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ABSTRACT

In recent years, organ-on-a-chip technology has developed rapidly in the biomedical field. Traditional single-organ chip provides a reliable experimental platform for disease modeling and drug development by simulating the physiological function of a specific organ, and some data generated by these platforms have been recognized by the Food and Drug Administration. Nevertheless, single-organ chip cannot fully simulate the complex interactions between body organs. To address these limitations, multi-organ-on-a-chip (MOOC) platform emerged. By using microelectromechanical system technology and 3D printing method, MOOC can integrate multiple organs with different structures/connections and precisely regulate the parameters of a micro-environment, such as fluid dynamics, chemical gradient, and mechanical stress. Moreover, the use of a biocompatible membrane and matrix gel materials enables the three-dimensional construction of cellular microenvironments, which enhance substance exchange and signal transmission between organs. Combined with a real-time monitoring system, the MOOC platform offers dynamic feedback and regulatory capabilities to simulate the complex interactions of human physiology more accurately. This paper recently reviews research progress in MOOC design strategies, construction methods, and their applications in drug discovery, disease research, and personalized medicine. Additionally, the technical challenges of MOOC technology and outlook of MOOC development trend are also included in this paper. In summary, MOOC technology represents an emerging platform with significant potential to improve disease modeling and early stage drug development. Furthermore, its integration with other frontier technologies may offer new opportunities to study disease mechanisms and explore novel therapeutic strategies.

I. INTRODUCTION

In vitro models are essential bridges between fundamental research and clinical applications, playing a pivotal role in advancing biomedicine. Traditional two-dimensional (2D) cell culture model is widely used for its simplicity and cost-effective. However, it fails to replicate the three-dimensional (3D) environment and the intricate cell–cell interactions in the human body. Although animal models can simulate a human environment to the greatest extent possible, there are significant physiological discrepancies between animal models and humans.[1,2] These differences not only impede the precision of research outcomes but also result in the inefficient expenditure of resources and time during the research process. In recent years, organoids have gained much attention; they still lack systematic approaches to fully reconstruct complex microenvironments.[3–5]

Nowadays, rapid advances in biomedical engineering have brought organ-on-a-chip technology to the forefront as a groundbreaking model system for *in vitro* culture. By integrating various cell types and structural components onto a miniature microfluidic platform, organ-on-a-chip enables precise control over key microenvironmental factors, including fluid flow rates, mechanical stresses, and chemical gradients to simulate the physiological functions and pathological processes of human organs.[6,7]

The early microfluidic organ-on-a-chip system dates back to 2004, when Sin *et al.* at Cornell University reported a microchip-based microculture system for pharmacokinetic/pharmacodynamic (PK/PD) modeling.[8] In 2010, a pivotal study by Huh *et al.* at Harvard University described a lung-on-a-chip model, which could precisely manipulate the lung microenvironment, including shear stress, tension, and pressure.[9] This breakthrough had a major

impact on the development of the field. Since then, various types of organ-on-a-chip have been engineered to replicate specific functions, such as liver-on-a-chip to model metabolism,[10,11] heart-on-a-chip to simulate beating,[12] and kidney-on-a-chip to mimic filtration.[13] As technology advance, an expanding array of organ-on-a-chip has emerged, including skin-on-a-chip,[14,15] intestine-on-a-chip,[16,17] and brain-on-a-chip,[18] which promoted the development of the organ-on-a-chip.

Nevertheless, the simulation of a single organ cannot fully reflect the complex interactions of multi-organ systems in the body. To address this problem, multi-organ-on-a-chip (MOOC) has been developed to provide a more comprehensive simulation of whole-body physiology and drug distribution *in vitro*. By integrating multiple organ units into a single platform with microfluidic control, MOOC enables the exchange of substances such as metabolites, signaling molecules, and immune cells between organs. This exchange facilitates more accurate simulation of *in vivo* conditions. Notably, Viravaidya *et al.* first demonstrated organ–organ interactions using a body-on-a-chip system by investigating lung–liver interactions to understand the mechanisms of naphthalene toxicity.[19] Building upon this, various groups have developed different multi-organ models to simulate the complex interactions between organs. Advances in stem cell technology are providing more physiologically relevant cell sources for organ-on-a-chip.[13] For example, induced pluripotent stem cells (iPSCs) and organoids now provide patient-specific stem cells that can be integrated and differentiated within MOOC platforms, enabling the creation of personalized preclinical models.

As shown in Fig. 1, this paper reviews the design principles, construction strategies, experimental protocols, and assays of multi-organ chips, while highlighting their significant biomedical applications. The review also discusses current challenges such as system complexity, scalability, and cost faced by the MOOC. It is important to consider both the advantages and limitations of the MOOC. MOOC technology is expected to gradually overcome these technical obstacles and expand into broader applications in the future.

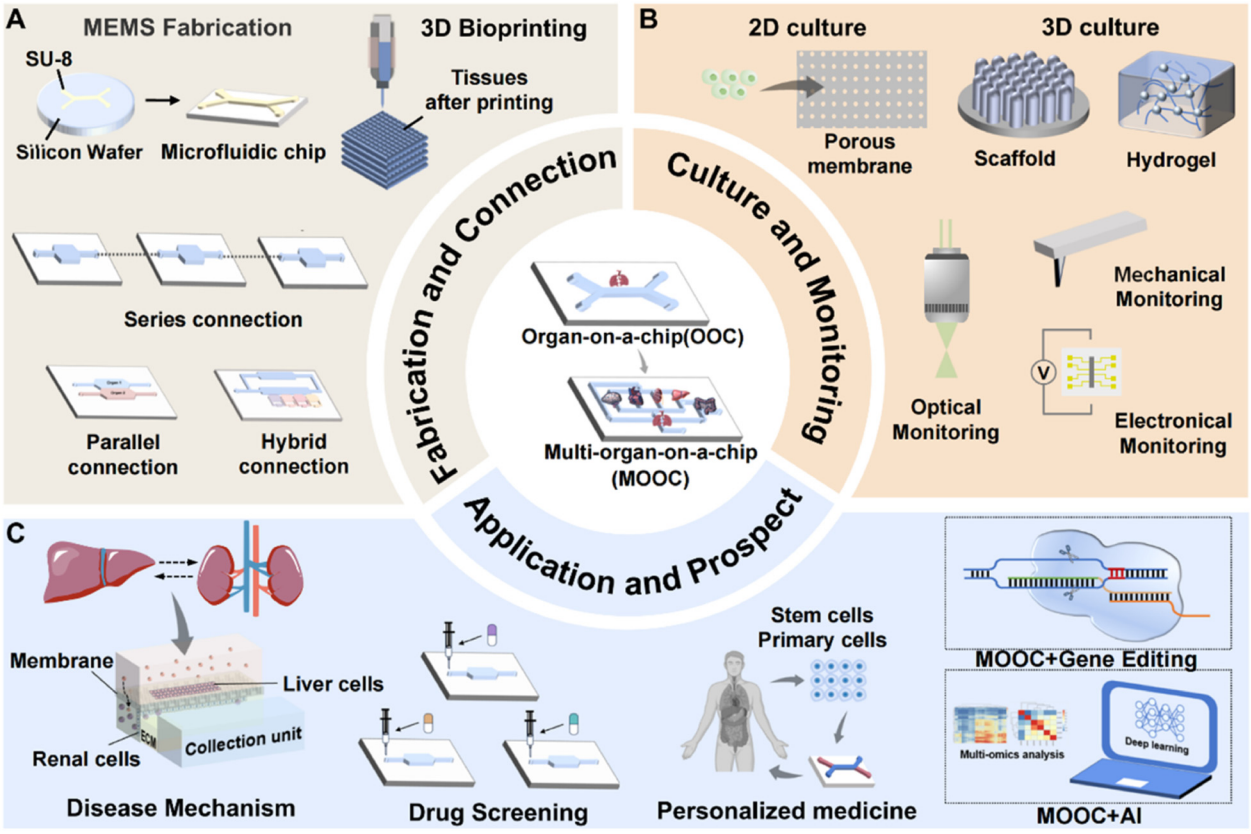

FIG. 1. Multi-organ-on-chip (MOOC) from fabrication to application. (a) MOOC fabrication and connection: MOOC are fabricated by microelectromechanical system (MEMS) technology or 3D printing technology. After that, different chips are connected mainly by series connection, parallel connection, or hybrid connection; (b) cell culture and chip environment monitoring: normally, there are 2D and 3D cell culture methods for option, detection technologies are based on physicochemical properties, such as optical, electrochemical, and mechanical properties. (c) Applications and prospects: MOOC can be widely used in disease models, drug screening, and personalized medicines. In future, MOOC will combine with advanced technologies, such as Artificial Intelligence (AI), nanorobots, and gene editing systems.

II. STRUCTURAL DESIGN OF MULTI-ORGAN-ON-CHIP

A. Single-organ chip is basic unit

Single-organ chip mimics the microstructure of an organ by designing tiny channels and chambers on the chip. After that, cells were cultured in these chambers to mimic the physiological behavior of the organ. As shown in Fig. 2, single-organ chip structures can be categorized into two types: "mono-layer structure" and "multi-layer structure."

Mono-layer structure: Fig. 2(a) shows a chip designed to mimic the structure and function of the blood–brain barrier (BBB). In this chip, pericytes and astrocytes, essential for the maintenance and support of the BBB in a physiological environment, were cultured in a central chamber resembling brain tissue. The surrounding circular area was blood vessels where endothelial cells were cultured. These two parts were connected by small capillary channels, the size of which only suitable for media exchange.

Multi-layer structure: Fig. 2(b) shows the three-layer structure with an upper cell layer and a porous membrane in the middle, and a lower layer contains different types of cells or substrates. The middle layer has a porous membrane that not only supports the upper cells, but also facilitates the exchange of nutrients and metabolites between the top and bottom layers. The system has a clear geometric topology for culturing various cell types and especially for membrane-structured organs, such as intestinal, pulmonary, and renal barriers.[13,20]

Based on structural dimensions, organ-on-a-chip technologies can also be categorized into two main types: micro-level (1–100 μm) and meso-level (100 μm–1 mm).

Micro-level chips, optimized for high-throughput screening, often rely on monolayer cell cultures and laminar flow control within their small-scale channels. In 2021, Bircsak *et al.* designed a microfluidic liver-on-a-chip platform using iPSC-derived hepatocytes for high-throughput hepatotoxicity with 159 tested compounds.[21] In contrast, meso-level chips focus on recreating physiological 3D tissue microenvironments. They often feature complex vascular networks or biomimetic scaffolds. Gallegos-Martínez *et al.* presented a user-friendly 3D-printed tumor-on-a-chip platform for culturing cancer spheroids in hydrogels, enabling long-term drug testing with the real-time monitoring of viability, metabolism, and cytotoxicity, while mimicking key tumor microenvironment (TME) features.[22] This dimensional and functional distinction underscores their

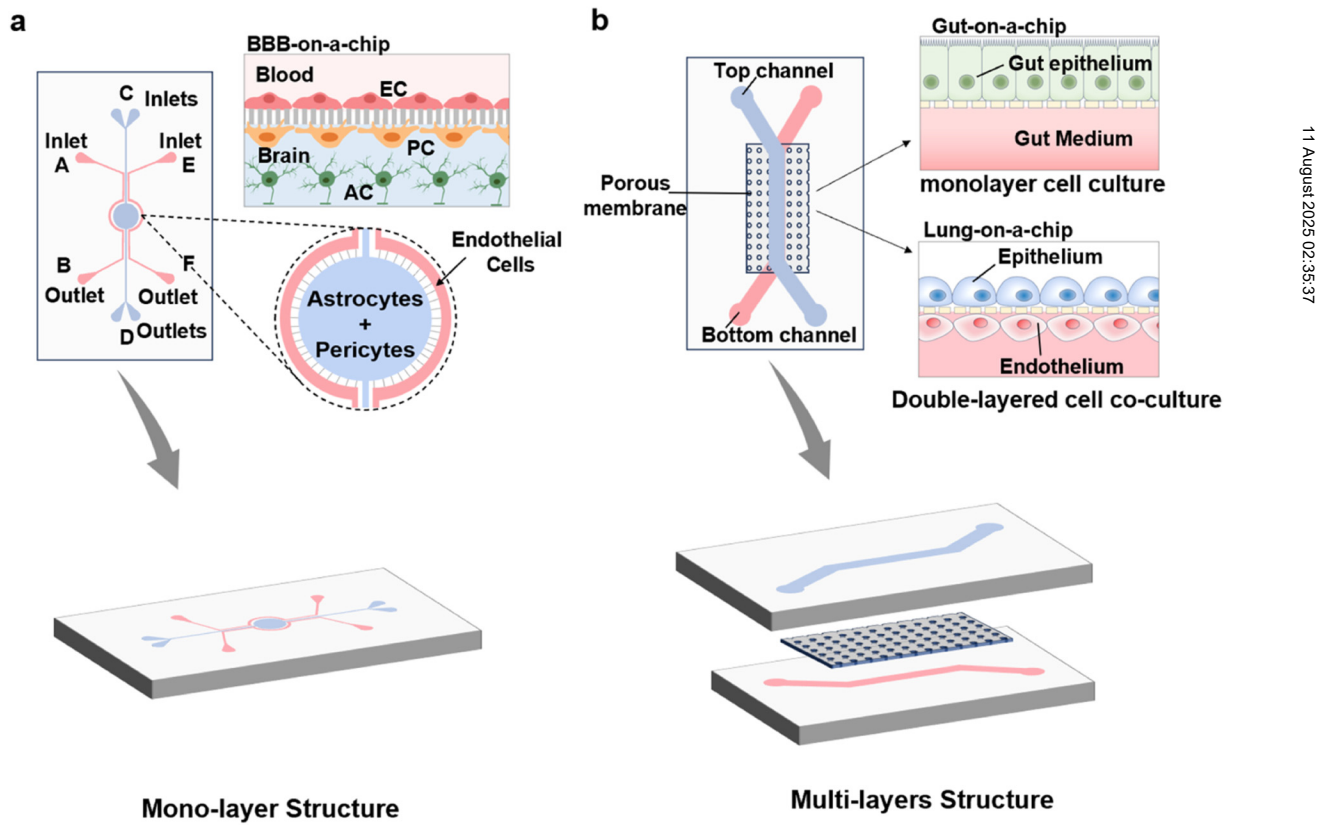

FIG. 2. Classification of single-organ chip. (a) Mono-layer chip: single-layer chip was designed according to the functional and structural requirements of a specific tissue or organ. (b) Multi-layer chip: sandwich construction, for example, with an upper cell layer and a porous membrane in the middle, and a lower layer contains different types of cells or substrates.

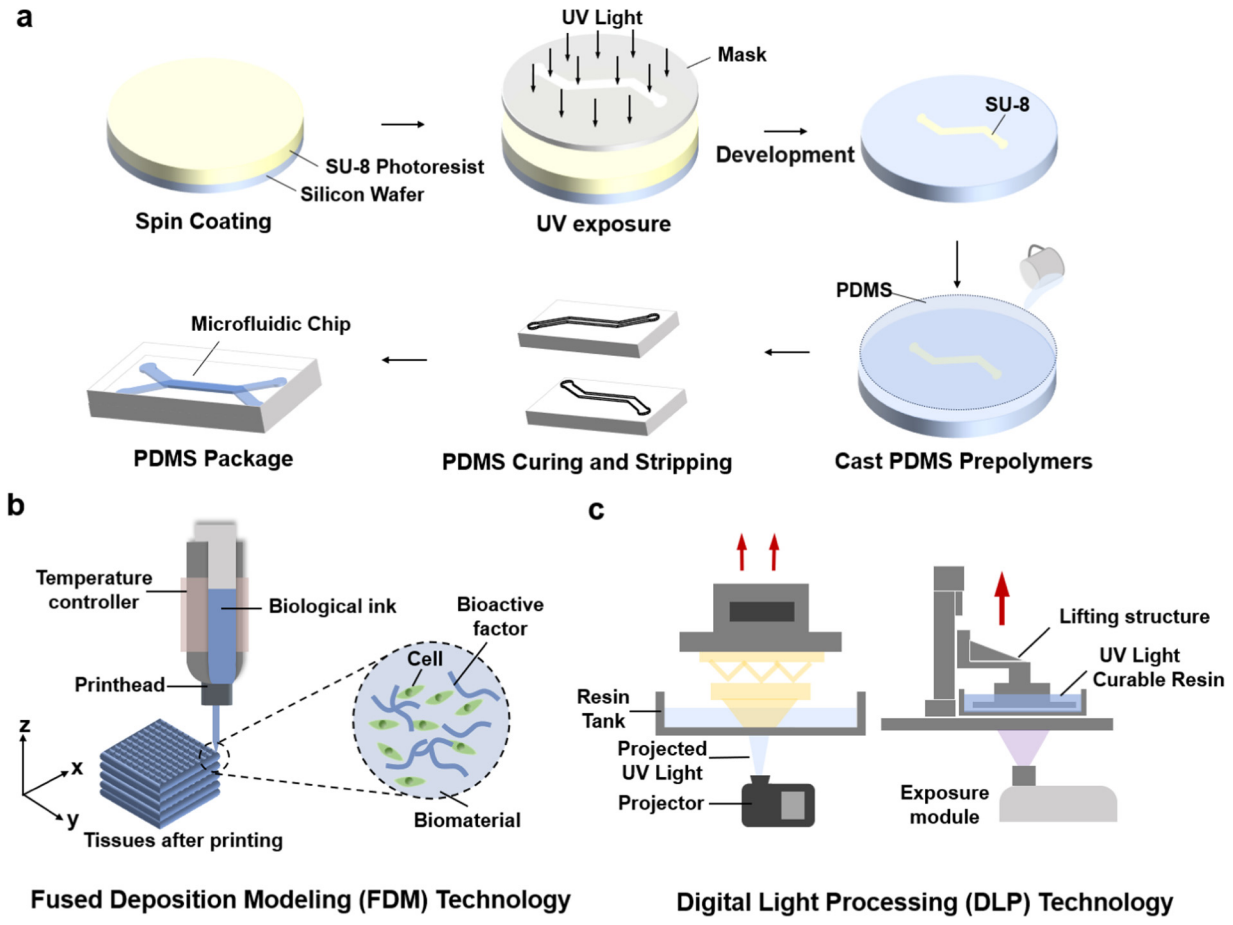

FIG. 3. Fabrication process of organ-on-a-chip. (a) MEMS technology: The designed microchannel structure was transferred from mask to a silicon wafer by using standard photolithography method. After that, a PDMS chip was made based on a silicon wafer mold. (b) FDM 3D printing technology: Direct printing of bio-inks containing cells, precisely depositing living cells in predetermined locations to form tissue-like structures. (c) DLP 3D printing technology: Used for bioprinting that requires high precision and complex structures, without the need for multiple photolithography and mold making.

complementary roles in preclinical research, from initial drug candidate triage to the mechanistic validation of therapeutic responses.

Organ-on-a-chip fabrication usually employs Microelectromechanical System (MEMS) techniques[23] [Fig. 3(a)]. The manufacturing process for organ-on-a-chip begins with Computer-aided Design (CAD) based on the required organ functions. To ensure that the final structure meets biophysical requirements, such as fluid shear stress (FSS) and culture medium pressure, simulation was conducted using multi-physics software like COMSOL. After design, photolithography technique was used to transfer the microchannel structure on the mask to a silicon wafer with a series of steps: photoresist coating, soft baking, UV exposure, postexposure, and photoresist development. After that, liquid PDMS (polydimethylsiloxane) prepared by mixing component A and component B was poured onto the photoresist mold, heated to cure, and peeled off to produce the PDMS chip.[24] Finally, the chip assembly was completed through PDMS chip and glass slide plasma bonding.

While traditional MEMS technology offers high precision and well-established manufacturing processes for organ-on-a-chip, but it is complex and costly, requiring equipment like photolithography machines and cleanrooms. 3D printing technology offers greater flexibility and efficiency to chip design and manufacturing. The "What You See Is What You Get" working mode was suitable for iterative improvement, especially in the fabrication of complex 3D structures.

3D bioprinting technology encompasses several methods.[25] Fused Deposition Modeling (FDM) is an extrusion-based 3D printing method that involves heating thermoplastic materials until they melt, then extruding the molten material through a nozzle, and depositing it layer by layer to construct a 3D object[26] [Fig. 3(b)]. FDM is compatible with a wide range of biomaterials and can print cell-containing structures or relatively simple scaffolds with favorable biocompatibility. However, its relatively low printing resolution poses challenges for constructing finely detailed microstructures.

For bioprinting applications requiring high precision and complex structures, Digital Light Processing (DLP) technology provides high precision. This light-curing 3D printing technique uses a reservoir of liquid photosensitive materials and projects light according to a pre-designed 3D model. When the light source irradiates specific areas of the liquid resin, it cures into a thin solid layer. The platform then moves incrementally, allowing each new layer to cure atop the previously solidified resin, building the 3D structure layer by layer [Fig. 3(c)]. Nevertheless, this technique has a relatively limited choice of materials, mostly photosensitive polymers, which are less biocompatible and usually require post-treatment (e.g., removal of uncured resins) that may impact cellular activity.

B. Connection single-organ chips to form multi-organ chips

Single-organ chip modules are connected in series or parallel through microfluidic channels to create a sealed fluidic network, forming a multi-organ chip. These channels enable the flow of nutrients, metabolites, and signaling molecules between different modules, simulating the circulation of blood and lymphatic fluids in the body. This integrated design allows for a more accurate replication of complex interactions and dynamic environments within the human body, such as liver–kidney, liver–gut, gut–brain, and skin–gut relationships. By precisely controlling the fluid flow rate and pressure, the physiological fluid dynamic environment between organs can be simulated. By integrating sensors, microenvironment parameters such as oxygen levels, pH, and chemical gradients can be monitored and regulated in real time. As shown in Fig. 4, the common connection modes are divided into series (fixed series or flexible series), parallel, and series–parallel hybrid connections.

1. Series connection

The fixed-series structure arranges single-organ modules in a predetermined sequence, connecting all modules through a single microfluidic channel [Fig. 4(a)]. Cells are seeded simultaneously into each chamber, and a common culture medium is provided.[27,28] This design is aimed at simulating specific physiological processes or drug metabolism pathways, ensuring that fluid flows sequentially through each organ model. For example, an intestine–liver–kidney arrangement can be used to model the entire process of drug absorption, metabolism, and excretion. A typical example of this structure is the compartmentalized microfluidic cell culture system developed by Zhang *et al.*[29] They selected four types of human cells (C3A, A549, HK-2, and preadipocytes) to represent liver, lung, kidney, and adipose tissue. By adding growth factors to the culture medium, the functions of different cells were optimized, with findings showing that TGFβ1 enhances the function of A549 cells but inhibits C3 A cells, indicating some degree of crosstalk between cell culture compartments.

The fixed series is suitable for unidirectional substance transfer. However, it cannot fully simulate complex physiological interactions and feedback loops seen in biological systems, such as those in hormone regulation within the endocrine system.[30] These interactions require bidirectional signaling and real-time feedback, which are limited in a fixed-series configuration with preset, unidirectional fluid paths.

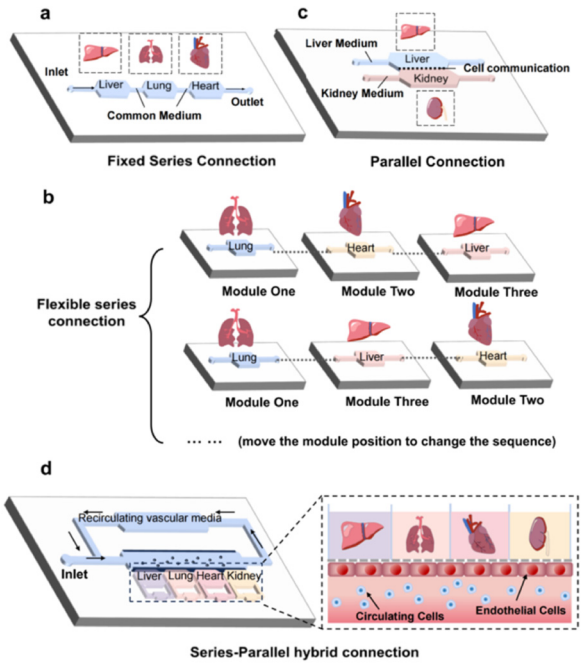

FIG. 4. Multi-organ chip connection methods. (a) Fixed-series connection: arranging individual organ modules in a predefined order and connecting all of them through the same microfluidic channel. (b) Flexible series connection: connecting individual single-organ chips that exist independently one by one. (c) Parallel connection: each organ module is connected through parallel microfluidic channels with porous structure in the middle. (d) Series–parallel hybrid connection: connecting multiple organ models in both series and parallel in the fluidic network.

The flexible series structure allows each individual organ module to have an independent culture system, enabling flexible arrangement and connection based on experimental requirements. As shown in Fig. 4(b), each organ module is cultured separately using an organ-/tissue-specific medium. After that, single-organ chip can be connected using adjustable micro-connectors or tubing, allowing researchers to modify fluid paths and sequences to simulate various physiological conditions and experimental needs. In 2024, Huang *et al.* applied this flexible series structure to connect kidney and liver chips to achieve the compound exchange and drug toxicity assessment.[31]

This highly flexible configuration allows researchers to independently control the function and interaction of each single-organ chip based on experimental requirements, providing higher experimental precision. However, the need for additional connections and coordination makes the experiment more complex. A flexible series structure relies on independent connections between multiple organ modules, requiring precise microfluidic design and stable connections to ensure consistent fluid transfer and interactions between organs. Currently, coordinating the microenvironments of multiple organ modules remains a significant challenge.

2. Parallel connection

As shown in Fig. 4(c), parallel connection is another common design for multi-organ chips, where each organ module is connected through parallel microfluidic channels with a porous structure separating different channels. The porous structure is typically micrometer-scale tubing or porous membrane, allowing biological fluids (such as cell culture media or blood simulating fluid) to flow between organ modules. Although the modules are connected independently through parallel channels, interactions between them can occur through a shared medium or chemical signal transmission. For instance, changes in metabolites in one module can affect other modules through fluid exchange, thus simulating physiological associations between different organs.

The parallel structure enables different modules to interact through shared media or chemical signals, allowing researchers to observe dynamic feedback and regulation between organs, thereby more accurately replicating *in vivo* organ complexity. However, accurately simulating complex *in vivo* feedback mechanisms remains challenging when multiple signal integrations are involved. Additionally, the parallel structure lacks the capacity to capture the sequential and continuous nature of physiological processes, limiting its ability to replicate certain metabolic pathways. Some physiological responses require a specific order, which this structure cannot fully achieve.

3. Series–parallel hybrid connection

The series–parallel hybrid structure connects multiple organ models in both series and parallel in the fluidic network [Fig. 4(d)]. This structure can simulate interactions between various organs or tissues in the body. The upper layer is designed with organ units that can be inserted or replaced, enabling flexibility to change or adjust the sequence according to experimental needs. The lower layer serves as a shared fluid input and output network for all organ models, simulating the flow and exchange of blood, lymph, or other bodily fluids between different organs. With this structure, researchers can study and observe interactions and communication between different organs or tissues. In 2022, Ronaldson-Bouchard *et al.* developed a body-on-a-chip system accommodating the heart, liver, bone, and skin, where fluid connections were achieved through a shared vascular flow beneath organ compartments.[32]

This configuration provides exceptional flexibility while ensuring fluid dynamics consistency and control. However, the complexity of manufacturing and operation requires high technical skill and experience operators. Additionally, ensuring uniform fluid distribution and dynamic adjustments between modules requires precise fluid dynamics control. Maintaining the functional stability and effectiveness of interactions between these modules remains a challenge that requires further attention.

C. Cell culture on chip: from two-dimensional to three-dimensional

1. Two-dimensional cell culture based on porous membranes

Currently, cell sources for constructing single-organ chips mainly include primary cells, cell lines, stem cells, and organoids, each offering unique characteristics to meet specific research needs. Primary cells are isolated directly from human tissues that retain the physiological properties of cells in the body, simulating organ function more realistically. However, primary cells are challenging to obtain, and have limited proliferative capacity and a short lifespan *in vitro*. Well-established cell lines, particularly immortalized ones, are widely used in organ chips due to their ability to proliferate indefinitely, relatively simple culture requirements, and ease of handling. Because of their rapid growth rate and low cost, researchers often use these cell lines for high-throughput screening and initial experiments. However, a major limitation of cell lines is that they can differ from *in vivo* cells, particularly in gene expression profiles and physiological functions. The development of induced pluripotent stem cells (iPSCs) technology provides an alternative cell source that more closely resembles the functionality of human organs.[33] This groundbreaking technique involves reprogramming somatic cells (such as skin or blood cells) into pluripotent stem cells capable of differentiating into nearly any cell type. As shown in Fig. 5, researchers can use iPSCs to produce specific organ cell types, such as myocytes, hepatocytes, and neurons, for organ function simulation.

Some organ-on-a-chip systems use a porous membrane as a middle layer and cell culture substrate. Cell cultures are typically grown on porous membranes, allowing cells to form monolayer or bilayer arrangements on the surface. Common types of porous membranes are polycarbonate (PC), polyethylene terephthalate (PET), and polydimethylsiloxane (PDMS).[34–36] To promote cell adhesion and growth on porous membranes, the membrane surface is coated with cell adhesion factors. These factors are usually extracellular matrix proteins, such as collagen, laminin, and fibronectin, which mimic *in vivo* cell–matrix interactions to enhance cell attachment and growth. After cell seeding, precise control of fluid flow and microenvironment conditions provides an optimal culture environment. The nutrient content and growth factors in the culture medium are adjusted according to cell type to support cell growth and proliferation.

Nevertheless, this 2D membrane structure on chips typically only models cellular behavior in a monolayer plane and cannot simulate multi-layered cell arrangement or 3D cell-to-cell interactions. For example, cell spheroids, a spherical-like structure, formed during cell proliferation due to cell–cell interactions and aggregation. Real organs possess intricate three-dimensional architectures, where cell–cell and cell–matrix interactions are three-dimensional, involving complex mechanical and biochemical signaling. A 2D membrane structure cannot provide this 3D information or fully replicate these interactions, which may lead to inaccurate predictions of biological responses *in vivo*.

2. Three-dimensional cell culture based on hydrogel fibrous scaffolds

Polymers such as polydimethylsiloxane (PDMS) are commonly used as base materials for organ microfluidic chips. PDMS has excellent optical transparency, which enables researchers to clearly observe cell and fluid dynamics inside the chip through equipment such as microscopes. Good gas permeability is another major advantage, which ensures that cells are supplied with

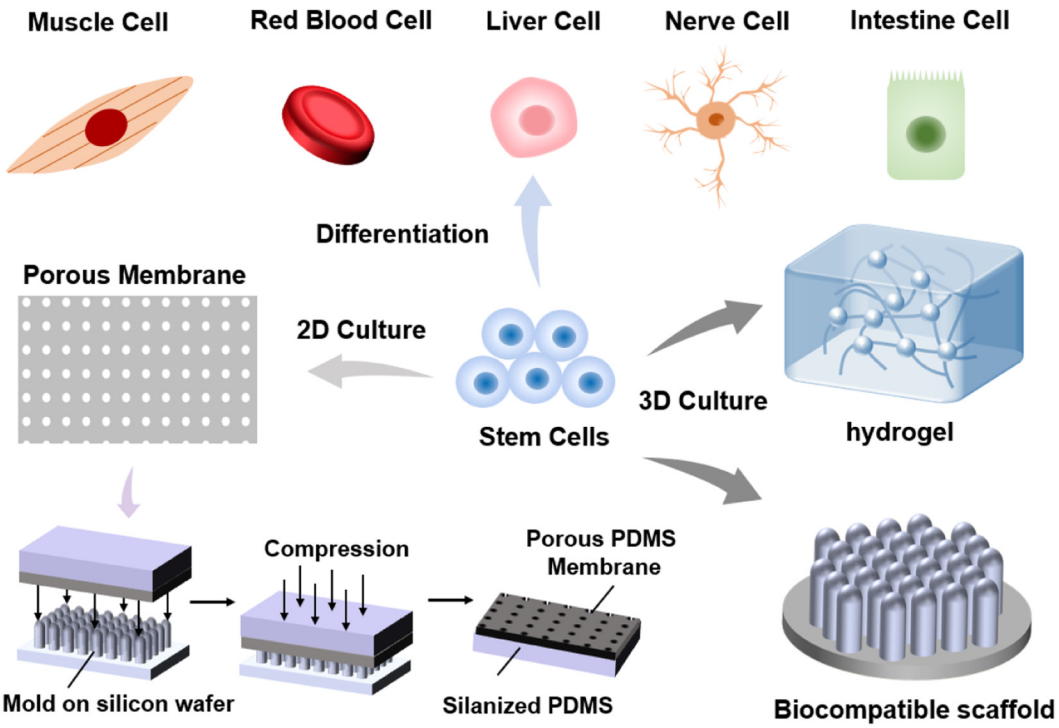

FIG. 5. Cell culture of organ-on-a-chip. Stem cells can differentiate into a variety of somatic cell types, such as myocytes, hepatocytes, and neuronal cells. Cell culture can be divided into two types: (1) 2D culture. Cells are grown on the surface of a porous membrane and form a monolayer or bilayer of cell layers. PDMS porous membranes are usually fabricated using photoresist templates on a silicon wafer. (2) 3D culture. Cells are grown in three dimensions based on hydrogels or fibrous scaffolds.

sufficient oxygen during the incubation process, thus favoring healthy cell growth and metabolic activities. In addition, it can be machined and molded to create complex and fine microfluidic channels and structures. The selection of biomaterials must be able to support cell growth and function with appropriate biocompatibility and mechanical properties.

Hydrogels, with high water content and excellent biocompatibility, can mimic the 3D structure of the natural Extracellular Matrix (ECM), providing cells with a growth environment similar to that observed *in vivo*.[37] Hydrogels can be classified into natural and synthetic types, which have their own advantages and disadvantages in cell culture. (Table I). Natural hydrogels are derived from nature, such as collagen, sodium alginate, hyaluronic acid, and fibrin protein. These hydrogels offer excellent biocompatibility and degradability, effectively simulating the natural environment *in vivo*, promoting cell adhesion, proliferation, and differentiation.

Notably, natural hydrogels have batch variability and potential immunogenicity issues.[37] Synthetic hydrogels, such as polyethylene glycol (PEG), poly (lactic-co-glycolic acid) (PLGA), and polyacrylamide (PAM), offer adjustable mechanical properties and chemical characteristics. Although the properties of these synthetic materials can be modified chemically, they lack natural cell adhesion sites, and some degradation products may negatively affect cells. As early as 2009, Sung *et al.* cultured cells embedded in 3D hydrogels in separate chambers representing the liver, tumor, and bone marrow, connected by channels simulating blood flow, to provide a more physiologically relevant environment than monolayer culture.[28] Recently, Nie *et al.* have developed a technique for pre-embedding cells in hydrogel tubular organ chips, resulting in branched and curved tubular structures and an initial vascular–nephron interaction model.[38]

Fibrous scaffold is a biomaterial with a fibrous structure. These scaffolds provide topological shape guidance to cells by mimicking the fibrous network structure of collagen fibers and elastin fibers, which guides cell alignment and differentiation, thus promoting tissue formation and functional restoration.[39] The combination of hydrogels and fibrous scaffolds can mimic natural ECM, providing favorable conditions for cell growth, differentiation, and tissue formation.[40]

Hydrogels are particularly suitable for modeling organs that require a 3D ECM, such as the liver, kidney, and brain tissue. Cartilage tissue physiology demands a matrix-rich environment, and hydrogels can support cartilage cell growth and cartilage regeneration by adjusting mechanical properties and degradation rates.[42] On the other hand, fibrous scaffolds are better suited for organs requiring mechanical strength and directional support, such as the heart, lungs, and blood vessels. Combining both hydrogels and fibrous scaffolds can provide comprehensive support for more complex organ models. For instance, hydrogels can simulate the dermal layer of the skin, supporting keratinocyte growth, while

TABLE I. Comparison of hydrogel materials for organ-on-a-chip.

Hydrogel	Materials	Advantages	Disadvantages	Reference
Natural hydrogel	Collagen	- Biocompatible - Strong cell adhesion - Degradable	- Low mechanical strength - Variations between batches	37 and 41
	Sodium alginate	- Biocompatible - Adjustable gel strength - Gentle gelling conditions	- Degradation products may be biologically active - No natural cell adhesion sites	37,42, and 43
	Fibrous protein	- Strong cell adhesion - Procoagulant - Degradable	- Weak mechanical properties - Complex gelation process - Higher costs	37 and 42
Synthetic hydrogel	Polyethylene glycol (PEG)	- Biocompatible - Adjustable mechanical properties - Non-toxic degradation products	- No natural cell adhesion sites - Degradation products may be biologically active	37,44, and 45
	Poly (lactic-co-glycolic acid) (PLGA)	- Adjustable degradation rate - Good mechanical properties - Suitable for multiple cell types	- Degradation product acidic - Can lead to localized acidosis	37,46, and 47
	Polyacrylamide (PAM)	- High mechanical strength - Adjustable porosity - Easily machined and shaped	- Toxicity issues - Long-term use may affect cellular health	37 and 48

fibrous scaffolds can mimic the mechanical strength and elasticity of the skin, making them suitable for wound healing studies and drug testing.

D. Monitor system for organ-on-a-chip

Selecting suitable detection methods is crucial during the culture process, as these provide important information about cell growth, function, and interactions. The growth status, morphological changes, and structural organization of cells can be observed and recorded via microscopy. As illustrated in Fig. 6(a), optical signals enable the detection of cellular biological processes. Fluorescent markers such as 4',6-Diamidino-2-Phenylindole (DAPI), green fluorescent protein (GFP), PE, and Cy5 are used to identify specific cellular components or molecules, including the nucleus, cell membrane, and particular protein expressions. For mechanical analysis, atomic force microscopy (AFM), as shown in Fig. 6(b), measures cell surface properties such as stiffness, elasticity, and adhesion.[49] AFM also detects interactions between cells and the matrix, delivering nanoscale mechanical data essential for understanding cellular biomechanics and cell–matrix interactions. Additionally, various biochemical sensors are also widely applied to MOOCs.[50] As shown in Fig. 6(c), electrochemical sensors can measure the concentrations of cellular metabolites (e.g., glucose and lactate), current changes, or other electrochemical signals, thereby assessing cell physiological states and providing real-time data on cellular metabolic activity and functional status.[51] In addition, as shown in Fig. 6(d), Trans-epithelial Electrical Resistance (TEER) sensors can be used to evaluate cell health and function by measuring the changes in membrane resistance, providing information on membrane integrity and electrophysiological properties.[52]

III. COMMON MOOC MODELS AND THEIR APPLICATIONS

A. Liver–kidney-on-a-chip

The liver and kidneys are essential metabolic organs that collaborate closely in the processing and excretion of drugs and metabolites. Drug metabolism generally follows liver–kidney pathway: the liver converts lipophilic drugs into water-soluble metabolites, which are then excreted by the kidneys. If one organ's function is compromised, the other organ often bears a heavier load, potentially leading to reduced metabolic efficiency or increased toxicity.[53] Moreover, certain drugs exhibit bidirectional interactions between the liver and kidney, which adds complexity to the regulation of drug metabolism.

As depicted in Fig. 7(a), the multi-layered liver–kidney chip design integrates cells from both organs. HepG2 cells, cultured in the upper layer, model liver tissue for drug metabolism, while glomerular endothelial cells (GECs) in the lower layer enable the evaluation of drug-induced toxicity on kidney filtration. Using this liver–kidney chip model, Li *et al.* simultaneously assessed drug metabolism in hepatocytes and the resulting nephrotoxicity within a single device.[54] Lin *et al.* used non-contact co-cultures of liver spheres and the renal proximal tubule barrier in this model to study the toxicity and metabolic mechanism of 14-day repeated dose cyclosporin A alone or in combination with rifampin.[55] Theobald's team used a multi-compartment microfluidic chip to culture HepG2 and RPTEC cells to simulate the liver and kidney, investigating the process of vitamin D metabolism in the liver and bioactivation in the kidney.[56]

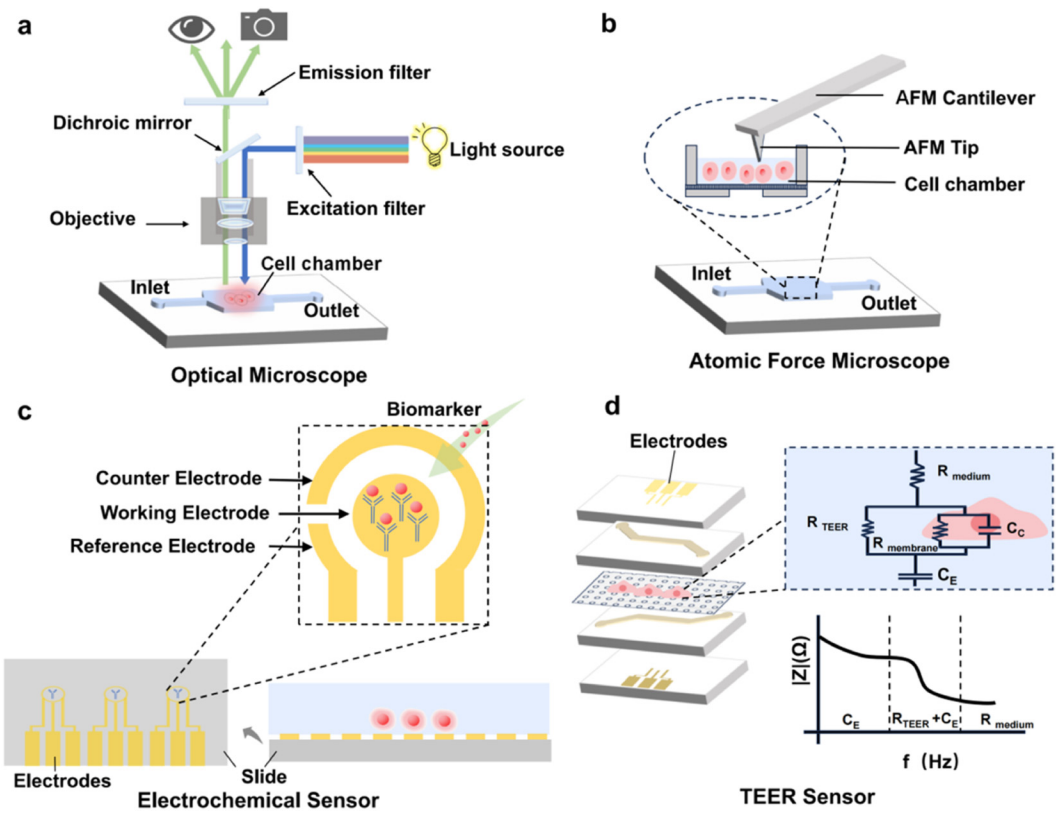

FIG. 6. Detection methods on MOOC. (a) Optical microscope. Observe the growth status, morphological changes of the cells. (b) Atomic Force Microscope (AFM). Measure the mechanical properties of the cell surface such as rigidity, elasticity, adhesion. (c) Electrochemical sensors. Measurement of changes in cellular metabolites by means of a three-electrode system. (d) Trans-epithelial Electrical Resistance (TEER) sensor. Measurement of cell state by electrical impedance changes.

B. Liver–gut-on-a-chip

Enterohepatic circulation is a vital physiological process in the human body. Bile acids, drugs, or their metabolites are first secreted by the liver into bile and then transported to the small intestine via the bile duct. In the small intestine, some of these substances are reabsorbed, re-entering circulation, and returning to the liver through the portal vein, thus forming a cyclic process. A liver–gut model can accurately simulate the entire process of drug absorption in the intestine, liver metabolism, and reabsorption. The gut–liver axis is significant in various diseases, such as non-alcoholic fatty liver disease, cirrhosis, and intestinal disorders.[57,58] Through the liver–gut model, researchers can study how gut microbiota, inflammatory factors, and metabolites influence liver health, thus uncovering disease mechanisms.

As shown in Fig. 7(b), the liver–gut chip typically consists of a dual-layer structure, with intestinal epithelial cells (Caco-2) in the upper layer and liver cells (HepG2) in the lower layer. In 2017, Choe *et al.* employed this chip model to evaluate the first-pass metabolism of apigenin, a flavonoid compound.[59] Hepatic steatosis, an abnormal accumulation of lipids in liver cells, is often induced by excessive alcohol consumption or obesity. In 2018, Lee and Sung used a gut–liver chip to simulate intestinal absorption and liver metabolism, demonstrating that fatty acids are absorbed through the intestinal layer and subsequently accumulate in liver cells.[60] In 2021, Jeon *et al.* confirmed that the liver–gut chip could replicate various aspects of hepatic steatosis. They assessed fatty acid absorption under different culture conditions and tested the anti-steatotic effects of XL-335 and metformin.[61]

C. Gut–brain-on-a-chip

The gut–brain axis refers to the two-way communication system between the gut and the brain, involving neural, endocrine, immune, and metabolic interactions. The gut microbiota, particularly bacteria in the gut, exerts influences on brain function and behavior through the gut–brain axis.[62] Gut microbes can affect the brain through a variety of mechanisms, including producing metabolites (such as short-chain fatty acids) that affect the synthesis of neurotransmitters or transmit signals through neural pathways, such as activating the vagus nerve. These microbial metabolites can cross the blood–brain barrier and directly or indirectly affect mood, cognitive function, and pain perception.

There are multiple signaling pathways between the gut and the brain, and several studies suggest that exosomes may act as vectors in this communication. Recently, Kim *et al.* developed a modular

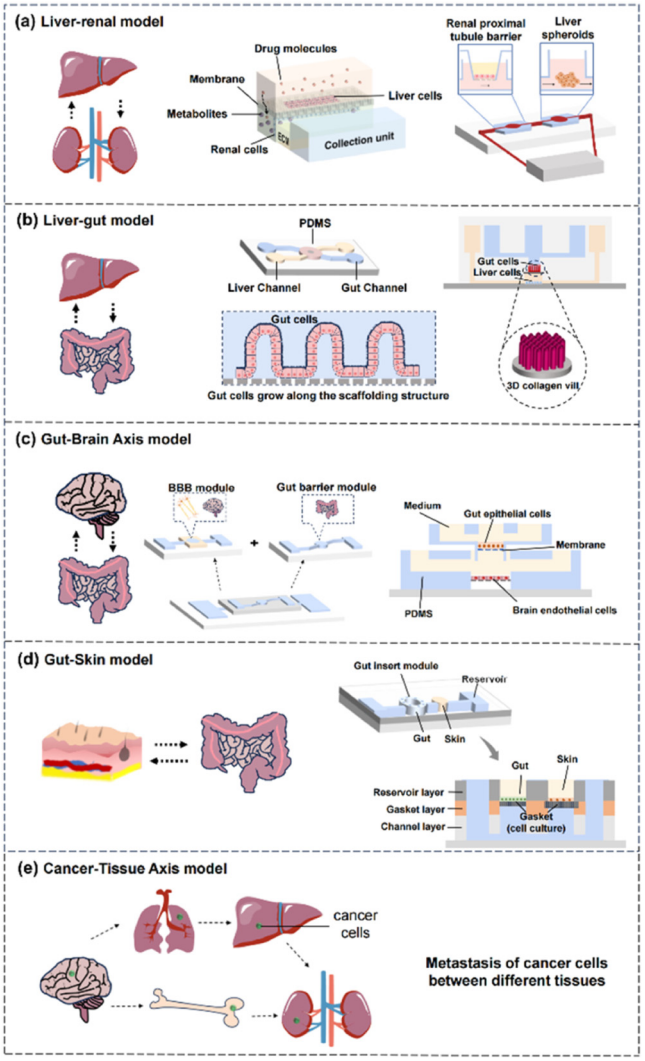

FIG. 7. MOOC models. (a) Liver–kidney model. Liver–kidney chip simulates the process of drug metabolism in the liver–kidney pathway for mechanism study and toxicity testing. (b) Liver–gut model. The liver–gut chip simulates the process of liver–intestine circulation *in vivo* and carries out research on the mechanism of non-alcoholic fatty liver, cirrhosis, intestinal diseases, and other diseases. (c) Gut–brain model. The gut–brain chip simulates the two-way communication system between the gut and the brain. Collagen scaffolds mimicking human intestinal villi are printed using 3D printing technology, thus providing cells with a three-dimensional organizational structure. (d) Skin–gut model. The skin–gut chip simulates the communication and regulation between the skin and the gut. (e) Cancer-tissue axis model. Metastasis of cancer cells between different tissues.

microfluidic chip, as shown in Fig. 7(c), in which co-cultures of intestinal epithelial cells and brain endothelial cells form the intestinal epithelial barrier and the blood–brain barrier (BBB), respectively, and are connected by microfluidic channels. These modules can be easily assembled and disassembled, and the co-cultured cell barriers form well under appropriate fluid flow conditions.[63] Metabolites or extracellular vesicles from the gut microbiota can cross the intestinal epithelial barrier or the blood–brain barrier and enter the systemic circulation. This phenomenon may induce physiological responses that directly or indirectly affect the central nervous system and its functions. Kim *et al.* used neurons derived from human iPSCs on a gut–brain axis chip to study the effects of that microbial-derived metabolites and exosomes on neurodevelopmental and neurodegenerative diseases.[64] The results suggest that microbial-derived metabolites and exosomes were promising strategies for the treatment.

D. Skin–gut-on-a-chip

The skin and gut communicate and regulate through several mechanisms, including the immune system, microbiota, and neuroendocrine pathways. The gut microbiota has important effects on immune regulation and inflammatory responses throughout the body. Gut dysbiosis can lead to systemic inflammation, which, in turn, affects skin health.[65] Nutrients absorbed from the gut and metabolites produced by gut microbes directly impact skin health.[66] Additionally, the gut and skin are also linked through the neuroendocrine system.[67] Neurotransmitters (in the gut, such as serotonin) not only affect gut mood but also affect inflammation and skin repair. As shown in Fig. 7(d), Lee has developed a MOOC whose modular design allows separate culture and differentiation of gut and skin tissue.[68] After assembly, the two organs are connected by the microfluidic channels, allowing perfusion and mass transfer. The results showed that the impairment of intestinal barrier function exacerbated the adverse effects of fatty acids on skin cells.

E. Cancer-tissue axis

Cancer-tissue axis usually refers to a complex system of interacting relationships between cancer cells and surrounding tissues that develop during cancer development [Fig. 7(e)]. Tumor-on-a-chip can simulate the microenvironment of tumors *in vitro*, including the ECM, vascular system, and immune cells, allowing researchers to observe tumor behavior in a setting more closely resembling *in vivo* conditions, namely, metastasis mechanisms and drug metabolism pathways. In 2024, Mohammed *et al.* develop a MOOC device to simulate the interactions between breast cancer and liver tissues, enabling more accurate PK–PD modeling and simulation.[69] In 2023, Fedi *et al.* developed a MOOC model: HepG2 cells were co-cultured as a monolayer in one chamber, while a 3D hydrogel model of ovarian cancer was co-cultured in a second chamber. Then, cisplatin was infused into the system to simulate systemic administration, facilitating the study of both the drug's efficacy and hepatotoxicity in a physiological condition.[70]

The tumor microenvironment (TME) is a highly complex ecosystem in which the interaction of immune cells and circulating tumor cells (CTCs) plays a key role in tumor immune escape and treatment resistance.[71–75] Cui *et al.* developed a patient-specific glioblastoma microarray to reveal the immunosuppressive features of the microenvironment of mesenchymal Glioblastoma (GBM) and enhanced the efficacy of PD-1 inhibitors by coadministering with Colony-Stimulating Factor 1 Receptor (CSF-1R) inhibitors, which provided a new platform for the screening of

personalized immunotherapy for GBM.[76] Onal *et al.* found that breast cancer cells and macrophages form an interactive loop through CSF-1 (paracrine) and membrane-bound Epidermal Growth Factor (EGF) (occluder) that macrophages chemotaxis to cancer cells, whereas cancer cells require a direct contact for the endocytosis of EGF and that macrophage migration of cancer cells is reversed in different matrices.[77] Using the MOOC platform, Kim *et al.* successfully simulated the migration of natural killer (NK) cells into neuroblastoma, the infiltration in 3D tumor stroma, and the activation of anti-tumor cells. There is a clear drop in CD16-positive NK cells within the population of cells that have migrated and infiltrated.[78] Recently, Argenziano *et al.* developed a β-cyclodextrin-based nanosponge containing gemcitabine that was functionalized with ICOS-Fc (ICOS-Fc-NS-GEM). In the MOOC platform, it was shown to inhibit the activity, proliferation, and invasive ability of pancreatic cancer cells.[79] These studies show that an immune-organ-on-chip to recapitulate the tumor-mediated infiltration of circulating immune cells within the 3D tumor model.

Notably, patient-derived organoids (PDOs) established from resected tumor tissues recapitulate the molecular and cellular characteristics of parental tumors, serving as physiologically relevant models for tumor microenvironment studies and drug screening.[80] The integration of PDOs with microfluidic platforms enables controlled recapitulation of tumor progression, metastasis, and responses to chemotherapy, targeted agents, or immunotherapies *in vitro*. Zou *et al.* established an organoid microarray system for the co-culture of HCC-PDOs and Peripheral Blood Mononuclear Cells (PBMC) with Mesenchymal Stem Cells (MSC) and Cancer-Associated Fibroblasts (CAF) to mimic the tumor microenvironment and predict the response of hepatocellular carcinoma patients to PD-L1 immunotherapy.[81] This organotypic co-culture platform provides an innovative and intuitive research tool for resolving the phenotypic features and dynamic behaviors of tumor-like organs when they interact with immune cells. While PDOs-on-a-chip technology primarily focuses on cultivating single tumor-like organs, it can partially reflect tumor tissue characteristics, but struggles to capture the dynamic communication between tumors, the immune system, and distant organs. This limits its assessment of systemic tumor effects and overall drug efficacy or toxicity.

F. Other MOOC models

Drug-induced liver injury (DILI) has been a vexing problem in the field of drug development and clinical therapy.[82] Liver–other organ-on-a-chip plays a key role in testing drug toxicity. For example, in 2020, Baert *et al.* used a MOOC to study the reproductive toxicity of cyclophosphamide.[83] They cultured primary adult testicular cells alongside hepatic spheroids, adding cyclophosphamide to the culture medium. The results showed that cyclophosphamide caused the upregulation of specific cytochromes in the liver spheroids and loss of germ cells, effects not observed in single testicular chip cultures.

The lung–liver-on-a-chip can be used to simulate and study the interaction between lung and liver diseases, such as the effects of chronic obstructive pulmonary disease (COPD) on the liver or the effects of liver cirrhosis on lung function.[84] Moreover, lung–liver-on-a-chip also can be used to study the distribution and toxicity of environmental contaminants between the lung and liver. By simulating the inhalation and metabolism of these substances, researchers can better understand their potential health risks. In 2020, Miller developed a multi-organ micro-physiological system comprising lung, liver, and breast cancer modules to simulate the effects of curcumin administered via inhalation therapy and intravenous injection, comparing the differences in effectiveness and potential toxicity between inhalation delivery and intravenous delivery of drugs.[85]

IV. LIMITATIONS AND CHALLENGES FOR MOOCs

Although current MOOC technology has great potential for applications, a few challenges remain in meeting the unique needs of normal physiological functions. These challenges include the variability in culture environments and conditions, the lack of vascular and neural networks, the complexity of connection methods, and compatibility of biomaterials. If these challenges are not adequately addressed, the broad application and further development of MOOC technology may be limited.

A. Variability of culture environment and culture conditions on MOOCs

The core of MOOC technology is its ability to simulate complex physiological environments *in vitro,* which requires highly precise culture environments and conditions, as each type of organ tissue has distinct requirements regarding temperature, oxygen concentration, pH, and nutrient supply. Meeting the diverse needs of different organs in terms of culture conditions is challenging. For example, endothelial cells proliferate at 37 °C, whereas renal podocytes proliferate at 33 °C and differentiate at 37 °C.[86] Cardiomyocytes are highly oxygen-sensitive and rely on sufficient oxygen supply to maintain normal function. Hypoxic conditions can lead to ischemic injury in cardiomyocytes, potentially resulting in serious issues such as myocardial infarction. In contrast, anaerobic microbes are essential for maintaining gut barrier function, preventing pathogen invasion, and promoting nutrient absorption.[87] Additionally, cells respond differently to fluid shear stress (FSS); either excessive or insufficient shear force can disrupt their normal functions. For instance, moderate FSS helps maintain vascular health in endothelial cells, while in tubular epithelial cells, FSS affects AQP2 positioning and function by altering cytoskeletal dynamics.[88] In constructing a MOOC platform, precise regulation of cell density and ratio across different chambers is essential. High podocyte density can impair their differentiation and functionality.[86] Within the tumor microenvironment, the proportions of tumor cells, cancer-associated fibroblasts, and macrophages significantly influence tumor growth, invasion, and immune response. To ensure the physiological relevance of *in vitro* models, these cellular ratios should be accurately replicated. In long-term cultured models, the stability of the model needs to be maintained by replenishing or removing specific cell types at the right time based on changes in cell growth and proportions that are monitored in real time.

To address these issues, researchers have developed perfusion systems and real-time monitoring systems,[89–91] but some limitations remain. For example, existing perfusion systems cannot

deliver uniform FSS across all organ modules, resulting in inconsistent cellular environments across regions. The high cost of precise sensors and monitoring equipment limits their broad application, potentially hindering the physiological functionality of some MOOCs. Using machine learning and artificial intelligence (AI) technology, creating adaptive control systems to automatically adjust culture conditions according to real-time monitoring data, achieving more uniform fluid distribution and shear force regulation, may be a solution.

B. Lack of vascular and neural networks on MOOCs

The vascular network in the human body is highly intricate, encompassing both large blood vessels and capillaries that facilitate nutrient, oxygen, and waste exchange between organs. Indeed, vascular requirements vary greatly among different organs on MOOC. For instance, the liver requires high vascularization to support its metabolic functions, while the heart needs blood vessels capable of withstanding high shear forces. Current technology is not yet able to accommodate these diverse vascular needs on a single chip. Some reports have described chips with vascular distributions, but these typically simulate the vascular environment of only a single organ or simple tissue.[92–94] Replicating the complex vascular networks that link different organs remains challenging.

Furthermore, vascular and neural networks are closely integrated in the body, jointly regulating organ functions. While some studies have begun attempting to integrate vascular and neural networks by introducing microvascular systems or biomimetic neural networks, these technologies are still in developmental stages and require further advancement. Without such integration, it is difficult to fully replicate organ function, and achieving functional interaction between these networks on MOOCs remains a significant challenge.

C. Standardization of MOOCs

The standardization of organ chips is one of the main obstacles to the wide application of MOOC technology. At present, the MOOC system developed by different research institutions and companies varies greatly in the interface design, material preparation, operation process, and other aspects.[95] This difference makes it difficult to compare and reproduce data between different laboratories, limiting the generalizability and credibility of outcomes. (1) Flow parameters: Establishing a unified design standard for chips, such as organ arrangement order, fluid channel dimensions, and culture medium flow rates, is essential to improve consistency and comparability of experimental results. (2) Chip materials: PDMS, polymers, and hydrogels perform differently across the batch effect. To ensure the consistency of MOOCs under different experimental conditions, the selection and use of standardized materials is also crucial, and the biological compatibility of materials needs to be considered together. (3) Operation process: the standardization of the

TABLE II. Comparison of different commercialized OOC/MOOC companies.

Commercialized companies	Core product	Technical characteristics	Advantages and limitations	Reference
TissUse	HUMIMIC	- Support—four to eight organ interconnection - Micropump-driven fluid circulation	- Highly bionic and suitable for multi-organ interactions studies - High cost and operational complexity	96 and 97
Emulate	Organ chip	- Integrated mechanical stress control (e.g., lung respiration simulation) - Supporting imaging analysis	- Highly standardized, good data reproducibility - Single-organ model-based, limited scalability	98–102
Mimetas	OrganoPlate	- Pumpless gravity-driven fluids - Support for co-culture and vascularization models	- High throughput (40–96 chips/plate), compatible with lab equipment - Lower precision fluid control	21,103, and 104
CN Bio	PhysioMimix	- Perfusion 3D culture - Support for primary cells and organoids - Integrated metabolic analysis module	- Closer to the metabolic functions of human physiology - Larger device, requires specialized training	105 and 106
InSphero	Akura	- Automated liquid handling - Focused tumor/hepatotoxicity testing	- High throughput, suitable for industrialized screening - Less physiological complexity	107–109
React4Life	MIVO	- Support eight organ interconnection - 3D culture (organoids, biopsies) with circulating immune cells - Unique dynamic shear stress control algorithm	- Suitable for oncology/Immune oncology with controlled flow of immune cells - High cost and limited throughput	78 and 79

TABLE III. Comparison of commercialized companies and "in-house" designed chips.

	Commercialized OOC/ MOOC companies	"In-house" designed chips
Standardization	✓	✗
Low cost	✗	✓
Flexibility	✗	✓
Technical complexity	✓	✗
Throughput	✓	✗

experimental operation process is an important link to ensure the consistency of the MOOC technology between different laboratories, namely, the density of cells and the frequency of the medium replacement. Furthermore, the standardization of MOOC technology also involves regulation and quality control. Regulatory authorities such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) may require these standards to ensure that the use of MOOCs is consistent with safety and efficacy requirements. Developing a globally unified regulatory framework and quality control standards will facilitate the standardized fabrication and application.

D. Commercialized MOOC platform

Some commercialized OOC/MOOC companies, such as Emulate, Mimetas, and TissUse, have carved out their own niches in terms of technical features, application areas, and market performance (Table II). Emulate, a world leader in organ-on-a-chip technology development, traces its origins back to the Wyss Institute at Harvard University.[9] Mimetas' OrganoPlate series leverages its unique PhaseGuide technology to enable precise cell distribution without the need for porous membranes.[104] TissUse's HUMIMIC organoid tandem microarray culture system stands out as a microfluidic micro-physiology system platform.[21] It is capable of maintaining and culturing microscale organoid

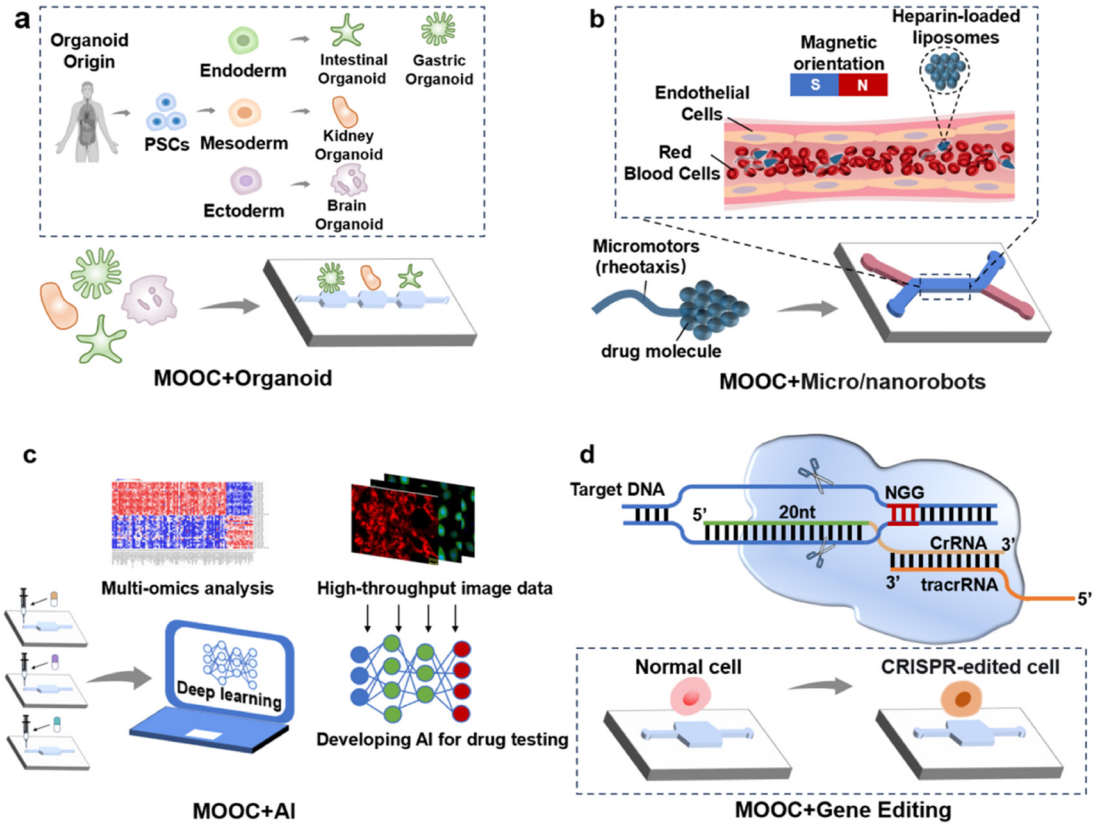

FIG. 8. Future development direction of MOOCs. (a) Combination of organoids and MOOCs. Organ-like cells derived from stem cells or pluripotent stem cells are combined with organ chips to form organoid-on-a-chip. (b) Combination of nanorobots and MOOCs. Precise control of the drug release location and dosage within the organ chip, thus simulating the drug delivery process in the body. (c) Combination of AI and MOOCs. AI handles a large amount of biological data generated by MOOCs. (d) Combination of gene editing and MOOCs. Cells or organoids that have been genetically edited can be implanted and cultured on MOOCs, and these edited cells are able to interact with other types of cells or tissues within the chip.

equivalents and has been recommended by the FDA to provide tandem culture solutions for different organoids. Although commercial OOC/MOOC platforms excel in standardization and reproducibility, in-house designed chips offer unique and irreplaceable benefits in terms of research flexibility, cost-effectiveness, and customized microenvironment simulation, particularly for cutting-edge exploratory studies or research teams with limited budgets (Table III).

V. FUTURE DEVELOPMENT DIRECTIONS

As an emerging technology, MOOC is rapidly developing and gradually being applied in the fields of drug screening, toxicity testing, and disease research. With technological progress, the future development of organ chips will likely involve integration with other cutting-edge technologies, such as organoids, artificial intelligence, nanorobots, gene editing, and more.[110–112] This cross-domain fusion will significantly increase the complexity, functionality, and application breadth of organ chips, enabling them to play a greater role in drug discovery, disease research, personalized medicine, and other areas.

Technology Optimizations: Future research will focus on developing improved culture systems, integrating vascular and neural networks, and establishing uniform standards to address challenges currently faced by organ chips in controlling culture environments, lacking vascular and neural networks, and standardization. These developments will promote the standardized growth of organ chip technology, facilitating its global application.

Application Scene Expansion: (1) Disease research. By simulating inter-organ interactions, MOOCs support the study of dynamic inter-organ interactions and metabolic processes, helping to deepen the understanding of disease mechanisms. (2) Drug development. MOOC allows for the assessment of drug efficacy and toxicity, reducing the reliance on animal testing and enhancing the efficiency of drug development.

Technology fusion: (1) Integration with organoids. As shown in Fig. 8(a), building more complex 3D tissue models, such as combining tumor organoids with chips, enables precise simulation of the tumor microenvironment, aiding personalized treatment and supporting research in developmental biology and regenerative medicine. This integration could provide new insights for reproductive health and organ transplantation. (2) Integration with Nanorobots: As illustrated in Fig. 8(b), this combination allows for precise drug release control on MOOC, studying drug distribution and metabolism, and optimizing dosing strategies. Nanorobots could also manipulate cellular and tissue structures while carrying sensors to monitor internal environmental parameters of organ chips in real time, such as pH, oxygen concentration, and metabolite levels, to support experimental adjustments. (3) Integration with Artificial Intelligence (AI). As shown in Fig. 8(c), AI processing enables the analysis of large volumes of biological data generated by MOOC systems (including multi-omics and imaging data) to extract biological patterns and drug response models, build predictive models, and dynamically adjust experimental conditions. (4) Integration with gene editing. As shown in Fig. 8(d), gene editing on MOOC can be used to study gene–environment interactions. By controlling microenvironmental conditions (e.g., oxygen levels, nutrient supply, mechanical stress), researchers can observe specific gene behaviors under various conditions and their relationship to disease progression, which helps understand how environmental factors influence health and disease through gene regulation.

In summary, MOOCs are rapidly developing as an advanced *in vitro* modeling technology and are being increasingly applied in drug screening, toxicity testing, and disease research. With continuous technological advances, the future development of MOOCs will integrate closely with other frontier technologies such as organoids, nanorobots, AI, and gene editing, greatly expanding the application potential of MOOC technology.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Xu Gao: Data curation (equal); Visualization (lead); Writing – original draft (lead); Writing – review & editing (equal). **Ting Wang:** Data curation (equal); Investigation (equal); Visualization (lead); Writing – original draft (lead). **Wanqiu Huang:** Data curation (equal); Investigation (equal); Supervision (equal). **Chenyi Liu:** Data curation (equal); Investigation (equal); Supervision (equal). **Zhaoqi Zhang:** Supervision (equal). **Yuliang Deng:** Writing – review & editing (equal). **Jian Huang:** Data curation (equal); Investigation (equal); Writing – review & editing (equal).

DATA AVAILABILITY

This review article does not include any datasets. All figures presented were originally created by the authors to illustrate concepts and mechanisms discussed in the text.

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