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From organoids to organoids-on-a-chip: Current applications and challenges in biomedical research

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Abstract

The high failure rates in clinical drug development based on animal models highlight the urgent need for more representative human models in biomedical research. In response to this demand, organoids and organ chips were integrated for greater physiological relevance and dynamic, controlled experimental conditions. This innovative platform—the organoids-on-a-chip technology—shows great promise in disease modeling, drug discovery, and personalized medicine, attracting interest from researchers, clinicians, regulatory authorities, and industry stakeholders. This review traces the evolution from organoids to organoids-on-a-chip, driven by the necessity for advanced biological models. We summarize the applications of organoids-on-a-chip in simulating physiological and pathological phenotypes and therapeutic evaluation of this technology. This section highlights how integrating technologies from organ chips, such as microfluidic systems, mechanical stimulation, and sensor integration, optimizes organoid cell types, spatial structure, and physiological functions, thereby expanding their biomedical applications. We conclude by addressing the current challenges in the development of organoids-on-a-chip and offering insights into the prospects. The advancement of organoids-on-a-chip is poised to enhance fidelity, standardization, and scalability. Furthermore, the integration of cutting-edge technologies and interdisciplinary collaborations will be crucial for the progression of organoids-on-a-chip technology.

Keywords: Organoids-on-a-chip; Organoids; Organ-on-a-chip; Drug testing; Disease modeling

Introduction

The pharmaceutical research and development sector urgently requires optimal preclinical screening models to effectively assess drug efficacy and side effects and minimize the risk of clinical trial failures. Although animal models can provide an *in vivo* environment, they exhibit species differences from humans and cannot adequately mimic the unique characteristics of the human body, leading to ethical controversies.[1–3] The advent of stem cell technology greatly facilitated the construction of human-derived models, with their self-renewal and differentiation potential providing excellent cellular seeds for the creation of organ/tissue models. Organoids are three-dimensional (3D) models that recapitulate the physiological functions and tissue organization of native tissues *in vitro* based on the self-assembly properties of stem, progenitor, or differentiated cells.[4,5] Compared to two-dimensional (2D) cell models, organoids contain a wider variety of cell types found within organs, offering several advantages for studying spatially organized structures and cell-cell interactions. Despite the immense potential of organoids in simulating physiology/pathology, personalized medicine, drug screening, and regenerative medicine, some limitations, such as high heterogeneity, low maturity, and uncontrollable conditions, greatly limit their widespread application in translational research.[3,6] Organs-on-a-chip technology is a paradigm that allows for precise control of cell culture parameters.[7,8] Recently, interest in combining organoids with organ-on-a-chip technology to create organoids-on-a-chip systems has been steadily growing. Engineered or microfabricated systems from

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organ-on-a-chip technology are utilized to culture organoids or induce stem cell differentiation into specific organoids within chambers.[3,6,9]

In this review, we first address the current challenges faced by both organoids and organoids-on-a-chip technologies, necessitating the development of organoids-on-a-chip systems. We then explore the applications of organoids-on-a-chip, particularly in simulating organ physiology, modeling disease pathology, and evaluating therapeutic methods. Details on design concepts, fabrication techniques, and representative organ/tissue types in organoids-on-a-chip have been extensively covered in the literature.[3,6,7,10] Our review emphasizes the rational selection of models for research needs, examines current challenges impeding development, and provides insights and strategies to overcome these barriers.

From Organoids to Organoids-on-a-chip

Organoids

Organoids typically arise from human pluripotent or adult stem cells that undergo a process of expansion, followed by *in vivo*-like differentiation and morphogenesis to emulate the cytoarchitecture and functionality of specific regions of organs. In some cases, differentiated cells or primary cancer cells with a certain level of cellular plasticity and self-organization could also form organoids.[11,12] The pioneering report on organoids described human adult stem cell-derived gut organoids featuring crypt-villus structures similar to the epithelium of native intestinal tissues.[13] Subsequently, the field of organoids quickly grew to encompass models of various organ tissues, including the retina,[14] brain,[15–17] liver,[18] lung,[19] kidney,[20–22] pancreas,[23] and heart [Table 1].[24]

The emergence of human organoids presents a model for investigating human-specific physiological processes that are not amenable to being replicated in largely inbred animal models.[25] For example, cortical organoids are capable of generating outer radial glia, which primarily undergo self-renewal and give rise to neurons in the outer subventricular zone.[26,27] This process is crucial for sustaining the expansion and folding of the human neocortex, a feature notably absent or rare in rodent models.[28] Given the differences in metabolism between humans and rodent animals with different growth rates, the distinct mechanisms by which drugs are metabolized in the liver can directly impact the outcomes of preclinical drug testing.[29] Ibuprofen and warfarin, which are toxic in rat models, are effective anti-inflammatory and anticoagulant drugs in humans, respectively.[30,31] Hence, human hepatocyte organoids are being increasingly applied in the study of drug metabolism and hepatotoxicity.[32–34]

Three-dimensional organoids outperform 2D cell models in replicating gene and protein expression, metabolic function, and microscale tissue architecture of primary human organs.[5] Organoids can simulate intricate phenotypes of organs with remarkable detail: Kidney organoids can resemble multiple segments of the nephron[21,22]; heart organoids can recapitulate chamber-like morphogenesis[24]; intestinal organoids can produce mucus[35]; and lacrimal gland organoids can produce a tear-like fluid in response to neurotransmitters.[36] Furthermore, organoids hold great promise in regenerative medicine due to their capacity to preserve organ microstructure and function, offering substantial potential for tissue replacement and repair in areas such as the biliary ducts, retina, and colon.[37–39]

Despite organoids offering a powerful platform for studying developmental biology, disease modeling, regenerative medicine, and drug discovery, several limitations still need to be overcome. The maturation level of organoids is not comparable to that of organs or tissues *in vivo*. As organoids grow, the central areas often undergo necrosis due to insufficient nutrient delivery, limiting their long-term culture viability. Organoids derived from human pluripotent stem cells typically fail to mature beyond a fetal developmental stage, making them less suitable for investigating aging-related changes or diseases. In addition, organoids cannot fully reproduce the functional characteristics of their corresponding organs, largely due to impaired cell-type fidelity. Factors such as *in vitro* culture stress, lack of vascularization, and the absence of an immune system may contribute to the limited diversity of specialized cell types in organoids.[40,41] Moreover, an uncontrolled culture environment and undefined animal-derived 3D matrix, along with the inherent stochasticity of *in vitro* self-organization and cell fate decisions, often result in substantial variability of organoid formation efficiency, morphology, and function. Current organoid culture systems also lack automated functional monitoring methods, hindering the timely observation of changes in metabolites, secreted substances, and electrical potentials [Table 1].[5]

Integrating organoids with organ-on-a-chip technology

Organ-on-a-chip, also referred to as a microphysiological system, is a microfabricated cultivation system designed to simulate the key functional units of human organs *in vitro*. This biomimetic system often employs perfused microfluidic channels lined with living cells. This helps replicate organ-level pathophysiology by controlling fluid flow and biophysical and chemical factors, as well as cell-cell or cell-matrix interactions.[2,8,42] Typically, organ-on-a-chip technology integrates several cell types, which are captured and anchored using a microporous membrane or pillar array, facilitating efficient nutrient and oxygen transport. Tissue fixation allows for precise control over the geometric arrangement of cell alignment, enabling multiaxial stretching to mimic the complex mechanical environment of the body. Organ-on-a-chip systems operate in conjunction with external devices, such as pumps, incubators, sensors, and microscopes, which are essential for maintaining, stimulating, and monitoring the cells within the organ-on-a-chip systems.[8] In addition, organ-on-a-chip systems allow for compartmentalization, where different cell types can thrive in their respective optimized microenvironments.[3] More details about the manufacturing process and organ types of organ-on-a-chip are available in some comprehensive reviews.[7,8,43]

Table 1: Overview of representative human organoids.

Organ types	Available cell types	Characteristics/function	Limitations
Brain	Neural stem/progenitor cells, radial glia, neurons, astrocytes, and oligodendrocytes[71,135,136]	Modeling development of specific brain regions, cortical layering, early neurogenesis, synapse formation, and spontaneous electrical activity	Size limit due to oxygen and nutrient diffusion constraints; lack of microvascular differentiation; absence of microglial cells; can not replicate complex neural connections and projection; lack of higher-order neural network functions
Spinal cord	Motor neurons, interneurons, progenitor cells, astrocytes, and oligodendrocytes[137–139]	Modeling the early development of the neural tube, axon growth, neurotransmission of sensory inputs and motor outputs	Limited maturation of spinal structures; incomplete myelination; lack of peripheral tissue integration; absence of immune response
Retina	Photoreceptors, bipolar, horizontal, amacrine, ganglion cells, and Müller cells[140–142]	Modeling simulating light signal processing	Lack of vascularization; limited optic nerve formation and incomplete retinal structure; lack a specific cone cell-rich region similar to the human central fovea
Heart	Cardiomyocytes, cardiac fibroblasts, endothelial cells, epicardial cells, and endocardial cells[143–145]	Modeling functional characteristics such as contractility, cavity formation, action potential propagation, and forming vascular-like structures	Incomplete heart chamber formation; limited electrical activity; insufficient integration with vasculature and lack of true perfusion through blood vessels
Liver	Hepatocytes, cholangiocytes, Kupffer cells, hepatic stellate cells, and liver sinusoidal endothelial cells[146–148]	Modeling the production of albumin and the secretion of bile acids and accumulating glycogen and producing albumin and bile acids	Limited bile duct formation and immune interactions; lack of full functional vascular network; lack of full metabolic complexity
Kidney	Multiple lineages such as nephron progenitors, ureteric buds, stromal progenitors, and vascular endothelial cells[21,73,149]	Modeling key renal functions such as glomerular filtration and tubular reabsorption	Lack of full functional vascular network and filtration systems; lack of distal nephrons (distal renal tubules and loops of Henle); insufficient maturation of the collecting ducts; limited nephron complexity
Lung	Basal cells, club cells, alveolar type 2 epithelial cells[150–152]	Replicating the functional capabilities of basal cells in self-renewal and differentiation, club cells in mucin production for airway protection, and alveolar type 2 epithelial cells in differentiating into AEC1s for gas exchange	Lack of terminal cellular maturity, particularly mature alveolar lineages, and progressive branching formation; absence of vascularization and immune cells
Pancreas	Islet cells, ductal cells, and acinar cells, which can differentiate into various pancreatic cell types, including β-cells, under specific culture conditions[153–155]	Replicating insulin secretion and endocrine function	Lack of islet maturation; low differentiation efficiency and long-term instability; lack of a complete vascular system and interactions with other pancreatic cell types and non-pancreatic tissues
Intestine	Intestinal stem cells, epithelial cells, including absorptive enterocytes and secretory cells like goblet, Paneth, and enteroendocrine cells[156–158]	Modeling the natural polarity and functionality of the intestinal epithelium	Lack of the full complement of the *in vivo* intestinal environment, including a complete immune cell community, neural cells, and microbiota
Blood vessels	Endothelial cells, pericytes, and vascular smooth muscle cells[159–161]	Modeling angiogenesis, vascular maturation, and the interaction between endothelial cells and pericytes; blood vessels can be formed within 2–3 weeks	Limited vessel maturation and connection structures; lack of perfusable capillary networks; insufficient integration with other tissues or organoids for perfusion

AEC1s: alveolar epithelial type 1 cells.

Although microfluidic and tissue engineering technologies have been established, organ-on-a-chip technology advanced in parallel with organoids. The first landmark study in this field was the lung-on-a-chip, which successfully replicated an alveolar-capillary barrier that mimics breathing motion.[13,44–46] To date, single-organ systems replicating the specific functions and structures of organs, such as the liver,[47] kidney,[48] gut,[49] heart,[50,51] bone,[52] and vasculature[53] have been successfully developed. In addition, multi-organ systems that integrate multiple tissues on a single chip facilitate the exchange of metabolites, secreted factors, extracellular vesicles, and circulating cells.[7]

Despite its name, organ-on-a-chip does not yet fully capture the concept of miniaturized organs cultured on a chip. Challenges such as complex experimental setups, a lack of intricate cellular architecture, and lower physiological relevance compared to organoids limit the broader application of organ-on-a-chip technology.[5] Organoids-on-a-chip have recently emerged as a synthesis of developmental biology, engineering, and physics. These systems aim to merge the strengths of organoids and organ-on-a-chip technology, thus addressing their respective limitations and achieving a higher level of complexity in modeling human organs *in vitro* [Figure 1 and Table 2].[3,54]

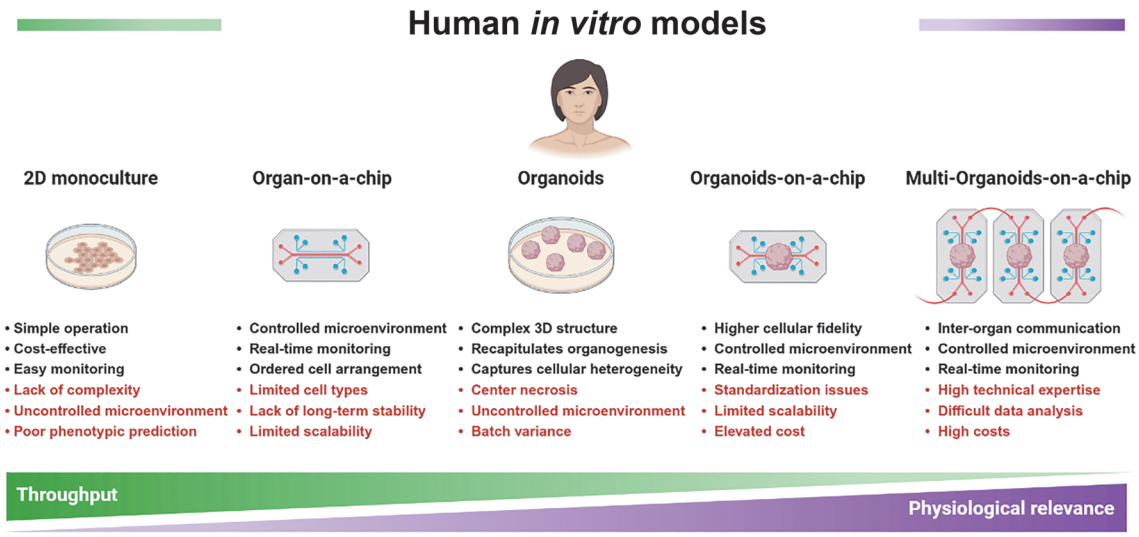

Figure 1: Comparative summary of advantages and disadvantages of human *in vitro* models.[3,6,7,10,55] Created in *BioRender.com*.

Application

Physiological simulation

Having incorporated the design philosophy of organ-on-a-chip technology, organoid culture systems are now endowed with a more physiologically relevant tissue microenvironment. This enhancement significantly boosts the physiological fidelity and functional intricacy of organoids, positioning them as superior equivalents for *in vivo* organs or tissues. To obtain a substantial quantity of more homogeneous organoids, micropillar array chips are employed during the initial stages of their differentiation to ensure the uniform size of embryoid bodies.[56–58] Organoids-on-a-chip with integrated microfluidic perfusion networks circumvent the problem of uncontrollable fluid shear stress encountered in traditional bioreactors, ensuring continuous and controlled nutrient and oxygen delivery and waste removal in defined chambers.[6] Thus, they replicate physiological organ flow environments and enhance differentiation and maturation consistency of organ-characteristic cell types.[6] For instance, culturing kidney organoids under controlled fluid shear stress within a 3D-printed millifluidic chip fosters the development of more mature podocyte and tubular structures, effectively mimicking the early stages of kidney development *in vivo*.[59] Furthermore, perfused cultures exhibited a greater variety of mature neuronal subtypes and more distinct hierarchical structures of the cerebral cortex in brain organoids.[60–62] Continuous fluid perfusion enabled intestinal organoids within the chip to generate the primary subtypes of gut epithelial cells, including Paneth cells, goblet cells, enterocytes, and enteroendocrine cells, all exhibiting cellular polarization. This polarized state is crucial for accurately mimicking the physiological functions of the intestinal epithelium and its response to external stimuli.[63,64]

Microfabricated compartments and microfluidic channels enable the dynamic regulation of biochemical and physical cues, such as morphogens, cytokines, mechanical forces, and electrical signals, creating a microenvironment conducive to organ development. Microfluidic chamber systems containing a high-concentration area (source) of chemical input diffusing toward a low-concentration area (sink) can generate chemical concentration gradients in microfluidic chips, which can be applied to establish the body axis during development.[6] Body axis formation is crucial for properly organizing and patterning developing organs in the human body, ensuring the correct spatial arrangement of tissues and structures during embryonic development.[65] Based on this, researchers have created concentration gradients of signaling molecules such as Wingless/int1 (WNT), fibroblast growth factors (FGF), and retinoic acid (RA) in microfluidic chips, which can simulate the internal signaling environment of neural tube development, enabling the regional patterning of the neural tube along the rostral-caudal and dorsal-ventral axis.[66,67] Furthermore, the development and maturation of organ structures and functions in organoids often require specific external conditions, including spatial constraints, mechanical stretching and contraction, fluid shear, stiffness, and topology.[68,69] For example, the human cerebral cortex enlarges and wrinkles to form gyri and sulci during development, which involves complex mechanisms, including neural stem cell proliferation, physical factors of mechanical instability, and cytoskeletal contraction.[70] Confining human pluripotent stem cell-derived cerebral organoids in microcompartments enables real-time observation of surface wrinkle formation, uncovering brain development physics absent in traditional *in vitro* organoid models lacking gyrus-like structures.[71] The process of food digestion and mixing in the stomach, along with the release and absorption of medications, is accompanied by rhythmic intraluminal flows and peristaltic movements. In the gastric organoids-on-a-chip model, a peristaltic pump generated pressure fluctuations by periodically compressing and relaxing the tubing, which was then transmitted to the microfluidic chip's chambers culturing human gastric organoids, simulating the peristaltic movements and physiological luminal flow of the stomach.[72]

Table 2: Overview of representative human organoids-on-a-chip.

Organ types	Cell sources	ECM	Chip design	Applications
Brain	hESCs[71]	Matrigel	A two-dimensional compartment constructed with an upper membrane connected to a media reservoir and a lower coverslip forming its base	Modeling the physics of the folding brain
	hiPSCs[56,57,62,84,85,162]	Matrigel	A high throughput micropillar array chip[57,85]	Modeling biological events during early brain development *in vitro*
			A perfusable chip system contained parallel multichannels[56,62]	
			Microfluidic fabricated hollow alginate fiber[84,162]	Covering the impact of prenatal exposure to environmental factors
Spinal cord	hESCs[163]	Laminin	A resin chip comprises a 3D-printed organoid holder and a polycarbonate (PC) membrane	Exploring nociceptive circuits for the development of pain therapeutics
Retina	hESCs[164]	Matrigel	A micro-millifluidic bioreactor is designed to be free from shear stress, comprising a series of linear, single-sided chambers alongside serpentine, alternating side chambers that enhance the mixing and distribution of cellular components within the system[164]	Exploring the impact of drug candidates on retinal health and validating the efficacy of AAV vectors for gene delivery in retinal treatments
	hiPSCs[98,165]	Matrigel	A chip contains an upper structure with four distinct tissue compartments interconnected through a microchannel, while the bottom is equipped with a perfused channel that is partitioned by a thin, porous membrane, facilitating the exchange of nutrients and waste products between the compartments[98,165]	
Liver	hiPSCs[57,58,166]	Nore, fibrinogen, and chitosan	A perfusable micropillar chip system[57,58]	Modeling human nonalcoholic fatty liver disease (NAFLD)
			A perfusable chip system with C-trap architecture[167]	
			All-aqueous droplet microfluidic device[166]	
Kidney	hPSCs[59,168]	Gelatin, fibrin, and Matrigel	A perfusable millifluidic chip by 3D bioprinting	Modeling the processes of glomerular vascular development and the morphogenesis of kidney organoids, investigate mechanisms of kidney diseases
	hASCs[169]	Collagen I	A three-lane OrganoPlate platform with parallel chips	Simulate BK virus infection and hereditary kidney diseases in a personalized manner
Lung	Tumor tissue[170]	Matrigel	A microwell array chip	Integrating *in situ* cryopreservation technology to preserve organoid viability for the drug sensitivity testing
	Tumor-free tissue[171]	Collagen I	A sandwiched chip with PET membrane	Development of more representative human preclinical models of the (diseased) alveolar compartment
Intestine	hiPSCs[63]	Matrigel	A microengineered multilayered device that incorporates a permeable membrane and supports the cultivation of epithelial cells harvested from intestinal organoids under dynamic flow conditions	Studying intestinal epithelial responses to external stimuli, such as cytokines
	hASCs[88,172,173]	Collagen I and Matrigel	A multilayer chip with a porous membrane enables cyclic deformation and supports the culture of epithelial cells within a microfluidic system[172]	Mimicking the human duodenum *in vivo*, enabling the study of intestinal function such as nutrient digestion, mucus secretion, and barrier integrity
			A microchip system equipped with crypt-like microcavities and a hydrogel scaffold supports the generation of epithelial organoids and the perfusable design allows the continuous removal of cellular debris[88]	Simulating *in vivo* crypt-villus architecture for host-microbe interaction studies
				Studying inflammation by stimulating immune responses with inflammatory cytokines
			A tailored 384-well IFlowPlate with an innovative "open-well" design supports the perfusion and vascularization of colon organoids without requiring external pumps[173]	
Breast	Colorectal liver metastasis tissue[108]	Fibrinogen and Matrigel	A multiplexed platform contains AngioTube scaffolds for vascular network formation and is integrated with a 96-well base plate for high-throughput analysis	Simulating of physiological drug delivery to tumors through a vascular network, providing a platform for investigating therapeutic responses in a clinically relevant timeframe
Pancreas/ islet	hiPSCs[174]	3D alginate and hydrogel	A perfusable multilayer chip comprises a top microwell array, a polycarbonate porous membrane, and a bottom PDMS layer	Recapitulating the key cellular composition and functions of islet, such as more sensitive glucose-stimulated insulin secretion and higher Ca^{2+} flux
	Pancreatic ductal adenocarcinoma cancer tissues[105]	Matrigel	A two-layer chamber chip, which can be reversibly clamped, features a 200-well array and an upper layer consisting of fluidic channels	Automated dynamic and combinatorial drug screening
Stomach	hPSCs[72]	Matrigel	A central compartment designed for organoid cultivation, accompanied by two adjacent chambers for media storage and exchange	Replicating the peristaltic movements in a manner that closely mimics *in vivo* conditions

3D: three-dimensional; AAV: adeno-associated virus; BK virus: human polyomavirus 1; ECM: Extracellular matrix; hESCs: human embryonic stem cells; hiPSCs: human induced pluripotent stem cells; hASCs: human adipose stem cells; PDMS: Polydimethylsiloxane; PET: polyethylene terephthalate.

Integrating organoids with organ-on-a-chip technology enables researchers to tackle critical challenges in both fields, improving modeling precision and reliability. Vascularization of organoids has been significantly advanced through strategies such as co-culture, which integrates endothelial cells with organ-specific progenitors; co-differentiation that directs pluripotent stem cells toward vascularized organoid formation; and organoid assembly, where preformed vascular and organ spheroids or organoids are fused.[18,73,74] Microfluidic technology from organ-on-a-chip enables the precise control of cellular microenvironments for the study of the intricate interactions between different organoids and their associated vascular networks under controlled conditions. The design flexibility of microfluidic devices makes them ideal for modeling complex physiological scenarios, including the crosstalk between various organoid types and their vasculature, thereby enhancing their physiological relevance.[75] There are three primary vascularization strategies: the top-down endothelial cell lining method, wherein endothelial cells are cultured to line microchannel walls; the bottom-up self-assembly method mimicking natural vascular development; and a hybrid approach combining both methods.[76] For example, a microfluidic platform leverages the precision of microfluidic technology to vascularize various types of organoids, including blood vessel organoids and pancreatic islet spheroids. The platform offers advantages such as controlled perfusion and the ability to simulate physiological flows, leading to enhanced growth, maturation, and functionality of the vascularized organoids.[77,78] In addition, bioprinting methods can be used to seed various vascular cells within channels, subsequently creating perfusable vascular structures for organoid culture. Three-dimensional printing technology offers customizable and intricate vascular network designs for organoid vascularization, thereby enhancing experimental precision and replication.[6] The engineering of human brain organoids with a functional vascular-like system using 3D-printed microfluidic chips has further underscored the potential for integrating neurovascular structures within organoids. This integration leads to the expression of blood-brain barrier markers, the formation of vascular networks that penetrate and interact with neural tissues, and the establishment of a neurovascular unit that mimics the *in vivo* brain microenvironment.[79]

To effectively replicate the *in vivo* microenvironment and preserve tissue structural integrity, extracellular matrix materials are vital in integrating organoids with organ-on-a-chip systems. As a multifunctional support system, the extracellular matrix offers a physical scaffold for cell attachment and growth, promoting the organization of cells into 3D structures that mimic native tissue architecture. Such 3D structures are crucial for simulating the physiological signals experienced by cells *in vivo*, thereby enhancing the functional maturity and differentiation capacity of organoids.[3] For instance, the microchip platform integrated with hydrogels directs the self-organization of stem cells into structures that closely replicate the crypt-villus architecture of the native intestine in intestinal organoids. This advancement facilitates the development of mini-intestines with enhanced physiological relevance, supporting complex biological processes such as cell turnover and tissue regeneration. Furthermore, by tailoring properties such as stiffness, porosity, degradation rate, and biocompatibility, hydrogels can be precisely engineered to support stem cell expansion, guide differentiation, and facilitate organoid formation, thus enabling greater control over developmental outcomes.[80,81]

Apart from single-organoid models, multi-organ systems containing assembloid and multi-organoids-on-a-chip platforms have also been successfully developed to simulate physiological phenotypes of organs. For example, preformed cortical, hippocampal, and thalamic organoids can settle into the microholes of microfluidic chips and fuse to form brain assembloids, maintaining active neural migration and interactions.[82] In addition, multi-organoid-on-chip platforms, such as the liver-islet, liver-heart, and liver-stomach-intestine axes, have been developed to mimic the complex physiological processes and systemic responses to external stimuli.[10]

Disease modeling

Building upon the increasing complexity of physiological phenotypes, engineered organoid-on-a-chip have provided new insights into disease pathogenesis. Precise microfluidic device design allows meticulous control of the organoid culture environment, enhancing the recreation of disease microenvironments and improving the reliability of pathological phenotype reproduction. Integrating organoids with organ-on-a-chip technology increases their *in vitro* survival, prolonging functional phenotypes and expanding the treatment window for drug interventions. This is particularly beneficial for long-term studies of disease progression and treatment effects. Organoids-on-a-chip constructed from patient-specific cells or tissues accommodate individual differences such as genetic diversity, ethnicity, sex, and age, facilitating personalized modeling of pathological features and in-depth exploration of disease mechanisms. In addition, multi-organoid-on-chip systems simulate interactions between various tissues and organs, which is essential for understanding the mechanisms underlying systemic diseases. The following examples of classic disease models will further illustrate these advantages.

Prenatal brain development is a complex and critical period, influenced not only by genetic factors but also highly susceptible to various maternal environmental conditions, such as maternal infections, drug use, radiation exposure, and malnutrition. Such adverse stimuli may increase the risk of birth defects and neurodevelopmental disorders, potentially altering DNA structure and chromatin function, leading to permanent changes in brain structure and function and heightening susceptibility to neurobehavioral disorders after birth.[83] Given the species-specific and structural complexity of the human brain, organoids are considered one of the most representative models that closely mimic brain development, capable of simulating key processes such as neurogenesis, neuronal migration, cortical layering, and neural circuit formation.[26] By integrating a 3D matrix with mechanical fluid dynamics, the brain organoids-on-a-chip system provides a controlled microenvironment for organoid development and differentiation. Moreover, through the

use of micropumps or pressure differentials, the system ensures that soluble drugs or exogenous substances (such as nicotine, alcohol, heavy metals like cadmium, and antiepileptic drugs like valproic acid) interact with the brain organoids at constant rates and concentrations.[62,84–86] These chip systems maintain dynamic culture conditions that mimic *in vivo* blood flow, nutrient exchange, and metabolic waste removal throughout the experiment, creating a more realistic biochemical environment. In addition, microscopy, electrophysiological recordings, and molecular biology methods facilitate real-time monitoring of morphological and functional changes in the brain organoids. These brain organoids-on-a-chip systems allow researchers to observe how exogenous substances disrupt neuronal development and brain organization, offering essential insights for preventing and intervening in fetal neurodevelopmental disorders during pregnancy.

The development of advanced organoids-on-a-chip that mimic a section of the digestive tract offers a unique opportunity to investigate the complex interactions between microbes and the host, which is crucial for understanding the pathogenesis of infections. However, traditional intestinal organoid models have several limitations. The development of intestinal organoids cultured in a 3D matrix is random, typically forming a closed cystic structure without an open lumen. This architecture prevents internal cells from effectively acquiring nutrients and oxygen. Moreover, it restricts the removal of waste, thereby limiting the size and duration of organoid culture, which is highly unfavorable for exploring the impact of exogenous additives such as microbes, viruses, and drugs on the intestinal epithelium.[13,81] Researchers have engineered a microfluidic intestinal organoid chip to overcome previous limitations. The chip features a hydrogel scaffold that guides stem cells to form epithelial tissues with crypt- and villus-like structures. Its microfluidic system ensures nutrient and oxygen delivery and waste removal, supporting organoid longevity and function. In addition, it permits the study of microbe interactions through continuous microbe infusion into the open chambers. *Cryptosporidium parvum* can induce fatal diarrhea in immunocompromised adults and infants, a condition that can prove fatal in its severe form.[87] Following extended co-culture with *Cryptosporidium parvum*, these advanced organoids continuously produced fresh oocysts and elicited an interferon response, making it an ideal platform for mechanistic studies of host-microorganism interactions and long-term infection dynamics.[88]

Mechanosensing pathomechanisms, a crucial aspect of disease modeling, can also be effectively explored using organoids-on-a-chip systems. These models provide a unique platform to study how cells sense and respond to mechanical stimuli within physiologically relevant microenvironments. Autosomal recessive polycystic kidney disease is a severe hepatorenal disorder characterized by enlarged kidneys and progressive loss of renal function, which lacks Food and Drug Administration (FDA)-approved treatments due to the absence of physiologically relevant models.[89,90] A representative study employed a kidney organoids-on-a-chip platform to simulate specific pathological processes in autosomal recessive polycystic kidney disease. This platform includes the dilation of distal nephrons and the formation of vascular networks, thereby inducing clinically relevant cystic phenotypes. The 3D-printed millifluidic chips, when subjected to controlled fluid flow, not only recapitulate the disease's microenvironment but also identify therapeutic targets that were previously elusive in less dynamic models, such as Rac family small GTPase 1 (RAC1) and Finkel-Biskis-Jinkins murine osteosarcoma (FOS).[91] This underscores the model's pivotal role in facilitating clinical translation for drug discovery and therapeutic development.

Organoids-on-a-chip technology extends beyond single-organ disease modeling to effectively study systemic diseases like type 2 diabetes mellitus (T2DM). T2DM is a chronic metabolic disorder characterized by insulin resistance and impaired secretion, which involves complex interactions between the liver and pancreas. A microfluidic multi-organoid-on-chip that replicated the human liver-pancreas axis was developed, allowing co-culture of liver and pancreatic organoids for up to 30 days under continuous perfusion. This model simulated key T2DM features, including insulin secretion under hyperglycemic conditions, glucose metabolism, mitochondrial dysfunction, and glucose transporter expression changes.[92] However, traditional pump-driven organoid chips face challenges on scalability, maintenance, and stability, as well as uneven resource distribution due to high medium-to-cell ratios and risks of protein binding to large internal surfaces. To overcome these limitations, a gravity-driven dual-organoid-on-a-chip platform was developed, utilizing gravity-based flow with specialized reservoirs and perfusion channels for directed recirculating flow, enhancing scalability, flexibility, stability, and ease of use. When free fatty acids and fructose were added, this model successfully reproduced key pathological features of obesity, T2DM, and metabolic dysfunction-associated fatty liver disease, including hepatic steatosis, insulin resistance, pancreatic dysfunction, and chronic inflammation. Thus, this model provides a powerful tool for studying disease mechanisms and evaluating potential therapeutic interventions.[93]

Evaluation of therapeutic approaches

The process of drug development leading up to FDA approval is long and costly. Many drugs have been withdrawn from the market due to unforeseen toxic reactions after being approved and widely used.[94] This is primarily because traditional preclinical models, including 2D cell models and animal models, fail to accurately simulate the complex physiology of the human body. As a result, scientists are striving to develop models that more closely mimic human physiological and pathological states for the accurate assessment of the efficacy and safety of therapeutic interventions. In drug development and clinical applications, integrating pharmacokinetics (PK) and pharmacodynamics (PD) optimizes dosing, predicts drug interactions, and ensures safety and efficacy while enabling flexible adaptation of treatment regimens in personalized medicine using patient-specific PK/PD data.[9,95] In this context, organoids-on-a-chip technology has

emerged as a cutting-edge biomedical research tool that precisely controls biophysical and biochemical factors to simulate the tissue microenvironment within the human body, providing a more realistic experimental platform for studying biochemical reactions of drugs. The uniform culture environment offered by this technology enhances the consistency and reproducibility of drug testing results. Therefore, organoids-on-a-chip technology demonstrates remarkable potential in preclinical high-throughput drug screening and evaluation of the efficacy and safety of treatment methods, providing robust support for drug development and personalized medicine.

In addition to drug testing, organoids-on-a-chip technology has also been utilized to validate the delivery efficacy of gene therapy vectors. Adeno-associated viruses have become the preferred tool for gene therapy research in retinal organoids due to their high transduction efficiency, low immunogenicity, high stability, and long-term gene expression. However, the development of a novel, efficient adeno-associated virus vector is slow and costly, largely due to the lack of suitable preclinical models.[96,97] By transducing various types of adeno-associated virus vectors into retinal organoids cultured on microfluidic chips, researchers can study the dynamic behavior of these vectors within the organoids, including their cellular entry, intracellular distribution, and temporal changes. Furthermore, analyzing the cell types preferentially transduced by adeno-associated virus vectors in retinal organoids provides insights into the specificity of the vectors. This platform also monitors whether adeno-associated virus vector transduction induces cytotoxicity, inflammatory responses, or immune reactions. Through this comprehensive assessment, researchers can identify highly efficient, specific, and safe adeno-associated virus vectors for delivering therapeutic genes, followed by evaluations of whether these genes can ameliorate pathological conditions or restore retinal function in retinal organoids.[98]

Microfluidic chamber designs allow for the separate cultivation of various organoids in distinct regions linked by microchannels or separated by porous membranes. These multi-organoid-on-chip technologies hold remarkable potential for drug development and screening by mimicking the interactions among different organs in the human body. Such systems can provide insights into how drugs are distributed and metabolized throughout the body, as well as any possible systemic side effects. The liver plays a crucial role in metabolism, which is why liver organoids equipped with normal metabolic enzymes are often central to multi-organoid-on-chip models.[99] Several liver-centered multi-organoid-on-chip models have been developed to assess the effectiveness and safety of drugs. In a microfluidic system co-cultivating liver and heart organoids, researchers can monitor the effects of drugs and their metabolites on various organs in real time. For example, the metabolism of capecitabine, an oral chemotherapy drug, can be evaluated in liver organoids along with its associated cardiac toxicity due to its metabolite, 5-fluorouracil (5-FU).[100] Similarly, clomipramine, an antidepressant, is metabolized by liver organoid enzymes into its active form, norclomipramine, which can significantly diminish the viability of co-cultured heart organoid cells, affecting heart function and calcium regulation.[101] Furthermore, a multi-organoid-on-chip platform can facilitate complex interactions, allowing the activity of one organoid to influence the responses of others. This functionality serves as a robust tool for drug development and toxicity assessment. For example, a system that incorporated six types of human tissues, specifically liver, heart, vasculature, lung, testis, and colon (or brain) organoids, was utilized to investigate the toxicity of capecitabine and cyclophosphamide after liver metabolism. The findings revealed that in the presence of liver organoids, both drugs were metabolized into active forms, resulting in toxicity in downstream organoids. In contrast, when liver organoids were absent, these drugs were not metabolized, leading to a lack of observed toxicity in the downstream tissues.[99,102] In addition to the liver, the intestine is also vital for drug metabolism and absorption, particularly with respect to the first-pass effect of oral medications. After ingestion, drugs are absorbed in the intestine before entering the liver through the portal vein. This process can significantly reduce the amount of drug that reaches systemic circulation, thereby impacting its effectiveness. To simulate and evaluate first-pass metabolism, a microfluidic chip utilized differences in height between wells to direct the flow of docetaxel-containing fluid from small intestine organoids to colorectal adenocarcinoma (HT-29) spheroids. The presence of small intestine organoids considerably reduced the cytotoxic effects of docetaxel on HT-29 spheroids, a phenomenon consistent with the drug's low clinical oral bioavailability, suggesting that similar mechanisms in the small intestinal environment may affect drug absorption, metabolism, and distribution, leading to both reduced cytotoxicity and low bioavailability.[103]

Furthermore, incorporating high-throughput and automation concepts into organoids-on-a-chip technology has greatly expanded its potential applications in biomedical research and drug development. High-throughput technology enables the simultaneous testing of multiple drugs and doses, thereby accelerating the drug discovery process. Automated workflows allow for real-time monitoring of drug responses in organoids while reducing manual intervention, which enhances experimental efficiency and speed.[40,104] In clinical treatment, the combination and sequence of drug administration significantly affect efficacy. However, the complexity of these treatments often surpasses the simulation capabilities of traditional screening models. By leveraging an automated microfluidic platform, precise control over drug concentration and delivery timing can be achieved, thereby dynamically replicating the drug administration sequences, dosages, and timings encountered in clinical practice. This platform can automatically dispense reagents at the correct times and in accurate amounts, thus eliminating errors commonly associated with manual operations and considerably improving the reproducibility and consistency of experiments.[105]

Tumor research

In tumor research, traditional 2D cell models lack physiological relevance and genetic heterogeneity, whereas

patient-derived xenografts better mimic clinical conditions but are resource-intensive and time-consuming.[106] Tumor organoids derived from cancer resections and metastatic biopsies retain the original tumor's genetic mutations and histopathology, reflecting clinical heterogeneity and demonstrating strong proliferative capabilities for stable culture and passaging. Initial drug sensitivity assays show that these organoids can replicate patient-specific responses to chemotherapy and targeted therapies, underscoring their potential for precision medicine applications.[107] Currently, various organ-specific tumor models have been established for cancers such as those of the colon, brain, prostate, pancreas, liver, breast, bladder, stomach, esophagus, endometrium, and lung. The common goal of these tumor organoid models is to enhance the *in vivo* relevance of the tumor microenvironment (TME) while maintaining the long-term viability and key pathological features of the tumor organoids.[10] For instance, tumor growth relies on an extensive vascular network for nutrient supply, with tumor cells promoting angiogenesis by secreting pro-angiogenic factors. To investigate this process, a tumor organoid-on-a-chip was developed to simulate the transport of biomaterials near the arterial end of capillaries within the TME. A stable perfused 3D microvascular network was pre-established in a compartment adjacent to the tumor organoid, followed by the implantation of breast cancer organoids. This setup allowed for real-time dynamic observation of cell proliferation, angiogenesis, cell migration, and tumor cell intravasation over consecutive weeks.[108] Furthermore, in the domain of 3D tumor modeling, organ-on-a-chip systems offer detailed microscale representations of TMEs by incorporating dynamic physiological processes. In contrast, bioprinted research models serve as robust tools for replicating the macroscopic architecture and heterogeneity of tumors, providing greater structural complexity and complementing the microscale precision of organ-on-a-chip platforms.[109,110]

Tumors are one of the most widely studied for drug screening applications using organoids, and many designs for tumor organoid chips apply to other types of organoids.[10,11] As cancer treatment shifts from a one-size-fits-all approach to personalized medicine, organoids-on-a-chip platforms enable researchers to test various drugs based on specific tumor characteristics from individual patients. This personalized approach can more accurately predict a patient's response to specific therapies, ultimately allowing for tailored treatment plans. Organoids-on-a-chip provide a realistic microenvironment for drug metabolism that facilitates controlled biochemical and physical stimuli while simulating tumor interactions with immune cells, stromal cells, and the vascular system. For example, ellipticine, a natural product with antitumor activity, interferes with DNA replication and RNA synthesis to inhibit tumor cell growth. Under simulated gastrointestinal peristalsis conditions on microfluidic chips, human colon tumor organoids displayed reduced uptake of ellipticine-loaded polymeric micelles, demonstrating the negative impact of physiological processes like peristalsis on drug distribution and absorption in tumor tissue, and potentially on anticancer efficacy.[111] Taking this into consideration is crucial for the design and evaluation of drug delivery systems and anticancer therapies, as their efficacy largely hinges on the effective delivery of drugs to tumor cells.[112] Failing to simulate these physiological conditions or relying solely on organoid testing in conventional culture dishes may lead to an overestimation of drug efficacy *in vivo*, ultimately affecting decisions in drug development and clinical applications. Drug resistance and insensitivity are major challenges that contribute to cancer treatment failure. Organoids-on-a-chip systems serve as powerful tools for investigating the mechanisms of drug resistance in tumor cells and their pharmacological sensitivity, as well as for testing new strategies to overcome such resistance. For instance, patient-derived colorectal cancer organoids cultured and expanded in a microfluidic device exhibited similar sensitivity to the first-line treatment drug 5-FU compared to organoids grown on a traditional Petri dish, indicating that organoid chips can serve as reliable drug screening models.[113] Moreover, conventional organoid cultures typically require extended periods to form and mature, making them unsuitable for rapid assessments of treatment responses, particularly in cases where tumors progress and metastasize quickly.[107] Organoids-on-a-chip technology enables rapid evaluations of tumor drug sensitivity within clinically relevant timeframes (usually <14 days), which is crucial for clinicians to promptly devise and adjust treatment plans. In addition, many traditional organoids lack functional microvascular networks, which restricts the simulation of mass transfer and drug distribution within the TME.[114] By integrating microfluidic technology with 3D microvascular networks, organoids-on-a-chip platforms can rapidly assess an individual breast cancer tumor's sensitivity to treatment drugs within clinically relevant timeframes (not exceeding 14 days).[108] Similarly, using an integrated superhydrophobic micro-well array chip was shown to help complete the entire process of deriving lung tumor organoids from patient samples, conducting drug sensitivity tests, and obtaining results within a week.[115]

Challenges

Organoids-on-a-chip technology represents an innovative model that combines the biological complexity of organoids with the precise control of organ-on-a-chip systems. However, several limitations remain that hinder its widespread adoption, primarily concerning technical complexity, cost-effectiveness, biological maturity of models, real-time monitoring capabilities, and challenges related to standardization and reproducibility [Figure 2].

Technical complexity and cost

The complexity and high costs associated with organoids-on-a-chip technology pose barriers to its implementation. Developing a microfluidic system to accurately simulate *in vivo* conditions requires specialized skills in microfabrication and bioengineering. Furthermore, the materials used, such as polydimethylsiloxane (PDMS), can be expensive, particularly when custom designs are necessary.[116] The need for precision equipment—peristaltic pumps,

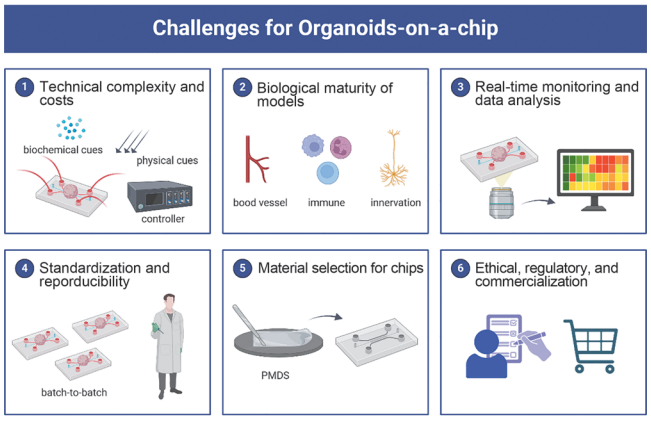

Figure 2: A schematic overview of challenges for organoids-on-a-chip technology.[3,6,120,121] Created in *BioRender.com*.

valves, and sensors—further elevates costs. In addition, organoids require specific growth conditions involving costly extracellular matrix materials, growth factors, and sterile environments, all of which contribute to the overall expense. Maintaining the functionality and structural integrity of organoids over time adds another layer of complexity and cost, as high-quality cell culture equipment and supplies are essential. Finally, sophisticated imaging and data analysis systems require substantial investment, limiting the accessibility of this technology in resource-constrained laboratories.

Biological maturity of models

While organoids-on-a-chip systems aim to replicate the 3D structures and functions of real organs, their biological maturity often remains insufficient. Current models frequently lack essential features such as functional vascular networks, neural connections, and the integration of immune cells. The current challenges in vascularizing organoid-on-a-chip models include the lack of fully functional vasculature, such as perfusion capabilities, and the limitations in accurately replicating the size and shape of blood vessels using existing methods. In addition, there is a need to address the heterogeneity introduced by different vascularization techniques and achieve long-term stability and functionality of the vasculature *in vitro*.[76] The absence of functional microvascular networks hinders the long-term culture and expansion of organoids and may limit the comprehensive evaluation of drug absorption, distribution, metabolism, and excretion.[75] Similarly, the absence of neural connections may prevent organoids-on-a-chip from accurately simulating responses to neural signals, impacting their application in neuroscience research. Furthermore, organoids-on-a-chip without immune cell integration may fail to accurately mimic immune responses, including those involved in inflammation and tissue repair regulation.[6,10] These limitations can affect the accuracy of organoids-on-a-chip in disease modeling, particularly in replicating diseases involving microvascular abnormalities, inflammation, or neural damage, increasing the unreliability of testing therapeutic interventions.

Real-time monitoring and data analysis

Although organoids-on-a-chip systems can integrate various sensors, limitations in their sensitivity, selectivity, and compatibility with the chip system restrict their monitoring capabilities, particularly for low-abundance biomarkers or multiplex assays.[117] In addition, real-time imaging may be constrained by speed and resolution, making it challenging to observe dynamic processes such as cell migration or tissue reconstruction. The data generated by these systems are often multimodal, requiring advanced analysis techniques, such as machine learning and artificial intelligence, to extract meaningful biological information. However, challenges such as data quality, sample size, and model interpretability can hinder the effective application of these techniques.[104,105,118]

Standardization and reproducibility

The lack of standardized procedures and assessment criteria in the organoid and organoids-on-a-chip fields presents considerable challenges. Variability in cell sources, culture conditions, and chip designs among different research teams can complicate comparisons across studies. The inherent heterogeneity of self-assembled organoids also contributes to this issue. Disparities in the genetic backgrounds and functional states of cells, variability in the quality of biological materials used, and differences in the manipulation of environmental parameters can all affect experimental outcomes. Despite successful laboratory-scale predictions of drug efficacy, translating these models into clinical applications has proven difficult.[1,7]

Material selection for chips

Choosing suitable materials for organoids-on-a-chip systems is critical for their performance. PDMS is commonly used due to its biocompatibility and processability. However, its hydrophobic surface may adsorb hydrophobic drugs, affecting their bioavailability.[119] Approaches such as surface modification or using inert materials like polycarbonate, glass, or poly (methyl methacrylate, PMMA) may help overcome these limitations, leading to more accurate drug evaluations.[10] Extracellular matrix materials used in organoid and organ-on-a-chip systems face several challenges, including batch-to-batch variability, limited mechanical stability, and a lack of biochemical complexity, which can undermine reproducibility and physiological accuracy. Many commonly used extracellular matrices, such as Matrigel and collagen, are derived from animal sources, which can lead to variability and potential immunogenicity, affecting the reproducibility of experiments.

Ethical, regulatory and commercialization challenges

The use of human cells in organoids-on-a-chip technology raises ethical and regulatory issues. A comprehensive framework for ethical review and regulation of organoids and organoids-on-a-chip systems is still under development. For instance, when using human-induced pluripotent stem cells, it is crucial to consider the ethical

implications of cell sourcing and ensure that informed consent is obtained from donors. In addition, research involving patient-derived cells may involve sensitive health information, necessitating strict adherence to data protection regulations to maintain privacy.[95] Given that organoids-on-a-chip technology spans multiple disciplines, such as biology, engineering, material science, and medicine, its regulatory framework must address the unique needs and standards of each field. Transitioning this technology from laboratory settings to industrial applications involves overcoming challenges related to technology transfer, scalability, quality control, and cost management, as well as establishing market acceptance and protecting intellectual property. Collaborative efforts across disciplines are essential to advance regulatory frameworks and cultivate a supportive market environment for the technology's growth and widespread adoption.

Conclusion and Perspectives

As the number of clinical trial failures based on animal studies continues to increase (e.g., thalidomide,[122] opicinumab,[123] and semorinemab[124]), the demand for preclinical human *in vitro* models has grown significantly.[94] The Food and Drug Administration (FDA) Modernization Act 2.0, enacted in 2022, has replaced the 1983 mandate that required potential drugs to undergo safety and efficacy testing in animals.[125] This new legislation allows the FDA to advance drugs or biologics to human trials following either animal or non-animal testing. Consequently, the FDA no longer mandates animal testing data for new drug evaluations, thereby reducing the reliance on animal research.[126] Recently, the first drug, TNT005, derived entirely from preclinical data obtained through organ-on-a-chip technology, has been approved by the FDA for clinical trials. This landmark decision signifies the first instance in which organ-on-a-chip experiments have officially supplanted traditional animal testing and received regulatory endorsement.[127] Importantly, organoids-on-a-chip address the limitations in biological complexity inherent to conventional organ-on-a-chip models by providing data that more accurately reflects human physiological and pathological conditions. This advancement could expedite the translation of drugs from laboratory settings to clinical trials, significantly reducing both development time and costs and demonstrating substantial promise in drug discovery and precision medicine.

Looking ahead, the technology surrounding organoids-on-a-chip will continue to progress toward enhanced simulation fidelity, standardization, and scalability. Beyond the imperative of improving organoid maturation and refining chip materials, living organoid biobanks are crucial for the integration of diverse biological resources. The establishment of the biobanks will facilitate the acquisition of samples with varied genetic backgrounds, disease subtypes, and personalized characteristics, thereby boosting the field of personalized medicine and targeted drug development.[128–131] In addition, the integration of cutting-edge technologies and interdisciplinary collaboration is imperative. Given the inevitability of technological evolution, it is essential to adopt a broad, cross-disciplinary perspective in incorporating suitable technologies into organoids-on-a-chip research. For instance, multi-omics analyses can yield detailed insights across various biological layers, including transcriptomics and proteomics. When coupled with organoids-on-a-chip technology, these analyses provide a deeper understanding of disease states at the molecular level, revealing complex molecular networks involved in pathological processes, identifying drug targets, and predicting potential side effects.[10,132] In addition, artificial intelligence and neural network-based methodologies can facilitate the analysis of organoids-on-a-chip data and integrate these findings with broader physiological and pathological datasets, thereby enhancing the predictive power of experimental outcomes and automating the optimization of experimental designs and dosing conditions.[104,118] 3D imaging streamlines automated high-throughput screening by enabling swift and standardized visualization of numerous organoids. This technology, when coupled with machine learning algorithms, can automate the detection and quantification of specific organoid structures, thereby enhancing the efficiency and objectivity of the screening process.[133] Recent advances in biomacromolecules have introduced alternatives for extracellular matrix materials, such as silk proteins. With structural and functional similarities to collagen, silk proteins are gaining recognition for their biocompatibility, tunable mechanical properties, and minimal immunogenicity, making them a promising solution for more reliable and reproducible 3D organoid culture and tissue engineering applications.[134] Moving forward, close collaboration between bioengineers, data scientists, biologists, and clinicians will be paramount to ensure accurate data interpretation and application. Furthermore, global regulatory bodies, industry stakeholders, academia, and patient advocacy groups should engage in continuous dialogue to address the ethical and regulatory challenges posed by varying standards across different countries, which may impact international research collaboration and the dissemination of technology.

In conclusion, organoids-on-a-chip technology is advancing rapidly in the biomedical field, garnering much interest from both the FDA and the commercial sector due to its vast potential for application in disease modeling and therapeutic method evaluation. However, it is crucial to recognize that this technology remains in its infancy, with ample opportunities for improvement. Advancing and refining organoids-on-a-chip systems will necessitate interdisciplinary collaboration and the collective efforts of researchers globally. The progress of this technology relies on the innovative integration of various methods, built on a solid foundation of organoid and organ-on-a-chip technologies. These foundational developments are crucial for successfully modeling complex biological systems. When selecting an *in vitro* model, researchers should align their choice with specific research objectives. Not every scenario warrants the use of more complex organoids-on-a-chip systems over traditional organoids, organ-on-a-chip models, 2D cell cultures, or animal models. The key lies in identifying the model that best meets the research needs. Although organoids-on-a-chip systems offer highly controlled biological microenvironments, their high costs, complex operational requirements, and

extended cultivation periods that include preparation and growth time may restrict their use in certain studies. Therefore, it is imperative to balance the pursuit of technological innovation with considerations of cost-effectiveness and practical feasibility. While the progress of organoids-on-a-chip technology is promising, ongoing exploration and refinement are essential to ensure the effectiveness and viability of research outputs derived from organoid technology.

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Conflicts of interest

None.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

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