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Strategies to overcome the limitations of current organoid technology - engineered organoids

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Abstract

Organoids, as 3D in vitro models derived from stem cells, have unparalleled advantages over traditional cell and animal models for studying organogenesis, disease mechanisms, drug screening, and personalized diagnosis and treatment. Despite the tremendous progress made in organoid technology, the translational application of organoids still presents enormous challenges due to the complex structure and function of human organs. In this review, the limitations of the translational application of traditional organoid technologies are first described. Next, we explore ways to address many of the limitations of traditional organoid cultures by engineering various dimensions of organoid systems. Finally, we discuss future directions in the field, including potential roles in drug screening, simulated microphysiology system and personalized diagnosis and treatment. We hope that this review inspires future research into organoids and microphysiology system.

Keywords

Organoid, organoids-on-chips, bioengineering, microenvironment, Microphysiological system

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Introduction

For a long time, human beings have been plagued with questions about the development of the human body from the time of the embryo, how diseases affect human organs, the impact of external environmental factors on the human body, and the development of new therapies and new drugs. Most research on these issues is currently limited to traditional two-dimensional cellular and animal models, but due to species differences and research costs,[1,2] there is an urgent need for a model that can more closely mimic the real environment of the human body and be cost-effective. In response to this need, organoid and organoids-on-chips technologies have emerged.

Organoids are self-organizing 3D tissues, usually derived from stem cells (pluripotent, embryonic, or adult stem cells), that mimic the key functions, structure, and biological complexity of organs.[3,4] Organoid research was fast-tracked in 2009 when Sato' team[5] cultured adult stem cells derived from the mouse intestine to produce the first miniature intestinal organoid with small intestinal crypt and villus structures. Although organoids show great potential to a certain extent, there is still a large gap between organoids and real human organs, which is due to the complex composition and many functions of real

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human organs. To date, a number of engineering strategies to support organoid culture and growth, proliferation, differentiation and maturation have been reported. For example, by applying engineering principles and design concepts to traditional organoid technologies, such as organoids-on-chips,[6] and 3D printing,[7] these engineering tools can be used to optimize organoid culture methods to ensure reproducibility as well as reliability, and these technologies enable precise control of the culture microenvironment so that organoids can more closely resemble real physiological environments. In addition to basic culture, precise analysis of organoids plays a crucial role, and engineering enables visualization and dynamic analysis of organoid microenvironments as well as biochemical metrics. On the basis of these insights, developing engineering strategies to precisely control organoid growth and development is important. In this review, we aim to highlight engineering strategies to alleviate the current organoid dilemma. First, some of the limitations of organoid systems in general are outlined. Next, strategies to overcome these limitations are detailed. Finally, we present the challenges and perspectives of using organoids in biomedical applications.

Limitations of current organoid systems

Limited survival time

Inadequate vascularization within organoids results in a limited nutrient and oxygen supply, which affects their long-term growth and long-term maintenance of their functional activities, and the shorter survival time of organoids is often a direct cause of their limited use for biomedical research. For example, blood vessels facilitate both nutrient supply and neuronal cell differentiation in brain tissues.[8] As the size of brain organoids increases, the lack of internal nutrients and the difficulty in removing metabolic waste lead to necrosis of the center of the organoid. This necrosis further interferes with its normal development and neuron migration routes, a problem that can be addressed, at least in part, through oscillating cultures.[9,10]

Although the above problems can also be solved by mechanically fragmenting the organoid, the phenotype of the organoid is disrupted by the constant fragmentation of the already-formed cellular tissue structure, all of which can affect the results of the study. Moreover, pluripotent stem cell-derived organoids are not fragmented or passaged.

New strategies to address nutritional accessibility are currently being developed, such as through brain slice culture.[11] The supply of nutrients is not the only problem; similarly, experiments often require the delivery of certain substances to specific areas of organs, but owing to the lack of a functioning vasculature system, these substances do not accurately reach the desired location.

Limited maturity

Many organoids have been successfully constructed, such as mammary organoids that secrete milk,[12] gastric organoids that secrete gastric acid,[13] and liver-islet axis organoids that respond to blood glucose stimulation.[14] However, no organoid has yet been successfully built that can completely replicate the full function of the corresponding organ. The vast majority of organoid models lack key specific cell types, and fail to fully recapitulate the complexity of human organs due to the lack of mesenchymal regions, immune cells, innervation, vascularization, corresponding flora microorganisms, lymphatic vessels, etc.

In addition to the influence of biological factors, the physical microenvironment also appears to be crucial for revealing the process of organogenesis and development in the human body, and the maturation of organs in vitro can be improved through the modulation of external environmental factors such as mechanical force stimulation, light stimulation, or electrical stimulation.[15–17] However, integrating these features remains a major challenge for current technology.[6] The long-term culture of organoids in vitro is also a major concern; the limitation of organoid lifespan does not mimic the development of natural organs very well; for example, epithelial organoids have a lifespan of approximately 1 week, which is far from the time it takes for stem cells to fully differentiate into the full range of cell types in vivo.[18,19] Brain organoids simulate only the phenotype of the fetal brain, and their mature development needs to be further facilitated if a model of the mature adult brain is to be obtained.[20,21]

Heterogeneity

Organoid cultures exhibit striking heterogeneity and variable complexity in terms of their cellular composition and are prone to poorly controlled morphogenesis during self-assembly, for example, morphological and structural differences between batches, functional differences,[22] however, these differences are usually due to the stochastic nature of in vitro self-assembly and organ developmental selection. Therefore, there is a great need for engineering tools, such as precise regulation of the medium composition and innovation of the extracellular matrix, to reduce the variability of organoids during development and increase the homogeneity of organoids, which in turn can lead to consistent conclusions in the study of disease mechanisms.

In addition, organoid construction methods are mostly manual, and some key factors, such as the number of cells, the proportion of types, and the proportion of extracellular matrix, can lead to differences in organoids when they are at the initial stage of organoid development. By increasing the means of automation, the use of robotic liquid handling systems during organoid culture can independently

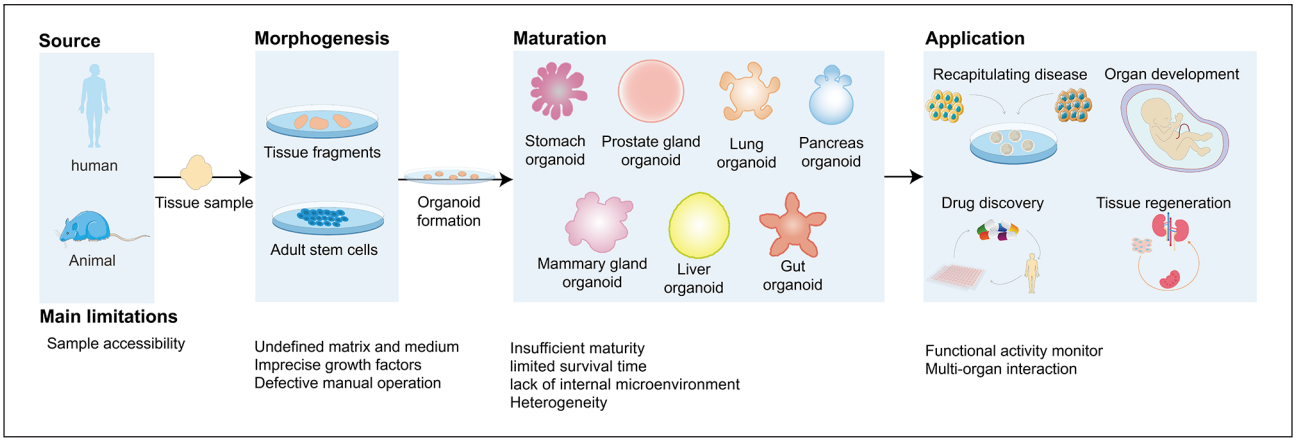

Figure 1. Schematic of the different organoids that can be derived from PSCs or tissue.

perform a series of precisely controlled tasks, including complex operations such as initial stem cell allocation, media addition and replacement, drug testing, and real-time analysis,[23,24] which are expected to increase the homogeneity and reduce the heterogeneity of organoids.

Insufficient function monitoring

Organoid reconstructions of various systems have replicated the development of different human systems and a portion of their functional and structural phenotypes, providing important insights into organogenesis and development as well as diseases of various systems. Therefore, it is particularly important for the monitoring of the structural-functional activity of organoids. Most of the common monitoring devices are traditional optical monitoring devices, but traditional optical microscopy monitoring provides relatively limited information. The use of conventional optical microscopes provides limited data due to situations such as individual three-dimensionally growing organoids being in different focal planes.[22] Similarly, traditional electrophysiological methods make accurate monitoring of the physiological properties of nerve organoids difficult because of their highly complex three-dimensional structure.

Recently, several studies have subdivided the physiological and pathological states of neural organoid by using techniques such as high-content imaging,[25] multi-electrode arrays,[26] optogenetics,[27] and biosensors.[28] Other organoids, such as liver organoids, can be used to assess the physiological or pathological function of the liver in vitro by analyzing the metabolites of exogenous compounds as well as endogenous substrates or the metabolic synthesis of bile and proteins.[29] However, the extremely low levels of some metabolites or compounds make it extremely difficult to monitor them. By incorporating miniature biochemical sensor technology, it is not only possible to achieve a high level of sensitivity, enabling the monitoring of concentrations up to the micromolar or nanomolar level, but also to minimize the impact on cellular activity.[30] Nevertheless, typical organoids are mostly cultured in Matrigel, which makes it difficulty in monitoring biochemical sensors. Therefore, to monitor the target with homogenized information, relatively precise control of the position and the shape of the organoids is necessary. High-throughput testing can be performed with increased automation, both when basic organoid cultures are performed and when high-throughput screening of organoids is performed.[31] These issues limit the further application of organoids in biomedical research. Hence, the modulation of some of the controllable factors of organoids (e.g. batch differences in Matrigel and growth factor content) may be beneficial in mitigating these limitations.[22] In response to some of these shortcomings, the use of engineering tools can help address some of the limitations of organoid platforms.

Engineered organoid formation

Organoid

Organoids are three-dimensional multicellular in vitro cultures formed by the self-organization of stem cells,[32,33] which have significant potential in research areas such as growth and development, disease modeling, and drug discovery and have become an important development direction in tissue engineering and regenerative medicine[34,35] (Figure 1).

Compared with traditional animal models, organoid cultures are more amenable to intervention and in-depth biomedical research. Organoids can be generated from pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), as well as from tissue samples. Currently, organoid technology is used in areas such as drug discovery,[36] personalized diagnosis,[37] and cell therapy.[19] In recent decades,

remarkable progress has been made in the development of various organoids (e.g. brain,[9] heart,[38] kidney,[39] lung,[40] stomach,[41] prostate gland,[42] pancreas,[43] mammary gland,[12] liver,[44] and others). Notably, organoids derived from tissues of tumor patients have also been successfully established.[45] These organoids offer a broad spectrum of applications, spanning from organogenesis and disease modeling to personalized medical interventions and drug screening strategies. Hereditary disorders are diseases caused by alterations in genetic material; these disorders are often accompanied by a wide range of birth defects, and owing to the complexity of their disorders, many genetic disorders lack effective treatment. Organoids have a wide range of applications for modeling such diseases. To date, numerous genetic diseases have been reproduced by organoids, such as human microcephaly,[9] polycystic kidney disease,[46] autism spectrum disorders[47] and other disorders, and these models provide new tools for further exploration of disease pathogenesis as well as therapeutic strategies. Additionally, various types of tumor-patient tissue-derived organoids have been used for research, such as gastroesophageal cancer organoids,[48] lung cancer organoids,[36] and pancreatic cancer organoids,[49] and these models provide abundant data for the treatment of the corresponding diseases. Similarly, substantial progress has been made in the study of microbial-host interactions, for example, gut flora-on-oncogene interactions,[50] gut microbial-host interactions in preterm infants.[51] Despite great progress in organoid technology, several limitations remain, including low maturity and high heterogeneity.

To minimize the impact of these problems as much as possible, more reliable organoids are being cultivated through the use of organoids-on-chips technology, biomaterials, microfluidics, biochemical sensors, and other technologies. As an alternative to the extracellular matrix (ECM), Matrigel was prepared from soluble basement membranes extracted from the tumors of Engelbreth-Holm-Swarm (EHS) mice enriched with extracellular matrix proteins. Nonetheless, the uncertainty of its composition and batch variability limit its application in biomedical research. Consequently, alternatives to Matrigel have been used, for example, for the spinal cord,[52] brain,[53] and alveoli.[54] These biomaterials can reduce the batch variability of organoid generation and increase homogeneity.

Similarly, the maturation of organoids is very limited because of the lack of a complex physiological microenvironment in vitro. Therefore, engineering the culture environment to mimic fluid shear forces in vivo, for example, is more conducive to organoid development and maturation and improves the reliability of organoids.

Organoids-on-chips

Organoids-on-chips refers to the organoid system based on microfluidic technology, which replaces of two-dimensional cells in organoids-on-chips with three-dimensional cells or organoids, and is an extension of traditional organs-on-chips methods in biotechnology,[6] which focus on the microfluidic chips technology by combining it with a variety of methods, such as cell biology, biomaterials, and engineering. We summarized the key components of the organoids-on-chips model, including the organoid type, cell source, extracellular matrix, chip design, and application (Table 1).

Furthermore, we also present a conceptual diagram of some organoids-on-chips (Figure 2). In microfluidic devices, cells and tissues can be cultured in miniature volumes and controlled microenvironments designed to mimic specific in vivo niche.[66–70] Owing to the characteristics of certain shear forces in three-dimensional culture environments, these cells mimic the physiological state of humans more closely and can be simulated in vitro to reconstruct a tissue and organ microenvironment that encompasses a variety of living cells, functional tissue interfaces, biofluids, mechanical force stimulation, and other complex factors, all of which respond to the primary structural and functional characteristics of the human body's tissues and organs.

To address the high heterogeneity in conventional organoid cultures, which is predominantly caused by complex manual manipulation, microfluidics can offer a solution by utilizing microstructures. Numerous microfluidic devices capable of controlling morphology have been employed to generate organoids. Additionally, microcolumns of a certain size can control the size of the organoid and improve the homogeneity of the organoid.[55] Similarly, retinal organoids can be standardized in a controlled manner in microporous arrays.[60] Inter tissue fluid in brain tissue plays an important role in nutrient transfer, metabolic waste removal, and intercellular communication; however, most current in vitro studies of neurological disorders suffer from the drawbacks of two-dimensional cell cultures and the neglect of inter tissue fluid flow. Park et al.[71] mimicked Alzheimer's disease (AD) through the use of a three-dimensional sphere-based microfluidic chip. In this study, the toxic effects of β-amyloid were tested via neurospheres cultured under dynamic and static conditions, and surprisingly, dynamic cultures appeared to have greater neurodestructiveness and deeper synaptic dysfunction than static cultures, characteristics that are highly consistent with the pathology of AD. Thus, brain organs-on-chips provide structural and functional support for reproducing Alzheimer's disease. Cui's research team[56] cultured cortical organoids exposed to valproic acid (VPA) on perfusable organoids-on-chips. An in vitro model outlined the injury features of prenatal exposure to valproic acid, including increased neuronal progenitor cells, suppressed neurons, and altered forebrain regionalization, and analysis of transcriptomic data revealed a high degree of similarity between VPA-exposed organoids and postmortem autistic brains and organoids originating from autistic patients. Thus, this brain organoids-on-chips microarray

Table 1. Summary of existing human organoids-on-chips models used for biomedicine.

Organoid	Cell sources	Extracellular matrix	Chip design	Application	Ref.
Brain	hiPSCs	Matrigel	A chip Consists of bottom layer micropillars and top layer ring structure	Simplifying brain organoid formation protocols and overcoming the potential limitations	Zhu et al.[55]
			A chip consists of patterned micropillars and ring structure	Modeling prenatal brain exposure to valproic acid	Cui et al.[56]
		Fibrinogen, collagen type Ⅰ	A chip consists of five parallel channels separated by micropillars, a removable polyester membrane	Modeling brain organoids vascularization and deciphering potential vascularization factors	Shaji et al.[57]
		Matrigel	A chip that integrates perfusable vascular chambers and organoid chambers	Modeling the interaction between brain organoid and vascular system	Salmon et al.[58]
	hESCs		A tubular organoid perfusion device (upper) and an organoid holder device (bottom)	Revealing a new method for inducing aging phenotype within brain organoids	Ao et al.[59]
Retinal	mESC	PEG hydrogels	A chip consists of U-shaped hydrogel microporous array and PDMS ring	Standardizing retinal organoids and reducing heterogeneity	Decembrini et al.[60]
Heart	hiPSCs	decellularized ECM hydrogels	A chip consists of three culture chambers and two medium chambers	Modeling Long QT syndrome and cardiac fibrosis	Min et al.[61]
Colon	Patient	Matrigel	A chip capable of perfusing vascularized colonic organoids	Modeling vascularization and inflammation within colon organoids	Rajasekar et al.[62]
Kidney	hPSCs	Matrigel	A chip capable of perfusing vascular networks	Modeling kidney organoids vascularization	Homan et al.[63]
pancreatic tumor	Patient	Matrigel	A chip that can support various cells to self assemble into 3D tissues in substantive space	Reproducing the microenvironment of pancreatic tumor	Lai Benjamin et al.[64]
Liver	hESCs	4-arm-PEG-maleimide	A perfusable chip composed of channels of different shapes and depths	Established human vascularized hepatobiliary organoids	Abbasalizadeh et al.[65]

hiPSCs: human induced pluripotent stem cells; PSCs: human pluripotent stem cells; hESCs: human embryonic stem cells; PEG: polyethylene glycol; PDMS: polydimethylsiloxane; mESC: mouse embryonic stem cells.

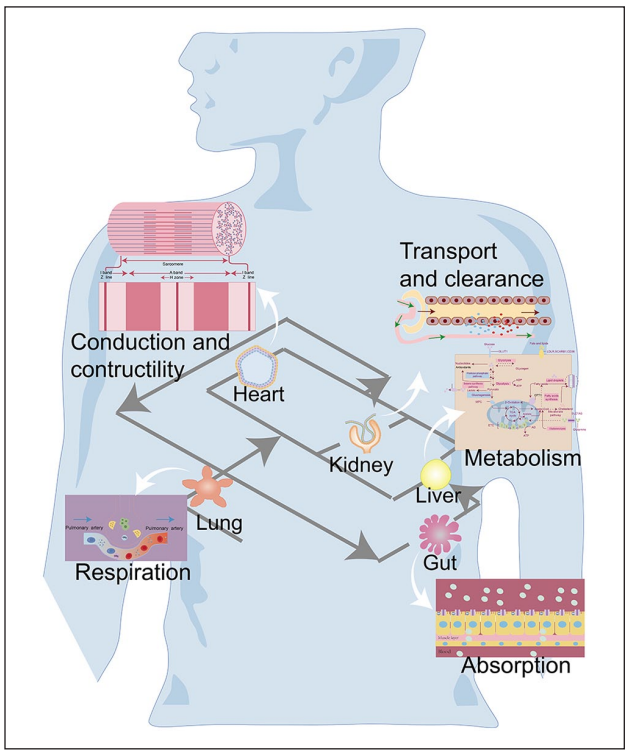

Figure 2. Conceptual schematic of a human organoids-on-a-chips.

model enables researchers to explore neurodevelopmental disorders under exposure to a variety of environmental factors and can be extended to applications in disease modeling and drug screening.

Apart from the nervous system, structurally and functionally mature cardiac tissue offers new tools for use in drug development and cardiotoxicity testing. By combining microfluidic microarray technology with decellularized heart extracellular matrix (HEM), Min's team[61] constructed a perfusable culture system based on pluripotent stem cells (hiPSCs)-derived cardiac tissues, HEM enhances the upregulation of genes involved in cardiac collagen deposition, fibrosis, and cardiomyocyte maturation in vitro. By stacking ductless chips on an axial shaker, large-scale culture of mature cardiac tissues can be achieved. Compared with conventional dynamic culture systems, more complex media flow was provided to cardiac tissue and facilitated electrical synchronization, and an improved ability to synchronize contractions of cardiac tissue was found. By transplanting cardiac tissues into rats, the generated cardiac tissues promoted functional and structural regeneration of the heart and achieved functional integration of the transplanted myocardium with the host myocardium. These cardiac tissues can also be used to assess contraction patterns and electrophysiological changes in drugs, as well as to model diseases such as long QT syndrome and cardiac fibrosis. In brief, these dynamic organoids-on-a-chips systems[72,73] can provide more reliable tools for basic medical research.

Furthermore, current organoid systems typically lack blood circulation, make it difficult for nutrients and oxygen to reach the core, have a continuously increase metabolic waste, cause extensive cell death and premature cell differentiation in the later stages of culture, resemble embryonic or fetal organs rather than adult organs in their developmental stages and functions, and lack relevant developmental signals,[9,74–76] which greatly limits the technological applications of organoids. Therefore, Shaji et al.[57] developed a three-layer, five-channel microfluidic device based on polydimethylsiloxane (PDMS) and directly cocultured brain organoids derived from human-induced pluripotent stem cells (hiPSCs) in a perfusable vascular bed generated by the self-assembly of human umbilical vein endothelial cells (HUVECs) to confirm the differentiation of the hiPSCs into brain organoids and to evaluate the stability and perfusability of the vascular bed during coculture. This study used brain organoids cultured on microarrays to identify genes involved in angiogenesis, and key members of the vascular endothelial growth factor (VEGF) signaling pathway were consistent with the angiogenic process observed in brain organoids cultured on microarrays.

Recently, in a study of the interaction of colonic organoids with HUVECs and fibroblasts, Rajasekar et al.[62] independently cultured up to perfusable vascularized colonic organoids in vitro and modeled colonic inflammation with innate immune function. This technique is useful for high-throughput drug screening, but the use of this customized well plate makes the user's use in terms of spatial control inflexible. Salmon's team[58] overcomes this limitation by printing microfluidic chips via 3D printing technology to realize interactions between organoids and the vascular system on a chip, and this 3D printing-based organoid chips were able to more accurately recreate the true physiological roles of vascular endothelial cells and brain organoids. Similarly, vascularized heart, pancreatic and liver cancer tissues can be fabricated, providing a new platform for organogenesis development, disease modeling and drug development.

Most organs, with the exception of the vascular system, possess tissue-resident immune cells. However, human organoids lack these immune cells, which restricts their applicability in modeling numerous normal physiological and disease mechanisms. Ranga, by developing a microphysiological analysis platform (MAP) for human brain organoids, reproduced the physiological interactions between monocytes and brain organoids derived from hiPSCs. By co-culturing young (20–30 years old) and old (>60 years old) primary monocytes with cultured human cortical organoids, it was found that primary monocytes from older (>60 years old) donors have a greater capacity to infiltrate cortical organoids and promote the expression

of proteins associated with senescence (e.g. p16). This platform provides a new potential method for inducing aging phenotypes within brain organoids without external genetic manipulation and offers new research avenues for studying immune-driven brain aging as well as restoring homeostasis in the aging brain. In addition to immune co-culture of normal organoids, immune co-culture of tumor organoids for immunotherapy of tumors is also a bottleneck that urgently needs to be overcome. Xu et al.[77] developed a novel culture of breast cancer organoids with a biomimetic matrix and immune environment. Initially, an alginate cryogel was prepared to serve as a biomimetic tumor matrix. Subsequently, tumor-associated macrophages (TAMs) were cultured within this matrix and induced to polarize in the M2 direction. Finally breast cancer cells were added to form an organoid model that was cocultured with immune cells. Direct co-culture significantly enhanced breast cancer organoid growth and cancer invasiveness phenotypes, a model that helps researchers understand gene expression, protein interactions, and other information pertinent to breast cancer progression. The same coculture idea can be applied to other tumor organoids (e.g. colorectal cancer, non-small cell lung cancer, and gastric cancer).

Currently, it is very difficult to coculture human organoids and immune cells in the long term. The coculture conditions of organoids are usually the best combination of the ratio of each cell type; however, each cell has its own specific culture conditions, and the long-term supply of nutrients to the organoids is a major problem, especially from the tumor tissues, to generate organoids in a relatively inefficient manner. It is hoped that future cross fertilization of technologies from various disciplines will help to bring organoid immune cell coculture technology closer to the real environment in the body as well as to realize long-term culture, thus broadening our understanding of the complex interactions between various organs in the human body.

3D printing

3D printing has shown very significant advantages in in vitro organoid modeling, including being able to explore the effects of a large number of variables on a system using a small number of samples in a highly efficient way and enabling organoids to maintain their original phenotype. It is faster, less costly, and easier to perform than traditional methods. For example, 3D-printed small rotating bioreactors reduce the media volume and incubator space compared with rotating bioreactors used for long-term maintenance of organoids.[10,78] In recent years, suboptimal culture conditions for most organoids have led to the overuse of plastic.

To address the above problems, Rezaei's team[79] developed a 3D-printed pipeline for the preparation of a personalized culture platform that spatially cultures individual brain organoids independently while ensuring fluid flow, an integrated platform that enables all culture steps such as cell aggregation, balloon growth, hydrogel embedding, and maturation of the organoids to take place in individual cell culture wells without the need for the organoids to be transferred. In the long run, this microprinted device could efficiently and dynamically culture brain organoids, provide sufficient nutrients and oxygen, reduce the risk of contamination, and be suitable for large-scale culture.

Similarly, to reduce the heterogeneity of retinal organoids (ROs) and efficiently generate homogeneous ROs, Sun et al.[80] developed a novel polydimethylsiloxane (PDMS) microwell platform in which ROs can be assembled in one step and remain homogeneous and maturationally differentiated for a long period of time (more than 25 weeks) without the use of BMP4 and Matrigel. The platform can be used for subsequent retinal disease modeling, drug screening, and the production of clinically translatable ROs. To construct a vascularized bionic chip, the central culture chamber for brain organoids is designed with "open holes" to support vascular network formation and vascular invasion.[58]

In addition to common hydrogels, decellularized extracellular matrix (dECM) is important because of its ability to have tissue-specific or organ-specific extracellular matrix properties. The dECM is able to support cell adhesion, migration, and proliferation due to its unique structural proteins, cytokines, signaling molecules, and other physical properties, such as mechanical properties similar to those of natural tissue.[81–83] In one study, human colonic organoids were derived by modifying colonic dECM for photopolymerization, which served as a bioink. These organoids were then encapsulated into hollow tubular structures also derived from colonic dECM. When compared to those cultured in traditional Matrigel, these organoids exhibited similar high cell viability and colon-specific morphology. Over time, they were found to possess a more complex cellular composition and the ability to promote self-renewal and differentiation, features that are not comparable to traditional Matrigel cultures.[84]

In addition, 3D printing is uniquely advantageous for reproducing the tumor microenvironment (TME), enabling precise control of biological, physical, and chemical cues in the TME and providing new possibilities for in vitro modeling of tumors. Using dual nozzles for 3D printing, glioblastoma cells and macrophages were integrated onto the gel after the hydrogel was printed, and the model reconstructed the role of tumor immunity, including the polarization of macrophages to glioblastoma cell-associated phenotypes and the invasiveness of glioblastoma cells to normal brain tissue. This integrated 3D printing system provides an advantageous tool for constructing other organs and tissues for vascularization or immune co-culture.[85] However, the reality is that monodisperse tumor cells rarely exist, and most current uses of monodisperse cells do not rebuild the tumor process very well.[86]

Table 2. Materials systems for organoids.

Type of ECM	Characteristic	Application	Ref.
Matrigel	High biocompatibility and stability, batch variability	Brain, kidney, etc.	Lancaster et al.[9], Huang et al.[94]
Decellularized ECM hydrogels	Good stability, high biocompatibility and high cost, widely used	Intestine	Giobbe et al.[93]
Hyaluronic acid	High biocompatibility and good stability	Kidney	Astashkina et al.[95]
Alginate	Low cost and high stability and reproducibility, low mechanical stiffness	Brain	Cassel de Camps et al.[96]
Collagen	Widely used and good biocompatibility, easy to degradation	Intestine	DiMarco et al.[97]
silk fibroin	Low immunogenicity and suitable mechanical stiffness	Breast cancer	Shi et al.[98]
Fibrin	High biocompatibility and easy to degrade	Intestine	Broguiere et al.[90]
Plasma-derived extracellular matrix	Optimum growth factors, chemicals, necessary physical cues and high repeatability	Hepatocellular carcinoma	El-Derby et al.[99]
PEG	Controllable physical environment and low immunogenicity	Intestine	Gjorevski et al.[100]
PCL	Good mechanical properties and stability	Brain	Rothenbücher et al.[101]
PLGA	High biocompatibility and undesirable degradation products	Intestine	Shaffiey et al.[102]
Synthetic peptide hydrogels	High biocompatibility, replenish regularly	Kidney	Treacy et al.[103]
PGA	High biocompatibility, suitable mechanical stiffness	cholangiocyte	Tysoe et al.[104]

PEG: polyethylene glycol; PCL: polycaprolactone; PLGA: polylactic-glycolic acid; PGA: polyglycolic acid.

By integrating bioprinted nozzles to allow individual tumor spheres to be placed in bioprinted scaffolds, this technique allows for the integration of other elements of the microenvironment with the tumor organoids and ensures high fidelity of printing.[87] The synergistic use of 3D printing, organoid, and organoid microarray technologies has an incalculable impact on tissue engineering and holds promise for solving future medical problems.

ECM for organoids

Typically, an organoid culture system is established by adding a single cell suspension of prepared stem cells to Matrigel. However, owing to the complex and ill-defined composition of Matrigel,[88,89] batch stabilization is difficult to achieve and may lead to organoid heterogeneity as well as unreliable reproducibility.

Furthermore, because Matrigel is derived from animal sources, it greatly limits its clinical use and human organoid transplantation. Therefore, the search for specific hydrogels that can overcome these problems has become particularly necessary. Currently, attention is being given to hydrogels composed of natural materials as well as synthetic ones, with fibrin,[90] hyaluronic acid,[91] or polyethylene glycol hydrogels[88,92] being able to reduce batch-to-batch variation as well as ethical issues compared with Matrigel of animal origin. The characteristics and types of different extracellular matrices and their application in organoids culture are summarized in Table 2. Giobbe et al.[93] reported a greater percentage of stem cells than crypts in small intestinal organoids by using an intestinal decellularized extracellular matrix for intestinal organoid cultures due to its favorable physiological and mechanical properties, demonstrating that organoid ontogeny and development are closely related to the surrounding environment.

Like other hydrogels, decellularized extra-tissue matrices alone have drawbacks such as batch-to-batch variability and a lack of bio-hardness, and to address these issues, De Santis et al.[105] introduced a natural extract, sodium alginate, into the dECM, which was shown to support the growth and differentiation of the human trachea, and the stabilization of tracheal structures for up to 28 days.

Conventional engineered hydrogels are difficult to characterize according to the different stages of organoid culture. Surprisingly, Urciuolo's team[106] developed a hydrogel-based method for the dynamic fabrication of hydrogels in organoids that can be personalized according to specific culture environments and incubation times, matching the conditions required for organoids at different stages. The method was validated for the characterization of multiple organoids and was found to direct the orientation of neural axons in spinal cord organoids, as well as to control differential cell migration in tumor organoids. It was also able to promote the ontogeny and development of liver organoids, small intestinal organoids, and lung organoids. In addition to some natural materials, synthetic biomaterials such as polyethylene glycol (PEG),[107] hyaluronic acid,[91] alginate,[108] can be less susceptible to batch-to-batch variations than Matrigel, and PEG can be customized to mimic the natural ECM on the basis of the respective culture characteristics of different types of organoids. For example, Tomaszewski's team[109] modified PEG with a

number of different kinds of peptides with basement membrane binders as an ECM for follicular organoids, and demonstrated that the modified PEG promoted follicular organoid maturation. Similarly, four-armed maleimide PEG (PEG-4-MAL) was used to design an ECM for organoid culture, and this ECM supports the developmental maturation of human intestinal organoids.[110,111]

To reduce the heterogeneity of intestinal organoids, Gjorevski's team[100] embedded intestinal stem cells in a hydrogel containing arginine-glycine-aspartic acid and photosensitive polyethylene glycol, which degraded and softened the matrix under 405 nm ultraviolet light, thereby controlling symmetry disruption and crypt formation in the organoids and achieving homogenization of the intestinal organoids. It is also expected that the heterogeneity of other types of organoids will be reduced through the development of a wider variety of biomaterials and the combination of different materials for different organoids. Other synthetic materials, such as hyaluronic acid and self-assembling peptides (PeptiGel Alpha4/5), have also been used to generate organoids.[98,103]

Artificial intelligence

To minimize the limitations of organoids in culture and application, many other modern technologies, including automation and artificial intelligence (AI), have been developed that can increase the reproducibility and reliability of organoids in culture as well as application. Traditional organoid culture is usually performed manually; however, frequent manual operations may introduce human error, which may affect the stability of the culture system.[5,22,112]

AI provides a new framework for organoid construction, multi-scale morphological and functional analysis of organoids, preclinical evaluation, and application, which can be used to explore the potential of AI-generated organoids in detail. In the construction strategy, the matrix gel is designed to more closely mimic the natural structure and functional properties of human organs. Machine learning provides a powerful solution to personalize the design of the chemical, structural, and biomechanical properties of synthetic Matrigel. The culture conditions including temperature, oxygen content, pH, and other factors are precisely controlled. The Parameters of the culture system were programed to improve the accuracy of organoids culture under various conditions; for example, Jiang et al.[31] developed an automated organoid culture analysis system, a platform that homogenizes organoid precursors by spiking the culture wells with a microfluidic droplet printer after mixing the stem cells with Matrigel. Similarly, the types of growth factors, which are crucial induction components, are optimally combined and concentrated in the most efficient and economical manner.

In addition to automation and improved homogeneity at the organoid culture stage, a number of advances have been made in the automation of complex operations in applications such as drug testing and real-time analysis of results.[23,24] By combining organoids with artificial intelligence, Renner's research colleagues[113] have established a process for executing organoid systems that is scalable, reproducible, and capable of high-throughput screening compared with traditional methods. Furthermore, the microfluidic system enables the integration of a nanosensing system to automate the monitoring of important parameters in culture such as oxygen and metabolites.[114] Moreover to improving the efficiency and accuracy of morphological analysis, machine learning can reduce the impact of human intervention.

Although the industry has not yet been able to reach a consistent standard, it is expected that an industry consensus will be reached in the near future.

Construction of physical and chemical microenvironments

Mechanical force

It is well known that physical microenvironmental factors in the body, such as mechanical forces, light stimulation, electrical stimulation, and other complex factors are essential for the development of human organs and tissues.[52,115,116] A variety of engineered materials are being explored for modeling the microenvironment of organoid cultures (Table 3). For example, by constructing a methacryloyl gelatin hydrogel system with tunable mechanical properties for use in the culture of cochlear organoids, Xia et al.[115] found that moderately stiff ECM stimulated the proliferation of cochlear progenitor cells (CPCs) through the mechanism of ITGA3/F-actin/YAP signaling, whereas an increase in stiffness inhibited the proliferation of CPCs and stimulated the differentiation toward inner ear hair cells (HCs). These findings suggest that ECM mechanical forces are the driving force that triggers intracellular signaling cascades that direct sensory epithelial generation.

In addition to cochlear tissue, Koser et al.[117] have studied the mechanical properties of spinal cord tissue. Similarly, not only is the ontogeny of normal brain tissue affected by the external environment during development, but the mechanical traits of brain tissue are altered in a number of common degenerative neurological disorders. For example, Alzheimer's disease (AD), the most common progressive neurodegenerative brain disease as well as the most common cause of dementia worldwide,[135] and β-amyloid deposition, a typical pathologic marker in AD patients, may affect the stiffness of brain tissue.[136] With age, the composition and physical properties of the extracellular matrix will alter the mechanosensitivity of neurons and glial cells, which in turn accelerates the progression of neurodegenerative diseases. This includes changes in the biomechanics of brain tissue associated with conditions such as Parkinson's disease (PD), stroke,

Table 3. Building physical microenvironment for organoids.

Stimulus type	Material	Stimulus form	Organoid response	Organoid	Ref.
Mechanical force					
	gelatin methacryloyl (GelMA)-HA-Arg-Gly-Asp (RGD) hydrogel	Controlling the stiffness of medium (1.5 kPa)	Stimulated CPC proliferation	Cochlear	Xia et al.[115]
	Alginate	Manipulating the percentage of the alginate gels	Mirroring the development sequence of the spinal cord in vivo	Spinal cord	Koser et al.[117]
	PEG	Transferring strains from silicone membrane to growing organoids	Intermediate matrix stiffness improved the efficiency of neural tube organoids	neural tube organoids	Abdel Fattah et al.[118]
	A two-dimensional compartment	Forcing to expand in only x and y dimension	Revealing the biomechanics that cortical folding during development	Brain	Karzbrun et al.[119]
	SpinΩ	Continuous low-shear	Enhance cell viability and promote maintenance of the stem cell niche and reduce cost	Brain	Qian et al.[10]
	Millifuidic chip	Fluidic shear stress	More mature vascular network	Kidney	Homan et al.[63]
	PEG-RGD hydrogel	Matrix stiffness	Modeling fibrotic liver mechanics	Liver	Sorrentino et al.[120]
Light					
	allyl sulfide hydrogels	Varying the duration of irradiation	More crypt formation	Intestine	Hushka et al.[121]
	strain-promoted azide-alkyne cycloaddition hydrogel	a photoinduced allyl sulfide exchange reaction	deforming intestinal organoid epithelial shape	Intestine	Yavitt et al.[122]
	multivalent click ligand	Mild Light stimulation	Prompting cell proliferation and steering macrophages toward an M2-like phenotype	Oral cancer	Xu et al.[123]
	Sulfur- and Nitrogen-Doped Carbon Nanodots	near-infrared light	Affecting organoids viability	Breast cancer	Roscigno et al.[124]
	Optogenetic regulation	Blue light-emitting	Inhibiting the release of the parathyroid hormone	Parathyroid gland	Liu et al.[125]
	Upconversion nanoparticles	Near-infrared light	Quantifying and imaging proteins	Hepatic ductal	Liu et al.[126]
	HD-Br	Near-infrared light	Tracking lysosomal and mitochondrial interactions dynamically	Liver	Fang et al.[127]
	CBPH	Near-infrared light	Inhibiting the migration, invasion, and regenerative capacity	Breast cancer	Li et al.[128]
	BPQDs@EXO nanospheres	Near-infrared light	Damaging genetic substance and ablating	Bladder cancer	Liu et al.[129]
Electricity					
	PEDOT:PSS, SEBS	Varying currents and pulse train frequency	Evoking calcium signals	Cortex	Li et al.[130]
	A modified SCHEEPDOG bioreactor	Varying the electric field strength	Increasing in volume	Intestine	Shim et al.[131]
	Pressure-sensitive transistor	Applying an electrical stimulation pulse	Changing in ECG, pressure and calcium flux	Heart	Kim et al.[132]
	Chips consisting of a porous inner well and a solid outer well	Applying an chronic electrical stimulation	Increasing in maturity	iMB-VOs	Dailamy et al.[133]
	3D MMF	Varying voltage pulses	Evoking corresponding field potentials	Brain	Park et al.[134]

PEDOT:PSS: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate; SEBS: poly(styrene-ethylene-butylene-styrene); C: cisplatin; BP: black phosphorus; HA: hyaluronic acid; BPQDs: black phosphorus quantum dots; EXO: exosome vector; iMB-VOs: myo-vascular organoids.

and AD.[137,138] Similarly, the developmental maturation as well as proliferation of cells can be facilitated by utilizing these subtle physical microenvironments. For example, the role of the ECM in neural development was investigated by Ranga et al.,[139] who reported that different stiffnesses of the matrix modulate the development of the neural tube.

In another study, Abdel Fattah et al.[118] developed a device that can apply mechanical external forces to promote the growth and development of neural tube organoid. Meanwhile, neural tube organoid was embedded in hydrogels with different hardnesses (soft 0.7 kPa, medium 2 kPa, and hard 8 kPa), respectively, and it was found that the developmental efficiency of neural tube organoid was increased in medium hydrogels compared with soft and hard hydrogels. This phenomenon was not found in hydrogels of other hardnesses. This suggests that the mechanical forces of the extracellular matrix play a regulatory role in the development of organoid organogenesis. In addition to the effects of mechanical forces on neural development, Karzbrun et al.[119] generated brain organoids in a microprocessed culture chamber and simulated the physical process of brain folding by forcing the organoids to expand in the *x* and *y* dimensions. Organoid culture combined with microfluidics has also been used to increase the viability of organoids, for example, SpinΩ.[10] In addition to brain organoids, kidney organoids,[63] and liver organoids[120] are also affected by shear stress.

Optical stimulation

In addition to the effects of mechanical stimulation, light factors and electrical stimulation[140] affect on the growth and development of organoids. Gabriel et al.[141] generated brain organoids containing optic vesicles through a staged cell culture protocol, and the structures could sense light while sending signals to other areas of the brain.

Jgamadze's team[142] transplanted brain organoids into the brains of rats with a damaged visual cortices; the grafts established synaptic connections with their hosts, and the hosts responded to visual stimuli such as flashing lights. Given the important role of light stimulation in the regulation of neurogenesis and development, further exploration of the mechanisms underlying the effects of light stimulation is urgently needed. Although stem cell-derived organoids currently provide a foundation for the study of organs in vivo, existing recording techniques have primarily focused on measuring localized and acute neural activity in a limited number of cells within the organoids. To address this technology gap, Wilson's team[143] combined electrophysiological recordings with optical imaging of the xenografted cortical organoids and the surrounding host cortex through the use of transparent graphene microelectrode arrays. By applying a visual stimulus and recording the subsequent electrical response, they demonstrated that the cortical organoids, after being implanted in the mouse brain, were able to establish synaptic connections with the surrounding host cortical tissues. These organoids received visual stimulus inputs from the mouse brain and generated corresponding electrophysiological responses.

Similarly, Osaki et al.[27] interconnected two brain organoids cultured in vitro via axon bundles to simulate the interconnections of different regions in the brain and used optogenetics to control specific neuronal populations. In addition to the direct effect of light stimulation on organoids, the fate of stem cells is regulated by manipulating scaffold properties through the incorporation of special biomaterials with photoactivity into biocompatible scaffolds.[144–146] For example, Hushka's team[121] optimized allyl sulfide hydrogels for use in intestinal organoid cultures and modulated the formation and structure of intestinal organoid crypts through optical stimulation. Similarly, Yavitt et al.[122] utilized light-induced hydrogel cross-linking exchange reactions to alter the curvature of epithelial cells for morphogenetic control of intestinal organoids and found that the effect of light-induced viscoelasticity on mechanotransduction pathways is regulated by time, which provides new ideas and directions for in-depth study of the pathogenesis of intestinal diseases as well as for the development of new therapeutics.

In addition to exploring the effects on organoids, Liu et al.[126] developed a photosensitive nanoprobe to detect and quantify protein levels in liver organoids. Fang 's team[127] also dynamically tracked lysosomes and mitochondria by using light-sensitive probes. Similarly, by incorporating light-sensitive materials into the culture of tumor organoids, researchers have developed many new therapies to treat various tumors, including oral cancer,[123] breast cancer,[124,128] and bladder cancer.[129] In addition to culturing organoids by photostimulation, Broguiere et al.[147] also innovated the two-photon pattern technique by using photopatterned nerve growth factor to guide nerve axons. This light-responsive platform could provide a valuable in vitro model for in-depth investigation of the mechanisms of early histomorphogenesis and the development of organoids, as well as for the study of the pathology and therapeutic measures of various systemic types of diseases.

Electrophysiology system

Electrical stimulation of stem cell differentiation and developmental maturation produces considerable effects.[148] In addition to having a similar cellular structure and organ function, the neural organoids were able to mimic the electrophysiology of the early human brain. Thus, electrophysiological recording of organoid activity plays a key role in the development of the organoid field.

Traditional fluorescence imaging methods such as calcium indicators are limited owing to phototoxicity and temporal resolution, and traditional electrophysiological

recording studies using membrane clamps or multi-electrode arrays to monitor electrical activity are often short-term or even require slicing of organoids and functional measurements in a planar, 2D format, which greatly limits functional monitoring of 3D cultures and greatly affects electrophysiological recording efficiency and reliability of electrophysiological recordings.[149] Park et al.[134] achieved the matching of electrode arrays to other 3D cultures, such as destination organoids, by utilizing reversible engineering control over the geometry of the cultures. Although breakthroughs have been made in the electrophysiological monitoring of 3D cultures, they can bring organoids into contact with the substrate, which in turn affects the normal structure and function of the organoids. On the basis of this, Yang et al.[26] developed an electronic recording platform called "kirigami," inspired by paper cutting and 3D folding. This platform can spontaneously transform into various 3D geometries in the suspended state, seamlessly integrate complete neurological organoids and assemblies, and support long-term recordings without interfering with the organoid's differentiation and development.

In addition to the monitoring of electrophysiological information in organoids, exogenous electrical stimulation also promotes the proliferation and differentiation of stem cells.[148] Tai et al.[150] have devised a technique that utilizes hydroacoustic excitation to induce the piezoelectric effect in electrospun poly-nanofiber scaffolds. This technique drives the generation of electrical charge, which in turn confirms the multidirectional differentiation of neural stem cells and controls their fate through exogenous electrical signals. Li et al.[130] developed a stretchable electrode system that can be tightly integrated with neural organoids to trigger calcium signals by applying electrical stimulation. The electrical properties of the organoid can be flexibly conditioned at different current amplitudes and frequencies. In addition to studying the normal nervous system, the 3D neural technology platform developed by Park et al.[134] has made significant contributions to exploring neurodevelopmental disorders. Other organoids also respond to electrical stimulation, Shim's team[131] also applied external electric field stimulation to 3D kidney and intestinal organoid models and observed that the electric field was able to cause significant tissue expansion.

Recently, electrophysiological tools with electronic devices have been used to study the electrical function of cardiac organoids during cardiac development. For example, Kim's team[132] embedded three-dimensional liquid metal electrodes with a low modulus and low impedance into the heart organoids to achieve ECG signal acquisition and electrical stimulation without restricting the development of the organoids. By applying chronic electrical stimulation to myovascular organoids, Dailamy et al.[133] demonstrated that electrical stimulation is a potential pathway for organoids to mature and vascularize skeletal muscle tissue.

Response mechanisms combining other physicochemical stimuli (e.g. temperature, magnetic fields, metal ions, enzymes, ultrasound, pH, etc.) can be engineered to promote physical cues for organoid development.[151–153]

Information acquisition and processing

Organoid imaging analysis

Due to the unique 3D structure of organoids, the application of conventional imaging techniques faces great challenges in maximizing the advantages of organoid systems. Thus, there is a need to utilize advanced imaging techniques to dig deeper into their complex structures and functions. With the aid of advanced microscopy techniques such as confocal microscopy,[154] light sheet microscopy,[155] and two-photon microscopy,[156] it is possible to acquire images with higher resolution than conventional microscopes.

3D imaging offers more detailed and comprehensive information about organoid structures, including cell types and shapes. Given the unique opacity of organoids, the tissue transparency technique is particularly suited for their study. This technique allows researchers to clearly observe the internal structures of tissues in three-dimensional space without damaging them. It involves removing or replacing light-scattering elements with special solutions to render the tissues transparent, and then using microscopes capable of capturing the structure of these transparent tissues. Currently, transparent solutions such as SeeDB[157] and ScaleA2[158] are more widely used. By introducing fructose glycerol into tissue transparency techniques, Rios et al.[154,159] obtained high-resolution volumetric images of multiple organ types, such as the intestines, mammary glands, and breast tumors, and the fructose glycerol reagent had greater transparency and quench resistance effects than conventional transparency protocols.

In addition to tissue transparency techniques, Khan et al.[160] present a positron emission microscopy approach that enables visualization as well as quantification of glycolytic activity in tumors by imaging a radiotracer (18F-fluorodeoxyglucose used here) in organoids. It can be used for personalized diagnosis and treatment with translational capabilities.

Biosensor

Electrochemical biosensors have also been used to some extent in organoid analysis, where they are immune to ambient light interference, are not dependent on microscopy, avoid the use of complex data analysis software, and are able to dynamically monitor the functional activity of organoids in real time, enabling the monitoring of multiple biomarkers through the use of small sample sizes, unlike microscopy image analysis.[22,32] Zhang et al.[161] achieved continuous monitoring of cardiac and hepatic organs by

integrating multiple biosensors into an organs-on-a-chips platform, which enables continuous and automated monitoring of a range of microenvironmental parameters (e.g. pH, oxygen, temperature, and several biomarkers) such as drug toxicity through the administration of adriamycin, acetaminophen, and biomarkers such as albumin, glutathione S-transferase alpha, and creatine kinase. Creatine kinases and other biomarkers were used to assess their toxicity. In addition to monitoring a single organoid, Lee et al.[162] constructed a way to culture breast cancer and cardiac organoids on the same platform, where they can communicate with each other, and by using electrochemical immunoadaptor sensors, they can noninvasively monitor biomarkers in the response of both to chemotherapeutic agents for the purpose of early monitoring and prediction of chemotherapy-induced cardiotoxicity. Similarly, Nguyen et al.[163] developed a dual-sensing nanoreporter capable of feeding back the activities of two key enzymes involved in T-cell-mediated target cell death during and subsequent to immune checkpoint antibody treatment, achieving efficient monitoring of dynamic immune processes in the tumor microenvironment and promising early prediction of treatment outcomes.

Although a wide variety of biosensors have been used in biomedical research, monitoring the different locations of organoids is still somewhat deficient because of their unique three-dimensional structure. To address this problem, Kalmykov's research team[164] developed a 3D self-recoiling biosensor array (3D-SR-BA) triggered by electricity, pH, magnetism, or other physicochemical factors that transforms 2D electrodes into 3D shapes. By evaluating the electrode arrays via cardiac analogs, phase coordination between calcium transients and potentials was observed, revealing a great deal of potential for monitoring the electrophysiological activity of the analogs. The accuracy of physiological signals from organoids still needs to be optimized due to differences in cell source and culture conditions as well as sensor design. Converting the physicochemical signals of organoids into generalized information still urgently needs further research. Additionally, the complex metabolites of organoids require more strict materials for sensors.

Conclusions and perspectives

This review provides an overview of engineering strategies to overcome the limitations of traditional organoids. Although these technologies have essentially optimized the structure, and function, there is still potential for improvement.

The development of organs in vivo requires a certain growth environment, in particular, various mechanical parameters such as stress-strain and forces of interaction between cellular tissues and between cells and body fluids. There is no unanimous consensus on the growth environment in which organoid development occurs. The external environment needs to be accurately regulated to better approximate the real in vivo physiological environment of the human body, which in turn guides the differentiation and developmental maturation of the organoid. By combining microfluidics and organs-on-a-chips technologies, the perfusion of organoids simulates the real fluid shear force, mechanical stress, and other factors in vivo and restores the environment in the human body, which can promote the differentiation and developmental maturity of organoids.

In addition to nutrient delivery, the design of the organoids-on-chips also considers that the simulated fluid must not generate excessive fluid force, as high pressure can damage tissue cells. Furthermore, measures are taken to ensure that the flow within the channels is not affected by bubbles or other factors and that the material of the chip does not absorb drugs or other chemicals. Currently, organoids still have certain limitations in replicating the complex in vivo environment. For example, the liver exhibits metabolic heterogeneity across different regions of hepatocytes, impacting its executive function. Similarly, establishing a gas-liquid interface culture for the respiratory tract has significant potential.

The complex and changeable external environment leads to a high degree of variability in the phenotype of organoids, which also restricts the clinical transformation of organoids to a certain degree. Additionally, simulating real organs with organoids suffers from a lack of vascularization and immune function, the complexity of Matrigel in mimicking the ECM, and heterogeneity. In addition to the limitations of the culture microenvironment, the blood flow and immune function of the organoids are also a formidable challenge. Consequently, the results obtained from biomedical experiments conducted solely on organoids often differ from those observed in the real in vivo environment. Therefore, the integration of multiple cell types and spatial control using 3D printing and microfluidics can maximize the reproduction of the real ECM environment in vivo. The use of 3D printed microarrays to enable precise manipulation of individual organoids has led to a significant reduction in the batch variation of organoids and enhanced their homogeneity. The use of organoid culture media and Matrigel also limits their further clinical translation due to their unspecified composition, which may cause structural-functional variability of organs as well as adverse toxic reactions. In the future, new alternatives that are closer to the condition of real organs in the human body, for example, for culturing brain organoids using a medium that is closer to the composition of cerebrospinal fluid, are expected to be developed.

Apart from issues in culture and application, real-time monitoring various functional indices of organoids, which requires an in-depth exploration of the complex structure of organoids and their fine cellular behaviors with the help

of engineering tools to reproduce the interactions between the organs and the real cellular ecological behaviors, is challenging. Smirnova et al.[165] introduced the term "organoid intelligence" (OI), which aims to expand the definition of biocomputing to include brain-directed OI computations, that is, to utilize the self-assembly mechanisms of brain organoids to memorize and compute inputs. The human brain is incomparable to computers in terms of information processing efficiency as well as energy efficiency. In the future, it is expected that the combination of the human brain and machines will be realized through the engineering and intellectualization of brain organoids into the currently emerging ChatGPT to mutually integrate with artificial intelligence and thus complement each other's strengths. The expandability, feasibility, and durability of the organoids are supported through the use of microfluidic devices that provide various types of external information, such as electrical, chemical, and machine-programed signals with purposeful information to the organoids, and high-resolution outputs of electrophysiological signals obtained from microelectrode arrays and implantable probes that are analyzed, can be used directly for computation, and can be used as biofeedback to facilitate learning, to facilitate organoid learning.

The convergence of organoids and AI provides new opportunities to elucidate not only the biological mechanisms of human cognition, learning, and memory, but also new tools for a deeper understanding of the pathogenesis of diseases in various systems and for the development of new therapeutic regimens to address the numerous clinical needs of the future.

While organoid models have been validated with some known drug groups, such as cardiac organoids, and routinely incorporate cardiac fibroblasts, challenges remain in achieving long-term stable, perfusable blood vessels and incorporating immune cells to better mimic inflammatory (myocarditis), autoimmune (lupus), and regenerative (treatment of myocardial infarction) conditions. More work is needed to explore the interactions between neurons and the myocardium to better model the effects of the autonomic nervous system, capture circadian rhythms and heart rate variability, and understand metabolic effects. In addition, further development of sex-specific models of hormone-treated and senescent organ models is needed.[166] Any patient's tissue can be used to create personalized organoids, although there are some differences in growth rates between patient tissue samples. Cells can be stored in biobanks and passaged in culture for long periods of time while retaining patient-specific phenotypes. This robustness is critical for standardization and clinical application.[167]

Microphysiological systems (MPS), including organoid and organ-on-a-chip technologies, can overcome the limitations of the two major experimental platforms, cell culture and model animals, which are widely used for drug efficacy and safety. Patel et al.[168] compared MPS cultures to static cultures and found that the model predicted a reduction in hypoxia and that the MPS platform maintained organoid viability and function. In addition to improving organoid survival, it also provides the ability to evaluate in situ parameters. Similarly, the MPS platform has revealed the gastric mucosal defense mechanism of H. pylori.[169] Other organ classes, such as kidney,[63] liver,[170] and renal cancer,[171] have also been explored for disease and drug screening through integration with MPS.

Engineered organoids represent a critical step toward high-resolution modeling of quantitative assays of complex biological processes. Bioengineered organoids are therefore a valuable addition to the library of in vitro models and open exciting prospects for translational research, especially in the field of drug discovery. The focus of the organoid should be on the purpose of the model, namely the biological process it is designed to capture and the question we want to answer. This purpose should guide our choices in terms of model complexity and throughput.

In conclusion, organoid research is still in its infancy, and addressing these challenges requires a multidisciplinary effort. We believe that the engineering of organoids represents a new era for realizing the translation of organoids from the laboratory to the clinic.

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