auxiliary compartment that controls the membrane deformation	Cardiac tissue	Systolic and diastolic movements	[67]
Electrical stimulus	Gold electrode patterning on PDMS microfluidic device	Cardiac tissue	Electrical pacing for function assessment	[73]
	ITO-IDA electrode glass substrates on PDMS chip	Muscle	Tissue contraction during exercise	[74]
	3D agar salt bridges and electrical circuit	Skin	Wound healing	[75]
Biochemical gradients	Tree-like gradient generator	Neural tube	Early neural tube regionalization mediated by WNT signaling gradient	[76]
	Placing the vasculature-mimicking lumen at one end of the chip	Solid tumor microenvironment	Acidic pH in tumor microenvironment	[78]
	Coating a gas impermeable film on the surface of the chip	Intestine	Steep oxygen gradient for healthy host-microbiome interactions	[79]

laminar flow that can reproduce the general laminar and streamlined blood flow in the vasculature (Fig. 4a). Moreover, the flow of the culture medium along the microchannel helps maintain the long-term culture of the cells in the device by continuously refreshing the culture medium. Previous studies have shown that different flow conditions, including magnitude and oscillation frequency, affect the barrier integrity and inflammatory responses of endothelial cells (ECs), leading to pathogenic events [62, 63].

Repetitive motions of organs, such as breathing motion of the lung and peristaltic motion in the gut, cause cyclic strain on the constituent cells. One representative OOC device mimicking cyclic strain motion utilized two side vacuum channels to stretch a porous membrane located in the center channel (Fig. 4b). This type of device was designed to construct various types of tissue models, including lung-on-a-chip [64] and gut-on-a-chip [65]. Another type of device which uses a micro-diaphragm that can be actuated by an electro-pneumatic setup was also developed to mimic 3D cyclic strain by breathing movements [66]. Moreover, the systolic and diastolic phases of the heart were replicated by pressurization of an auxiliary compartment that controls the membrane deformation between posts to induce homogeneous uniaxial cyclic strain [67].

Electrical stimulation is important for the development and regeneration of several tissues including neural [68], cardiac, and bone tissues [69]; wound healing [70, 71]; and cancer metastasis [72]. In the cardiac tissue chip model, gold electrodes were patterned and connected to the single generator MyoPacer for pacing cardiac tissue to assess cardiomyocyte function [73]. To construct an in vitro skeletal muscle tissue model of exercise training, a polydimethylsiloxane (PDMS) chip was bonded on glass substrates with indium tin oxide (ITO)-interdigitated array (IDA) electrodes that provide electrical stimulation [74]. An integrated microfluidic platform that can monitor real-time cell behavior under electrical stimuli and shear stress was designed to study the cellular migration of normal human dermal fibroblasts at the wound site [75]. In this device, 3D agar salt bridges were embedded in the platform at the end of the cell culture channel and connected to a single-loop electrical circuit to generate and control a constant electric field across the channel.

Microfluidic technologies enable the control of the spatiotemporal distribution of biochemical molecules to reconstitute various types of biochemical environments in tissues (Fig. 4c). A tree-like microfluidic gradient generator, one of the most widely used designs to form biochemical gradients, was integrated with a porous membrane-based double-layered microfluidic device for drug screening at

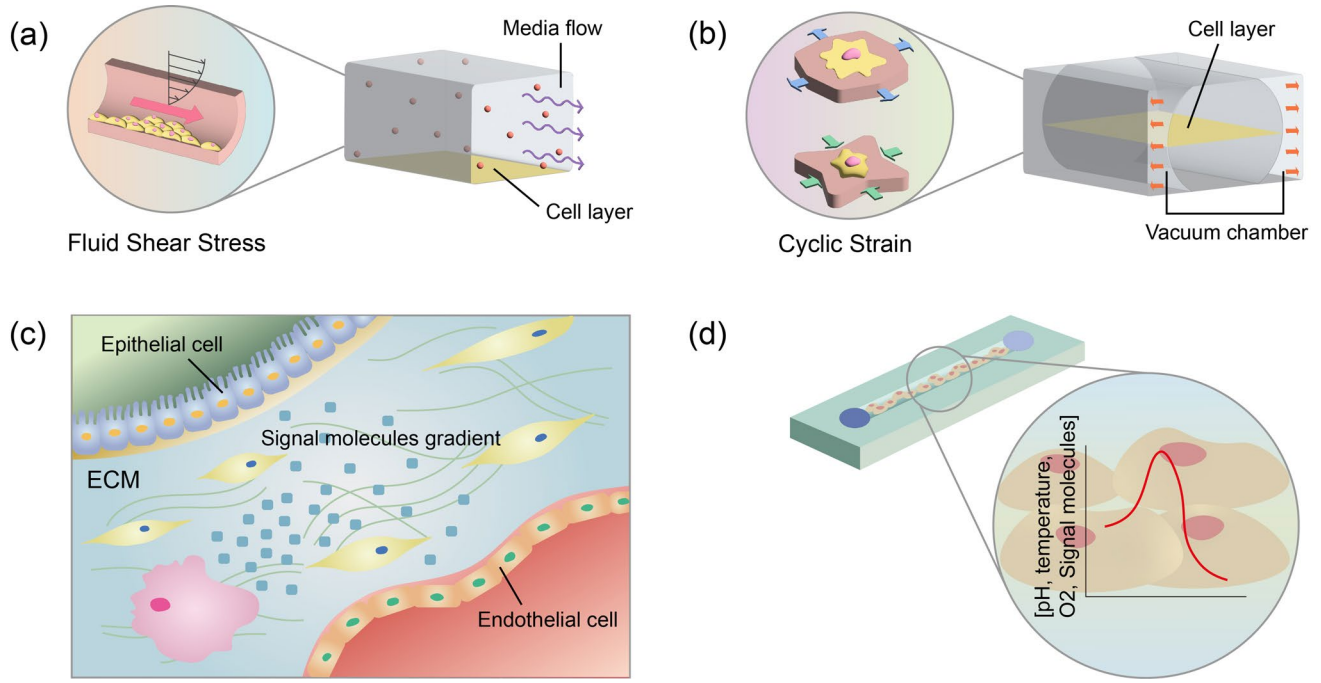

Fig. 4 Mechanical and biochemical microenvironments of tissues. **a**, **b** Fluid shear stress **a** and cyclic strain **b** reproduced on a chip. **c** Biochemical microenvironment of tissues. (d) pH, temperature, and oxygen concentration gradients generated in OOC to mimic biochemical cues in tissue

different drug concentrations. Integration of the tree-like gradient generator has also enabled the modeling of neural tube development by providing a cranial rostral-to-caudal neural axis gradient [76]. Moreover, chemical gradients can be embedded into hydrogels to replicate the immobilized and soluble factor gradients incorporated in ECM [77].

Several other biochemical parameters, such as pH, oxygen, and temperature, control the physiological and pathological functions of cells and tissues (Fig. 4d). The pH acidification of the tumor microenvironment was recreated on a tumor-on-a-chip model by placing the vasculature-mimicking lumen at one end of the device to induce asymmetric distribution of nutrients and tumor cell viability [78]. In another study, a physiologically relevant hypoxic microenvironment was created by coating the surface of the OOC with a gas-impermeable film [79].

4 Functional readouts for integrated systemic analysis

Integration of analytical biosensors into OOCs allows for real-time and on-chip monitoring of cells and their microenvironment; this is useful to measure the function of cells and tissues, analyze biological responses, or control the tissue microenvironment on the chip. Various types of physical and chemical sensors have been combined with OOCs to monitor the physiological and pathological conditions of tissues. For example, a muscular thin film (MTF) technique was integrated into a heart-on-a-chip to measure the contractility and action potential propagation of the anisotropic ventricular myocardium [80, 81]. Moreover, an organ-on-an-electronic-chip implementing 3D self-rolled biosensor arrays (3D-SR-BAs) was developed to record electrophysiological signals from human cardiac spheroids [82] (Fig. 5a). The liver-on-a-chip device was equipped with tissue-embedded oxygen sensors and connected to a sensor unit containing electrochemical sensors for real-time monitoring of the dynamics of mitochondrial dysfunction by measuring glucose and lactate metabolism [83].

The electrical activity of electrogenic tissues such as neuronal, muscle, and cardiac tissues can be recorded using microelectrode arrays (MEAs) [84–86]. In vitro MEAs contain more than 10,000 microelectrodes that record extracellular action potentials and stimulate cells at the single-cell level [87]. To minimize the mechanical mismatch at the interface between the cells and the electrodes, MEAs have been coated with bioactive hydrogels or fabricated with soft electrode materials [88]. Further, 3D printing technology has enabled the fabrication of high-resolution MEAs on soft substrate [88] and 3D microtower MEAs [85] in a time- and cost-efficient manner.

The barrier function of the tissue layer can be monitored using TEER in a real-time and non-invasive manner [89]. Many types of OOCs mimicking tissue barriers such as the BBB [9], lung epithelium [90], and intestinal barrier [65]

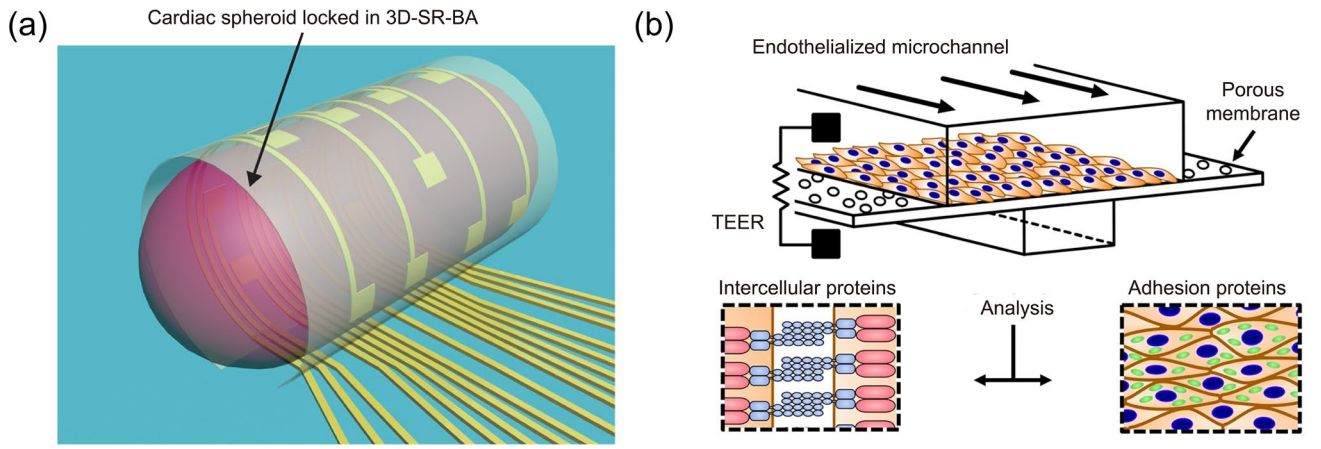

Fig. 5 Monitoring tissue function on a chip. **a** 3D-SR-BAs which monitors electrophysiological signal transduction of human cardiac spheroids. Reproduced with permission from [82]. Copyright (2019) AAAS. **b** TEER implemented chip that monitors barrier permeability on a chip in real-time. Reproduced with permission from [63]. Copyright (2017) Nature

have implemented TEER to monitor tissue barrier function over time. Previous studies measured TEER by repeatedly inserting electrodes into microfluidic channels [65, 90], which can cause large measurement variability. Further, the integration of electrodes within OOCs by coating the electrodes on the chip substrates has enhanced the stability and reliability of TEER measurements [63, 91] (Fig. 5b). One recent study showed a spatial-TEER-integrated OOC that can move along the length of the microchannel for localized measurements of TEER [92].

With the increasing demand for OOC platforms in the pharmaceutical field, several attempts have been made to integrate drug-screening tools with OOCs for on-chip analysis. An integrated microfluidic system consisting of a cell culture chamber, biochemical sensors, and fluid channels was developed to detect glucose and lactate production, pH, and oxygen levels in response to candidate drugs [93]. In one study, a microfluidic human serum albumin (HSA) immunosensor was embedded in a liver-on-a-chip to monitor albumin levels in diabetic disease modeling and drug treatment [94]. The cardiac muscle tissue chip integrated with MTF showed great potential for high-throughput drug-screening applications [95].

5 Advanced engineering technology in organs-on-chips

OOCs have been used to understand pathogenesis or pathophysiology of various diseases by mimicking pathological conditions of the diseases using iPSCs, disease-specific microarchitecture, or physicochemical microenvironments. For example, a 3D human Alzheimer's disease (AD) model that recapitulates the key features of AD (beta-amyloid aggregation, phosphorylated tau accumulation, and neuroinflammation) has been developed by culturing iPSC-derived AD neurons, astrocytes and microglia [96]. A 3D stenosis model with different constriction geometries that can recapitulate flow disturbances was developed to understand atherogenic flow-mediated endothelial dysfunction [97]. A non-alcoholic fatty liver disease (steatosis) has been modeled by culturing spheroids consisting of human hepatocellular carcinoma cells and human umbilical vein endothelial cells with free fatty acids supplementation [98].

As multiple organs interact with each other to control many biological processes in the human body, multiorgan-on-a-chip (MOOC) systems that combine multiple OOCs have been developed [99, 100] (Fig. 6a). These models have great potential for modeling drug adsorption, distribution, metabolism, excretion, and toxicity (ADMET) on chips. Various types of MOOCs, such as liver-cancer-heart-on-a-chip [101], liver-heart–lung-on-a-chip [102], and liver-cardiac-skeletal muscle-neurons-on-a-chip [103] were developed to predict drug efficacy and toxicity. MOOC platforms can be used not only for drug discovery but also for studying a variety of multiorgan metabolic activities. A microfluidic platform that reconstructs the human female reproductive tract and peripheral tissues by integrating the ovary, uterus, fallopian tube, cervix, and liver was developed as a tool for studying hormonal signaling during the human menstrual cycle and pregnancy [104]. These MOOCs need to incorporate interconnecting flow system between the multiple organ microenvironments. A gastrointestine (GI)-liver-on-a-chip has implemented the oscillatory bidirectional fluidic flow system that utilize gravity to obtain a wide range of flow rates depending on the dimension of a microchannel [105]. Moreover, interconnection via vascularization and scaling of

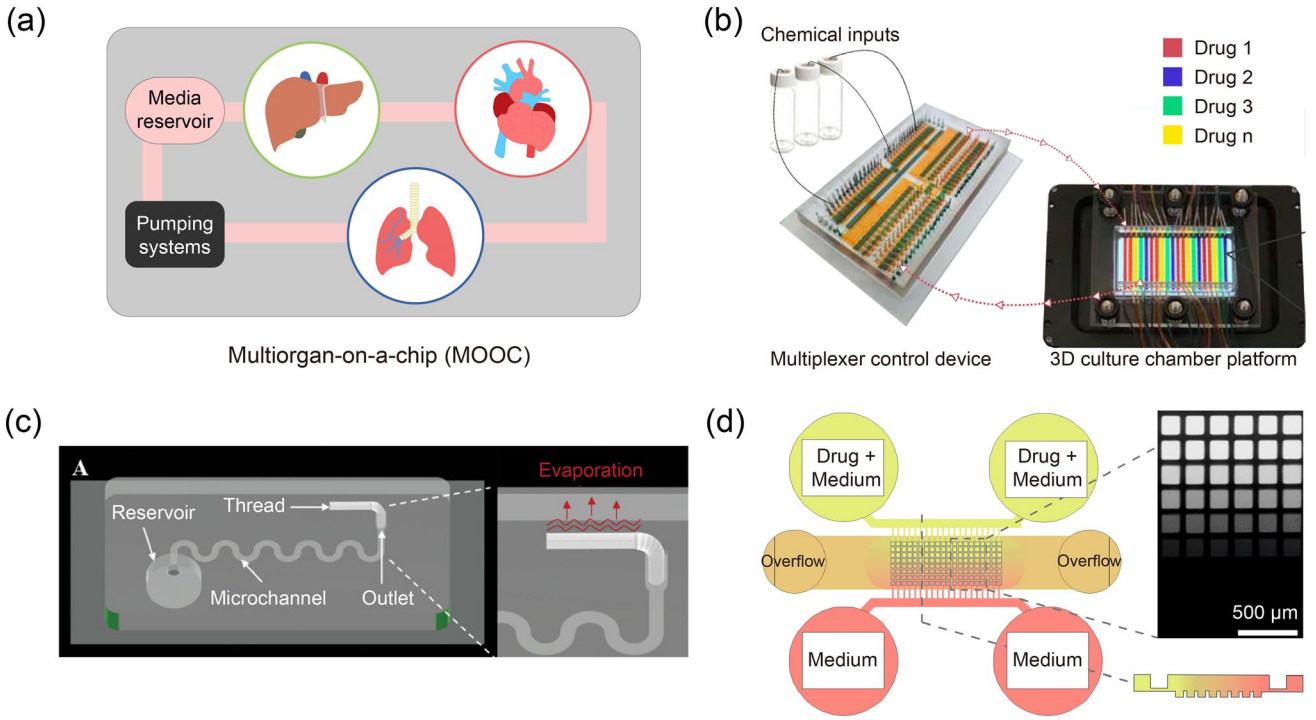

Fig. 6 Advanced engineering technology in OOCs. **a** Schematic illustration of liver-heart–lung-on-a-chip. **b** Automated microfluidic platform for high-throughput drug screening of tumor organoids. Reproduced with permission from [110]. Copyright (2020) Nature. **c** Pumpless and tubeless microfluidic chip that controls flow rates using adjustable hydrophilic thread. Reproduced with permission from [114]. Copyright (2020) Wiley. **d** A self-generated microfluidic concentration gradient chip for drug screening. Reproduced with permission from [115]. Copyright (2018) Nature

coupled OOCs are crucial factors to design MOOCs [106, 107].

The increasing demand for OOCs has led to the need for the automation and commercialization of the system. Recently, a robotic microfluidic platform "interrogator" was developed to automatically perform cell culture, perfusion, medium addition, fluidic linking, sample collection, and imaging for MOOC that links up to ten vascularized OOCs [108]. Moreover, a robotic liquid transfer system was used to fluidically link multiple OOCs for pharmacokinetic (PK) and pharmacodynamic (PD) modeling [109]. Another example of an automated and high-throughput system was developed for the dynamic and real-time analysis of growth, morphological changes, cellular apoptosis, and death of organoids. This system consisted of a multiplexer control device, 3D culture chamber, and custom software to facilitate personalized drug screening [110] (Fig. 6b). For the technology to be commercialized, large-scale, high-throughput, and automated fabrication processes must be satisfied [111]. Injection molding has been widely used for the large-scale production of OOCs owing to its advantages including low cost and time efficiency. Once the device design and fabrication steps are optimized for injection modeling, the entire production process becomes considerably simpler and faster than conventional soft lithography. An injection-molded plastic array platform mimicking the 3D tumor lymphatic vascular network has shown great potential for high-throughput drug testing [112]. However, the range of materials that can be used for injection molding is limited [113].

Recent advances in microfluidic technologies have led to the development of user-friendly OOCs. For example, recent studies have suggested microfluidic devices that can introduce flow into microfluidic channels without using external instrumentation. A pumpless and tubeless OOC platform could obtain different flow rates by adjusting the length and diameter of the hydrophilic thread [114] (Fig. 6c). In a multilayered microfluidic device, the resistive and capacitive microfluidic network and overflow ports could create long-lasting self-generated microfluidic concentration gradient [115] (Fig. 6d). A long-lasting, self-generated drug concentration gradient could be formed across an array of 240 tumor spheroids in a device to perform drug screening [115].

6 Summary and perspectives

Recently, significant advances have been made in the field of OOC technologies, demonstrating great potential as a complementary tool to preclinical animal studies. Cell biology, in conjunction with the development of microengineering and tissue engineering, has contributed to rapid progress in OOC research. Design of the microscale devices and dynamic control of physicochemical cues in a device have provided tissue-specific microenvironments to precisely recapitulate the key features of tissues on chips. Moreover, integration of biosensors has allowed for monitoring of the biological responses of the tissue models.

Currently, OOC research, from cell culture to functional analysis, requires technical skills and training to handle with the microscale devices. One of the major challenges in the field is a high user dependency that causes low reproducibility of the device. With the increasing demand of OOCs in various fields, systemized experimental procedures need to be established to minimize the user dependency. Moreover, high-throughput and automated systems could open the way for manufacturing end user-friendly OOC systems for practical applications. It is also challenging to reconstitute the complex and dynamic 3D microenvironment of tissues which can be precisely controlled in real-time. Introducing smart biomaterials such as stimuli-responsive biomaterials [116] and 3D spatiotemporally manipulative biomaterials [117] into OOC devices may better reconstruct the dynamic 3D microenvironment that regulates a variety of events in tissues [117–119].

OOC platforms can be utilized in a wide range of applications as preclinical tools, mainly in drug screening and pathophysiological studies. In addition to the newly developed drugs, nanoparticle-based drug delivery systems can be evaluated for their toxicity [120] and targeting efficacy [121] using OOCs [122]. Recently, iPSC technology has opened a new door to establishing personalized OOC models to develop personalized medicine [4]. Moreover, OOC devices can be used to study the pathophysiology of highly infectious illnesses such as SARS-CoV-2 [123] and influenza virus infection [124]. In this regard, the OOC technology may provide a breakthrough in fighting future pandemic respiratory viruses. Another potential application is the preclinical testing of implantable devices. The convergence of microsensors and bioelectronics into OOCs can mimic the tissue-implant interface, permitting better evaluation of the safety and efficacy of devices before implantation.

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Declaration

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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