Review

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Organoids as preclinical models of human disease: progress and applications

Abstract: In the field of biomedical research, organoids represent a remarkable advancement that has the potential to revolutionize our approach to studying human diseases even before clinical trials. Organoids are essentially miniature 3D models of specific organs or tissues, enabling scientists to investigate the causes of diseases, test new drugs, and explore personalized medicine within a controlled laboratory setting. Over the past decade, organoid technology has made substantial progress, allowing researchers to create highly detailed environments that closely mimic the human body. These organoids can be generated from various sources, including pluripotent stem cells, specialized tissue cells, and tumor tissue cells. This versatility enables scientists to replicate a wide range of diseases affecting different organ systems, effectively creating disease replicas in a laboratory dish. This exciting capability has provided us with unprecedented insights into the progression of diseases and how we can develop improved treatments. In this paper, we will provide an overview of the progress made in utilizing organoids as preclinical models, aiding our understanding and providing a more effective approach to addressing various human diseases.

Keywords: organoids; human disease; regenerative medicine; drug screening; tumor

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Introduction

Over the past half-century, there has been remarkable progress in the field of stem cell research. In 1961, Professor Ernest A. McCulloch made a groundbreaking discovery, finding that cells from the bone marrow of mice could differentiate into various cell types. He coined the term "pluripotent stem cells (PSCs)" [1]. Professors Rheinwald and Green described the first successful serial cultivation of human epidermal cells [2]. It wasn't until 1981 that scientists successfully isolated karyotypically normal embryonic stem cells from the inner cell mass of mouse blastocysts for the first time [3] and co-cultivated them *in vitro* with mitotically arrested fibroblasts. This co-cultivation was hypothesized to provide essential trophic factors for maintaining embryonic stem cell pluripotency [4]. In 1998, James Thomson and his colleagues established an embryonic stem cell line from human *in vitro* fertilized blastocysts that could be stably cultured *in vitro* with factors maintaining self-renewal and inhibiting differentiation [5]. Stem cells are known for their self-renewal and differentiation potential, allowing them to develop into specific cell types under suitable environmental conditions or through artificial induction [6]. For example, in 1980, Green and his colleagues successfully used autologous

keratinocyte sheets to treat two patients with third-degree burns at the Peter Bent Brigham Hospital [7]. These advancements collectively have fostered the idea of using human stem cells to culture three-dimensional (3D) organs, holding great promise for disease treatment and drug screening.

In 1907, Professor Wilson HV achieved the first *in vitro* regeneration of isolated sponge cells into complete organisms through self-organization [8] (Figure 1). Subsequently, scientists generated organ-like tissues from isolated chicken embryos through dissociation-reaggregation experiments [9]. In 1978, Li et al. found that mammary epithelial cells cultured on EHS substrates can form 3D ducts and lumens capable of synthesizing and secreting milk proteins, compared to two-dimensional (2D) cultures [10]. In 2009, scientists used adult stem cells for the first time to generate 3D gut-like organs through self-organization in a matrix [11]. Organoids can be derived from both embryonic stem cells and adult stem cells [11, 12]. Milestone achievements in somatic cell reprogramming technology have significantly enriched the sources of stem cells and organoids [13], facilitating the establishment of *in vitro* 3D organ processes. In recent decades, the field of organoids has made impressive strides. The term "organoid" commonly refers to different 3D tissues in *vitro* culture, ranging from cell aggregation to organ-on-chip systems [14]. Organoids represent complex 3D tissues that mimic the structure and function of organs *in vivo* and are derived from stem cells specifically differentiated through a self-organizing process [15].

In the past few decades, the field of organoids has made remarkable progress (Tables 1 and 2). As an emerging research platform, organoids have successfully addressed the limitations of both 2D cell culture and animal models [16]. Conventional monolayer cell cultures lack essential cellular interactions, organization, and complexity, and the immortalization of 2D cells can result in significant genetic alterations. While genetically engineered animal models have been widely used for disease modeling, they cannot fully replicate the genetic background and physiological conditions of humans. Human-derived organoids have the capacity to faithfully replicate the intricate pathological and physiological processes of *in vivo* organs. This capability holds great promise for applications in organ development, disease modeling, precision medicine, and drug discovery. In this context, we will describe the current development status of adult cells, stem cells, and tumor-derived organoids, and discuss their diverse applications in basic biology and preclinical research, covering the latest advances in organoid research. Finally, we will emphasize the key challenges that organoids face and their potential prospects for various applications.

Advances in organoid culture environments

Most organoids require a matrix gel to support the formation of 3D structures during development, and the most traditional and commonly used substrate is Matrigel, also called Cultrex or Engelbreth-Holm-Swarm (EHS) matrix. Matrigel, an EHS mouse sarcoma extract [17], contains
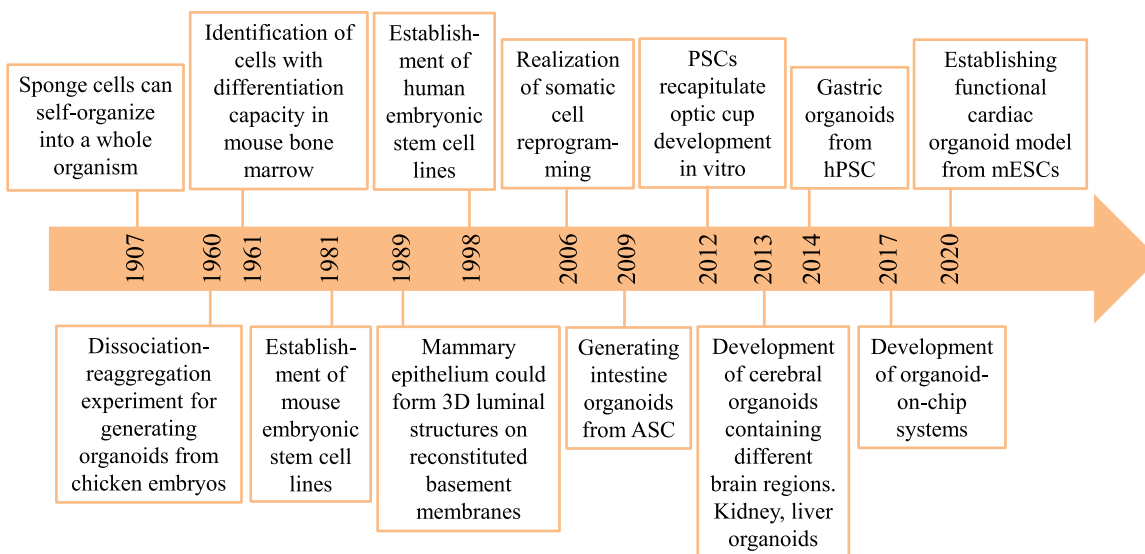

Figure 1: Timeline for the development of different types of organoids. Summary of research on critical landmarks in the establishment of various organoids. 3D, 3-dimensional; ASC, adult stem cells; hPSC, human pluripotent stem cells; mESC, embryonic stem cells.

Table 1: Summary of organoid models.

Organoid models	Organoid source	Species	Applications	References
Intestine	AdSCs	Human	Modeling of SARS-CoV-2 infection, drug screening	[37, 149]
	iPSCs	Human	Drug screening, toxicology research	[146, 147, 151]
Liver	iPSCs	Human	Gene editing, transplantation, drug screening, modeling of HBV infection	[194, 236]
	ESCs	Human	Drug screening, personalized treatment, modeling of alcohol-related liver disease	[141, 142, 237]
	AdSCs	Human or mouse	Drug screening, transplantation modeling of liver cancer	[238]
Lung	AdSCs	Mouse	Modeling of adenocarcinoma of lung, virus pathology, drug screening	[49, 56, 185]
	ESCs	Human	Modeling of lung differentiation and disease	[156]
	iPSCs	Human	Drug screening, transplantation	[159]
Heart	ESCs	Mouse	Regenerative medicine	[62]
	iPSCs	Human	Gene editing, modeling of disease	[63, 64]
Brain	ESCs	Human	Modeling of disease, gene editing; single-cell sequencing, assembly technology, vascularization technology	[67, 75–78]
	iPSCs	Human	Modeling of microcephaly, Parkinson's disease and amyotrophic lateral sclerosis disease	[92, 123, 124, 131]
	AdSCs	Human	Modeling of Alzheimer's disease	[113, 114]
Kidney	Npcs	Human or mouse	Gene editing, modeling of disease, drug screening	[239]
	iPSC	Human or mouse	Transplantation	[240]
	ESCs	Human	Toxicology research, transplantation	[241]
Pancreas	AdSCs	Human	Modeling of pancreatic cancer, drug screening, transplantation	[43, 242, 243]
Retina	iPSC	Mouse	Modeling of advanced retinal degenerative disease	[163]
	ESCs	Human or mouse	Transplantation	[25, 244]
Breast	AdSCs	Human	Drug screening, personalized treatment	[198, 201]
Inner ear	ESCs	Human or mouse	Modeling of disease	[169, 170]

AdSCs, adult stem cells; iPSCs, induced pluripotent stem cells; ESCs, embryonic stem cells; HBV, hepatitis B virus.

Table 2: Summary of media compositions for different organoids culture.

Organoid culture	Small molecule compounds	Cytokines
Cerebral organoids	Y-27632, MK-2206, GDC-0068, dorsomorphin	FGF-basic, Noggin, DKK-1, EGF, BDNF, GDNF, R-Spondin 1
Intestinal organoids	Y-27632, SB-202190, A 83-01, gastrin, nicotinamide	EGF, Noggin, R-Spondin 1, Wnt-3a, FBS
Liver organoids	Y-27632, A 83-01, DAPT, forskolin, gastrin, nicotinamide, prostaglandin E2	BMP-4, EGF, FGF-basic, FGF-10, HGF, Noggin, Wnt-3a, FBS
Pancreas organoids	Gastrin I, A 83-01, nicotinamide	FGF-10, EGF, Noggin, R-Spondin 1, Wnt-3a, B27
Prostate organoids	Y-27632, SB-202190, A 83-01, nicotinamide, prostaglandin E2, testosterone	EGF, activin A, FGF-basic, FGF-10, Noggin, R-Spondin 1, Wnt-10b
Lung organoids	CHIR-99021, SB-431542	Activin A, FGF-basic, FGF-4, Noggin, N2
Mammary organoids	Y-27632, phosphorylethanolamine, isoproterenol, hydrocortisone	Here gulin β-1, R-Spondin 1, R-Spondin 2, Noggin, EGF, FGF-basic, FGF-10, Wnt-3a, prolactin
Kidney organoids	CHIR-99021, retinoic acid, hesparin	BMP-2, BMP-4, BMP-7, FGF-basic, FGF-9

FGF, fibroblast growth factor; EGF, epidermal growth factor; HGF, hepatocyte growth factor; BMP, bone morphogenetic protein.

laminin and a number of growth factors, which are extensively used in organoid cultures, such as gastric [18], mammary glands [19], intestinal [11], and brain [20]. Due to the uncertainty of the compositions of Matrigel and the limitations of clinical applications, more types of matrix gels were subsequently developed. One approach was to produce

matrix gel by tissue decellularization. It was demonstrated that hydrogel substrates fabricated from decellularized porcine small intestine could provide better support for the growth and expansion of endoderm-derived organoids, overcoming the limitations of Matrigel tumor sources [21]. Residual animal protein constituents in matrix gels may trigger an immune response in the host, whereas synthetic macromolecular polymer matrix gels prevent this problem. Sorrentino et al. used PEG as a skeleton to integrate key extracellular matrix (ECM) proteins found in the liver, such as laminin-111, type IV collagen, and fibronectin, to create a fully chemically synthesized matrix. It was successfully employed for liver organoid culture [22]. Curvello and his colleagues developed a plant-derived matrix gel, 0.1 % plant nanocellulose fibers, which successfully cultured small intestinal organoids [23].

Cultures of nervous system-like organoids like cerebrum [20], hippocampus [24], and optic cup [25] are mostly cultured in suspension, allowing the stem cells to self-organize into 3D cell aggregates in liquid suspension. Recently, suspension culture methods have also been used for the development of other types of organoids. Capeling and her colleagues found that simple suspension cultures promoted the development of serosal mesothelial in human intestinal organoids. A comparison of suspension cultured organoids, human tissue and matrix gel cultured organoids revealed that suspension cultured organoids were more similar to human tissue at the molecular level.

Organoid chips are microfabricated cell culture devices that mimic the functional units of human organs *in vitro* [26]. Recent studies have demonstrated that the use of organoid-on-a-chips to construct and simulate human tissue micro-environments can recapitulate the specific physiological functions in some organs [27–29], and has a wide range of applications in the fields of organ development, regenerative medicine, drug screening, and disease modeling in the future.

Modeling human disease with organoids

Organoids derived from adult stem cells

There are two primary sources of organoids: adult stem cells (AdSCs) and PSCs, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Organoids derived from AdSCs are obtained directly from human or mouse adult tissues and are further recapitulated within a tissue-like environment by suspending them in ECM and supplementing them with growth factors. In comparison to organoids derived from PSCs, AdSCs-derived organoids demonstrate superior performance in mimicking physio-logical and pathological conditions [30, 31].

Adult stem cells derived small intestine organoids

The first AdSC-derived organoids were developed by Hans Clevers' research group. They established the first long-term *in vitro* culture of single crypts in Matrigel and maintained the crypts' structure by supplementing them with epidermal growth factor (EGF) and R-Spondin1 [32]. These generated organoids consisted of over 40 crypt domains surrounding a central lumen lined with a villus-like epithelium, referred to as the 'villus domain.' This crypt structure was maintained for a remarkable 8 months. Moreover, they successfully identified and isolated the specific population of AdSCs capable of retaining their stem cell properties and forming large crypt organoids. Lgr5$^+$ cells were found to retain these stem cell properties, and individual Lgr5-expressing stem cells could spontaneously create self-organizing crypt-villus organoids in the absence of non-epithelial niche cells [11]. Lgr5 crypt stem cells exhibited the capacity to undergo thousands of cell divisions, and a 'mini-guts' culture system was established, involving both whole crypts and single Lgr5$^+$ stem cells. This system utilized a serum-free medium supplemented with three recombinant proteins: R-Spondin1 (a Wnt signal amplifier and a ligand for Lgr5), EGF, and the bone morphogenetic protein (BMP) inhibitor Noggin [33].

In contrast to mouse intestinal crypts, which are equipped with Paneth cells capable of secreting some niche growth factors [32], human gut organoid cultures lack fully functional Wnt-secreting niche cells and necessitate the addition of exogenous Wnt3a to the culture medium [34]. For the sustained maintenance of human gut organoids through multiple passages, both a TGFβ pathway inhibitor and a p38 MAPK pathway inhibitor are essential. However, the removal of the p38 MAPK inhibitor is a prerequisite for the emergence of secretory cells [35]. These inhibitors were subsequently replaced by insulin-like growth factor 1 (IGF-1) and fibroblast growth factor 2 (FGF-2). Organoids faithfully recapitulate the characteristics of their respec-tive tissues compared to traditional 2D culture, offering significant potential in drug discovery, the study of the tumor microenvironment, exploration of tumor heteroge-neity, predictive modeling of responses, and personalized medicine [31, 36].

Furthermore, the intestine, often referred to as the "second immune system", plays a substantial role in microbiome infections. Intestinal organoids have proven invaluable in understanding the pathogenesis of colorectal

cancer caused by bacterial agents. In addition to the lungs' susceptibility to SARS-CoV-2 infection, human intestinal organoids can also be infected by SARS-CoV-2, leading to the production of substantial quantities of infectious viral particles within the intestinal environment [37].

Adult stem cells derived hepatic and biliary organoids

Hepatic and biliary (HPB) organoids can be generated through step-wise methods using either PSCs or iPSCs. Additionally, these organoids can be generated from adult human or mouse tissues. These HPB organoids are identified based on their specific tissue source, and their *in vitro* cell types closely mirror the guidelines outlined by Hans Clevers [38]. Hepatic organoids typically consist of cells or tissues derived from hepatocytes and cholangiocytes. LGR5, recognized as a marker for intestinal stem cells, has also been identified as a stem cell marker in various tissues. In the context of carbon tetrachloride (CCl4)-induced injury, Lgr5+ cells emerge in proximity to bile ducts. Furthermore, individual Lgr5+ cells from the damaged mouse liver can be clonally expanded as organoids when cultured in an Rspo1-based medium over an extended period. These cultured organoids, under the aforementioned conditions, express multiple markers associated with progenitor, hepatocyte, and cholangiocyte characteristics, suggesting a bipotential nature [39].

For the purpose of inducing hepatocyte differentiation, the culture medium was modified by eliminating R-Spondin and FSK, and substituting them with the Notch inhibitor DAPT, FGF19, dexamethasone, and BMP7. This alteration facilitated the expression of hepatocyte markers [40]. In 2018, Huch and colleagues developed a protocol for the long-term culture of mouse hepatocytes and human fetal liver cells as organoids. This protocol marked a departure from previous methods as it exhibited a morphological resemblance to cholangiocyte organoids. To achieve this, they employed small-molecule inhibitors, including Wnt agonists like R-Spondin1 and CHIR99021, the TGF-b inhibitor A83-01, and growth factors such as EGF, FGF7, FGF10, and hepatocyte growth factor (HGF).

The mouse Hepatocyte Organoids (mHOs), generated through this protocol, displayed a characteristic 'bunch-of-grapes' structure. These organoids expressed specific hepatocyte markers, including Alb, Hnf4a, Cyp1a2, Cyp3a11, and the fetal hepatocyte marker Afp. Importantly, they did not exhibit markers associated with cholangiocytes or progenitor cells, such as Krt19, Tbx3, and Sox9. Additionally, these mHOs bore a resemblance to hepatocytes observed after partial hepatectomy [41].

Hepatocyte organoids, aside from Wnt-mediated cultures, have been successfully established using an inflammatory cytokine-mediated expansion protocol for long-term cultured mHOs. In the context of liver regeneration, tumor necrosis factor-alpha (TNF-α) activates a series of transcription factors, including nuclear factor kappa B (NF-κB), Janus kinase (JAK/STAT), activator protein 1 (AP-1), and Yes-associated protein (YAP), which enhance cell proliferation. This culture condition presents an innovative approach to expanding primary mouse hepatocytes in 3D culture [41]. Under these expansion conditions, mHOs could be sustained for at least 8 months and exhibited similarities to proliferating hepatocytes following partial hepatectomy (PHx) [42]. This study introduces a novel approach to establishing hepatocyte organoids. The hepatocyte organoids culture system could be extended to propagate primary liver cancer (PLC) organoids, covering three of the most prevalent PLC subtypes: hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and combined HCC/CC (CHC) tumors [43, 44].

Adult stem cells derived lung organoids

Lungs are complex structures characterized by a branching network of airways and blood vessels, resembling a tree-like configuration. This intricate system begins with the trachea and extends into branching airway tubes, ultimately culminating in millions of air sacs known as alveoli where gas exchange with the vasculature takes place [45, 46]. The most proximal airway, the trachea, bifurcates at the carina, giving rise to the left and right main stem bronchi. Each main bronchus further subdivides into secondary or lobar bronchi, which subsequently branch into progressively narrower airways until the smallest bronchioles connect to the alveoli [47]. Various cell types are present in human lungs, encompassing epithelial cells, endothelial cells (comprising vasculature and lymphatics), pleura/mesothelium, airway and vascular smooth muscle cells, pericytes, fibroblasts, neurons, and immune cells such as alveolar macrophages [48].

Lung organoids can be generated from various cell types, including basal cells, airway secretory cells, and alveolar type II cells (AEC2 cells). Two primary approaches to obtain target cells are lineage-tracing and surface marker-based cell sorting. The initial organoids derived from mouse tracheal basal cells were referred to as "tracheospheres." These cells were isolated from airway basal cells expressing Trp-63 (p63) and cytokeratins 5 (Krt5) and Krt14. Basal cells play a crucial role in generating differentiated cells not only during postnatal growth but also in adults under steady-state conditions and during epithelial repair [49, 50].

Organoids obtained from isolated basal cells may be classified as either tracheospheres or bronchospheres, depending on whether the basal cells originate from the trachea or the larger airways. Typically, these organoids comprise TRP63$^+$ KRT5$^+$ basal cells, functional multiciliated cells, and secretory goblet cells [51, 52].

Organoids derived from airway secretory cells are primarily generated by isolating secretory Club cells based on surface markers. Lung epithelial cells that fulfill specific criteria, being CD45 (*Ptprc*)neg, CD31 (*Pecam1*)neg, EpCAM high, CD49f (*Itga6*)pos, CD104 (*Itgb4*)pos, and CD24 low, are capable of forming spheres. These spheres can be categorized into three general groups based on their morphology after 14 days of culture [53]. To generate 'alveolospheres', a lineage-tracing approach was initially employed [54]. In the case of human AEC2s, isolation is typically achieved using a monoclonal antibody that specifically targets human AEC2s, known as HTII-280 [55]. Throughout the COVID-19 pandemic, human lung organoids have proven to be a valuable preclinical model for investigating virus pathobiology and advancing therapeutic development. SARS-CoV-2 productively infects AEC2s, leading to the initiation of an innate immune response. The infection triggers both cell-autonomous and non-cell-autonomous apoptosis, potentially contributing to alveolar injury [56, 57].

Organoids derived from pluripotent stem cells

Cardiac organoids

Cardiovascular diseases are the leading global cause of death. It has long been difficult to obtain satisfactory and ideal cardiac models for cardiovascular disease studies *in vitro*. Recent advances in cardiac organoids have provided access to more accurate models with complex structures and functional maturation for unveiling cardiac developmental biology, modeling cardiovascular diseases, establishing relevant drug screening platforms, and exploring transplantation therapies.

A large body of studies has provided insights into methods of constructing heart-like organs and cardiac developmental biology. In the past decade, scientists have successfully employed tissue engineering techniques to facilitate the formation of cardiac chambers and emulate organ complexity, including hydrogels and biomaterial-based scaffolds [58]. Recent studies have made significant progress in generating cardiac organoids that can simulate heart development *in vivo* in a spatial and temporal manner through self-organization. These organoids are powerful

tools for studying cardiac development [59, 60]. The first functional 'mini-heart' organoids induced by mouse embryonic stem cells (mESCs) were reported in 2020 [60]. mESCs undergo a self-organized event to form heart-like structures when exposed to FGF4 and LN/ET. These structures showed considerable similarity to the developing heart *in vivo*, possessing four intact chambers and a conducting system that exhibited myocardial contraction and action potentials [60]. In a pioneering study published in 2021, Professor Mendjan and his group developed the first *in vitro* self-organizing human cardiac organoid model. This model spontaneously forms a cavity and beats on its own without the need for stent support. They found that cavity morphogenesis is governed by a mesodermal WNT-BMP signaling axis, and the cardiods can autonomously mobilize cardiac fibroblasts to migrate and repair damage after injury [59].

Cardiac organoids, which possess a 3D structure allowing them to mimic interactions between different cell types and complex pathophysiological processes, serve as an excellent platform for cardiovascular disease research. These miniature models are invaluable for investigating conditions like myocardial infarction, arrhythmia, and genetic cardiac diseases. Complementing pre-clinical models, cardiac organoids help uncover pathogenic mechanisms and detect drug toxicity and side effects. In a recent study by Richards et al., they created cardiac organoids that leverage nutrient transport principles and stimulated them with the neurotransmitter noradrenaline to mimic the post-myocardial infarction structure of the human heart *in vitro*. Through transcriptomic analysis and structural and functional validation, they successfully established an *in vitro* 3D model of myocardial tissue after myocardial infarction [61]. Voges and colleagues demonstrated that human cardiac organoids resembling fetal heart tissue could model acute myocardial infarction following cryoinjury with a dry ice probe. These cardiods exhibited an endogenous regenerative response, achieving full functional recovery within 2 weeks of acute injury. This study highlights the regenerative capacity of immature human heart tissue in response to injury [62]. In a recent study, Hofbauer et al. utilized an advanced hPSC-derived self-organizing cardiac organoid model to assess the effects of freezing injury on the heart. They observed that cardiac fibroblasts migrated toward the injury site and produced proteins to repair the damage [59]. Cardiac organoids are also utilized to study arrhythmia. Goldfracht et al. developed an arrhythmia model based on atrial engineered heart tissues and demonstrated its effectiveness by applying relevant pharmacological interventions. They confirmed the ability of the antiarrhythmic agents flecainide and vernakalant to

terminate re-entrant activity in the atrial organoid model [63]. Today, the combination of gene editing technology and cardiac organoid disease models holds promise for addressing genetic heart diseases. Mutations can be precisely corrected and mitigated to provide personalized treatment for patients. For instance, Chengzu Long and his colleagues corrected Duchenne muscular dystrophy (DMD) mutations through exon skipping in 3D engineered heart muscle. This approach led to restored dystrophin expression and the corresponding mechanical force of contraction. Their work demonstrated that single-site genomic editing in just 50 % of cardiomyocytes is sufficient to rescue mutant engineered heart muscle phenotypes to near-normal control levels [64]. However, it is important to note that current cardiac organoids remain relatively simplified, lacking the tissue microenvironment found *in vivo*, such as the immune and nervous systems. Bridging this gap between *in vivo* and *in vitro* applications of cardiac organoids will require further efforts and advancements.

Cerebral organoids

Studying and effectively treating diseases of the nervous system is a formidable challenge, primarily due to the intricate nature of the human brain and its inaccessibility. The human brain, arguably the most complex organ in our body, comprises an extensive array of highly specialized cells that function in an interconnected manner [65]. While there have been promising advances in investigating the molecular and biological mechanisms of neurological development and neuropsychiatric disorders using monolayer neuronal cultures derived from human pluripotent stem cells and animal models, there are limitations. The former lack precise cellular connectivity across various regions of the brain, as well as many distinguishing features and species differences that hinder animal models in neurobiology research [66]. Brain organoid techniques offer a solution to the shortcomings of animal models and 2D cellular models. They provide a more accurate representation of *in vivo* conditions by reproducing the diversity of human neural cells, the physiological environment of nervous system development, and the intricacy of neural networks.

The brain organoid technique, first established in 2013 [20], originated in Knoblich's laboratory. They developed a 3D organoid culture system using human pluripotent stem cells to generate cerebral organoids. These brain-like organoids have the ability to develop various discrete but interdependent brain regions. The cerebral cortical regions within these organoids faithfully recapitulate features of human cortical development, including apical-basal polarity, dorsal cortical cytoarchitecture, interkinetic nuclear migration during radial glial division, and the pattern of neural migration [20]. Cerebral organoids typically originate from pluripotent stem cells, including ESCs derived from early embryos and iPSCs obtained through somatic reprogramming. PSCs can be cultured and differentiated into various types of human cerebral organoids within 3D environments, either through the intrinsic self-organizing capacity of aggregated cells or by induction with specific developmental regulators [67]. To date, researchers have successfully established a variety of cerebral organoids, including whole-brain and specific brain region organoid systems, such as those modeling the cerebral cortex, midbrain, cerebellum, ventral telencephalon, thalamus, hypothalamus, striatum, and hippocampus [24, 68–73]. Numerous studies have shown that cerebral organoids effectively simulate *in vivo* processes like neurogenesis, neuronal migration, cortical stratification, and the establishment of neural circuits. They have significantly advanced our understanding of neurodevelopmental disorders and neurodegenerative diseases *in vivo* [66]. Substantial progress has been made in the study of conditions such as Miller-Dieker syndrome (MDS), microcephaly-related disorders, autism spectrum disorder (ASD), tuberous sclerosis complex, brain tumors, and Alzheimer's disease (AD), among others [74]. Notably, an increasing number of cutting-edge technologies, such as gene editing, single-cell sequencing, assembly techniques, and vascularization technologies, have been applied to cerebral organoids. This holds great promise for the advancement of research and treatment in the field of neurological disorders [75–78].

Neurodevelopmental disease

Early neurodevelopmental abnormalities can lead to malformations of the cerebral cortex, giving rise to disorders such as lissencephaly, microcephaly, Timothy syndrome, and tuberous sclerosis complex, as well as neuropsychiatric disorders, including ASD and schizophrenia (SZ). Human cerebral organoids have emerged as a promising platform for the study of neurodevelopmental and psychiatric disorders.

Lissencephaly. MDS is a severe cortical malformation characterized by defects in cortical folding and radial migration. It is commonly associated with microcephaly, cognitive impairment, mental retardation, and intractable epilepsy [79, 80]. MDS is caused by multiple heterozygous deletions of human chromosome 17p13.3, including PAFAH1B1 (LIS1 protein) and YWHAE (14-3-3ε protein) [81]. In research published in 2017, both Bershteyn et al. and Iefremova et al. developed forebrain organoids to deepen

our understanding of MDS. Studying MDS in animal models is challenging because mice naturally have lissencephalic brains [82, 83]. Outer radial glia (oRG) progenitors, known for their robust proliferation potential, produce large numbers of neurons and offer divergent tracks for neurons migrating along their basal fibers, facilitating cortical folding [84, 85]. When compared with control organoids, hiPS-derived telencephalic organoids from MDS patients displayed increased apoptosis, horizontal division of neural stem cells, and impaired neuron migration. Moreover, prolonged mitosis of oRG suggests that oRG may play a role in the pathogenesis of lissencephaly [82]. Additionally, the N-cadherin/beta-catenin signaling axis was impaired, resulting in defective non-cell-autonomous expansion of radial glial cells in the ventricular zone. Pharmacological activation of Wnt signaling ameliorated the abnormal growth of MDS patient-derived organoids [83].

Microcephaly. Microcephaly is a neurodevelopmental disorder characterized by abnormal brain development, resulting in a significant reduction in brain volume in affected individuals [86]. Gene mutations can lead to primary microcephaly, and extensive studies have connected several genes with autosomal recessive primary microcephaly (MCPH), such as CDK5RAP2, ASPM, NARS1, and IER3IP1 [87–89]. The first cerebral organoid was established to study microcephaly. Mutations in the CDK5RAP2 gene, which encodes centromere protein, can lead to autosomal recessive primary microcephaly in humans [90]. When cerebral organoids were generated from MCPH patients carrying heterozygous mutations in the CDK5RAP2 gene, they exhibited premature neuronal differentiation at the expense of the progenitor cell pools, resulting in smaller-sized brain organoids. Overexpression of the CDK5RAP2 gene could rescue the reduced size of cerebral organoids, and the phenotype could be reproduced by RNAi-mediated knockdown of CDK5RAP2 [20]. The most common cause of MCPH is mutations in the ASPM gene, which are critical for mitotic spindle function [85]. Cerebral organoids with biallelic mutations in ASPM exhibited less neuroepithelial organization, fewer vRG and oRG cells, and defective layer lamination, indicating developmental defects in the early stages. In later stages of development, calcium imaging experiments revealed fewer mature neurons and less synchronization of neuronal activities in organoids with ASPM mutations [91]. iPSC-derived cortical brain organoids with NARS1 mutations also displayed a substantial reduction in organoid size, linked to a decrease in neural rosette size and neuron number after mitosis, which may elucidate the pathogenesis of microcephaly [92]. To date, cerebral organoids have been employed to screen for genes

contributing to microcephaly. It was found that IER3IP1 could modulate brain growth by regulating the unfolded protein response [93].

In addition to primary microcephaly, scientists have harnessed brain organoids to understand microcephaly caused by pathogens. It has been discovered that cerebral organoids show reduced proliferation of neural progenitor cells and undergo cell death mediated by cysteine asparaginase when exposed to the ZIKV virus [94]. The NS2A protein encoded by the ZIKV virus disrupts the formation of adhesion junctions and impairs the proliferation of radial glial cells in human forebrain organoids [95]. In human fetal neural stem cells infected with ZIKV, two other proteins, NS4A and NS4B, synergistically inhibit the Akt-mTOR signaling pathway, blocking neurogenesis and leading to abnormal autophagy. This may provide insights into the pathogenesis of ZIKV virus-induced microcephaly [96]. Furthermore, organoid models of ZIKV virus-induced microcephaly have been used to test and screen potential therapeutic drugs [97, 98].

Autism spectrum disorder. ASD refers to a heterogeneous neurodevelopmental disorder caused by mutations in multiple genes [99]. ASD patients typically exhibit childhood-onset symptoms, including repetitive behaviors, restricted interests, and impaired social interactions and communication [74]. Understanding the pathophysiology of ASD has been challenging due to the extreme complexity of its pathogenesis, which involves interactions with multiple inherited factors and environmental influences.

Emerging evidence demonstrates that cerebral organoids are excellent tools for unraveling the mysteries of ASD. In a study published in 2015, scientists developed telencephalic organoids derived from iPSCs from severe idiopathic ASD patients and family controls [100]. Transcriptome and gene network analyses of ASD-derived organoids revealed upregulated genes associated with cell proliferation, neuronal differentiation, and synaptic assembly. Compared to control organoids, they found that the cell cycle was accelerated, and GABAergic inhibitory neurons were overproduced in ASD-derived organoids. Significantly, aberrant upregulation of the transcription factor FOXG1 was identified as responsible for the overproduction of GABAergic neurons through RNA interference experiments, and knockdown of FOXG1 rescued this phenotype [100]. In another study, CHD8, which encodes a chromatin remodeling factor, was found to affect GABAergic neuron development in ASD-derived telencephalic organoids by modulating DLX gene expression [101]. More recently, a pioneering study explained how different risk genes consistently contribute to the characterization of ASD phenotypes in human

cerebral cortex organoid models [102]. Evidence suggests that mutations in three ASD risk genes – SUV420H1, ARID1B, and CHD8 – can lead to asynchronous development of two major cortical neuronal lineages: γ-aminobutyric acid-releasing (GABAergic) neurons and deep-layer excitatory projection neurons. The expressivity of these mutations is closely regulated by individual genomic context, suggesting the pathological basis for the varying clinical manifestations of ASD patients and emphasizing the importance of future ASD treatment focusing on common pathways influenced by ASD risk genes [102].

Neurodegenerative disorders

Neurodegenerative diseases (NDDs) constitute a group of neurological disorders characterized by the progressive loss or degeneration of neurons in the nervous system, leading to memory, sensory, cognitive, and motor dysfunction [103, 104]. Some well-known NDDs include AD, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). Despite substantial efforts to understand the pathogenesis of NDDs, much remains unknown. Major pathological mechanisms include pathological protein aggregation and autophagy defects, aberrant mitochondrial energy metabolism, neuronal cell death, neuroinflammation, and dysfunction of synapses and neuronal networks [103]. There is an urgent need for effective therapeutic approaches and drugs for NDDs. Human cerebral organoids can significantly advance NDD research by overcoming the limitations of animal models, which possess different genetic backgrounds from humans, and simple 2D cell cultures that lack multiple cell types needed to replicate the *in vivo* environment.

Alzheimer's disease. AD is a neurodegenerative disease characterized by its insidious onset and progressive loss of higher neurological functions [105]. A hallmark pathology of AD is the deposition of neuronal extracellular amyloid-beta (Aβ) and the formation of neurofibrillary tangles, which consist of intracellular aggregates of hyperphosphorylated tau (Phosphorylated Tau, P-Tau) protein [106, 107]. In 2016, the first cerebral organoid derived from patients with familial AD carrying a repeat mutation in the APP gene was established [108]. Subsequently, familial AD brain organoid models with mutations in PSEN1, PSEN2, and MAPT genes, as well as sporadic AD brain organoids, have also been reported [109–111]. All of these models successfully recapitulate the pathological features of AD, including amyloid aggregation and abnormal hyperphosphorylated tau protein. In a study published in 2018, Joseph et al. introduced a new 3D human AD triculture model. This model

incorporates neurons, astrocytes, and microglia within a 3D microfluidic platform to facilitate microglia recruitment and the secretion of pro-inflammatory cytokines and chemokines, providing a more accurate organoid model of the human brain [112].

Human brain organoids derived from AD patients offer profound benefits for advancing mechanistic studies and therapeutic treatments. The APOE gene stands as a major risk factor for AD. Scientists have discovered that APOE4 astrocytes increase the formation of neuronal lipid droplets and cholesterol accumulation in chimeric organoids of astrocytes and neurons, promoting the onset of AD [113]. The co-occurrence of APOE4 astrocytes and neurons is essential for elevating neuronal tau protein levels, underscoring the significance of both neuronal and astrocytic APOE4 in p-tau pathology [113]. Pérez et al. constructed mitochondrial protease 1 (PITRM1) knockout cerebral organoids and observed that the early activation of the mitochondrial unfolded protein response (UPRmt) led to mitochondrial dysfunction, resulting in AD-related phenotypes [114]. There is evidence suggesting that isogenic conversion of the AD-causing gene APOE4 to APOE3 attenuates APOE4-related phenotypes in brain organoids from AD patients [115]. Furthermore, AD cerebral organoids also serve as an effective platform for the development and screening of therapeutic drugs for AD patients. The histone deacetylase 6 (HDAC6) inhibitor CKD-504 can degrade pathological tau proteins in cerebral organoids and ameliorate the pathological phenotype of AD [116]. Park et al. have developed a network-based drug-screening platform by integrating mathematical modeling and pathological characterization of organoids from AD patients, aiming to provide a strategy for precision medicine [117].

Parkinson's disease. PD, the second-most prevalent neurodegenerative disease globally, has become the fastest-growing neurological disease in terms of prevalence, disability, and mortality [118]. Similar to AD, the definitive cause of PD remains unclear, with research in recent years suggesting connections to genetics, the environment, and aging factors [119]. Among its pathologies, progressive loss of midbrain substantia nigra dopaminergic neurons and the accumulation of intracytoplasmic Lewy Bodies, eosinophilic inclusions, lead to the severe symptoms of PD [119, 120].

In 2016, Jo et al. cultured midbrain organoids containing neurons of distinct layers and detected electrically active and functionally mature midbrain dopaminergic neurons (mDAN) with the ability to produce dopamine [72]. In recent years, scientists have successfully replicated the typical pathological features of PD in midbrain organoids harboring different genes associated with PD. In iPSC-derived midbrain

organoids from PD patients carrying a triplication of the SNCA gene, increased expression levels of α-synuclein, a major component of Lewy bodies, were observed, and the accumulation of alpha-synuclein over time was linked to a selective loss of dopaminergic neurons [121]. Furthermore, midbrain organoids carrying mutations in the LRRK2 gene from PD patients exhibited neurodevelopmental defects, resulting in a decreased number of mDAN compared to controls [122]. Additionally, the induction of gene-edited iPSCs into midbrain organoids recapitulated hallmark features of PD [123, 124], underscoring the utility of established disease models for PD research.

Midbrain organoids derived from PD patients are promising models for therapeutic research and drug screening. Midbrain organoids with LRRK2 mutations from PD patients displayed increased LRRK2 activity, reduced dopaminergic neurons, and higher levels of autophagy [122]. Treatment with the LRRK2 inhibitor PFE-360 rescued the phenotype by increasing dopamine release and improving the function of dopaminergic neurons [122]. In another study, treatment with 2-hydroxypropyl-β-cyclodextrin ameliorated neuronal autophagy and mitophagy capacity in PD patient-specific midbrain organoids, leading to an increase in neuronal dopaminergic differentiation [125].

Amyotrophic lateral sclerosis. ALS is a neurodegenerative disease characterized by progressive muscle weakness due to the death of motor neurons in the brain, brainstem, and spinal cord [126]. Clinical symptoms of ALS patients include progressive muscle atrophy and cognitive decline, with ALS patients typically succumbing to respiratory failure within 5 years [127]. The etiology of ALS is not yet fully understood. Approximately 15 % of ALS cases are familial ALS (fALS), while the remainder are sporadic ALS (sALS) [128]. To date, approximately 40 genes linked to ALS have been identified, primarily influencing protein homeostasis, DNA damage repair, RNA metabolism, vesicle transport, and mitochondrial function [129]. Some of these genes are also associated with other neurological disorders, such as CCNF, ANXA11, TBK1, and SQSTM1, which are related to frontotemporal dementia (FTD) [129].

Scientists have harnessed the potential of human cerebral organoids to delve into the pathogenesis and treatment of ALS. For instance, Szebényi et al. developed a cerebral organoid slice model derived from human iPSCs, displaying early molecular pathology of C9ORF72 ALS/FTD [130]. Combined with single-cell sequencing, they observed protein disorders in astrocytes, including the early accumulation of the autophagy signaling protein P62 and the toxic dipeptide repeat protein poly (GA), along with cell death occurring in deep layer neurons. Furthermore,

GSK2606414, a repressor of translational inhibition caused by UPR, could pharmacologically rescue the phenotype [130]. Researchers have also recently generated microglia-containing cerebral organoids derived from iPSCs from ALS patients [131]. Impaired microglia-mediated autophagy and down-regulated expression of the type I interferon signaling pathway were detected in patient-derived cerebral organoids compared to controls, suggesting that microglia are also involved in the pathogenesis of ALS [131]. Additionally, Pereira et al. have generated sensory-motor organoids with physiological neuromuscular junctions (NMJs) and applied this model to different subgroups of ALS, contributing to a better understanding of the pathophysiological mechanisms of ALS [132].

Liver organoids

The liver, one of the essential organs in the human body, performs various functions, including metabolism, synthesis, detoxification, and bile secretion [133]. However, liver disease remains a significant global public health concern. According to the World Health Organization's statistics, 350 million people worldwide suffer from liver disease, with more than 1 million people succumbing to it annually. The primary causes are the scarcity of organ donors and an incomplete understanding of the pathological mechanisms [134]. Therefore, the study of liver development, regeneration, and pathogenesis holds immense significance in regenerative medicine and disease treatment.

Traditional methods for studying liver diseases have their limitations. For instance, in a 2D cell culture system, isolated primary human hepatocytes struggle to maintain viability over an extended period *in vitro* and have limited expansion capabilities. This system is suitable only for short-term studies, and the cells' functions deteriorate rapidly after 48 h. Conventional animal models may not be applicable to all diseases, with variations among species and potential ethical constraints [135, 136]. The development of liver organoids offers a more physiologically relevant and *in vivo*-like environment for studying liver diseases [137].

The induction of iPSCs toward mesoderm or endoderm, particularly for hepatocyte and cholangiocyte differentiation, involves the use of specific cytokines such as fibroblast growth factor (FGF), BMP, HGF, and Wnt. Liver organoids are subsequently formed by embedding these cells in Matrigel under the influence of specific factors. Another approach for liver organoid formation, pioneered by Takebe and colleagues, involves the spontaneous formation of 3D aggregates known as iPSC-liver buds (iPSC-LB). These liver buds are created by mixing human pluripotent stem cell-derived hepatocytes, human

mesenchymal stem cells, and human endothelial cells on a Matrigel layer, resulting in an organ-like *in vitro* liver model with functional vascular-like endothelial networks. Subsequent experiments involving the implantation of liver buds into mouse models of liver disease have demonstrated their potential for reducing mortality in mice with liver injuries [138]. Studies have shown that hPSC-derived liver organoids not only secrete human albumin and alpha-1-antitrypsin (A1AT) but also synthesize urea and regulate cytochrome P450 (CYP) enzymes *in vitro*.

Liver organoids have become invaluable tools in various therapeutic fields. For instance, HCC often results from chronic parenchymal liver damage due to monogenic diseases. Liver organoid models are indispensable for studying this disease [139]. It has been reported that hepatocyte-derived organoids, derived from iPSC-derived cholangiocytes in 3D culture, can excrete bile acids and exhibit functional secretion, mimicking conditions such as Alagille syndrome [140]. iPSC-derived liver organoid models of steatohepatitis hold the promise of providing personalized drug treatment options for reducing lipid accumulation, potentially mitigating the impact of Wolman's disease on patients [141]. Additionally, iPSC-derived liver organoids from patients with specific diseases can simulate various inherited metabolic disorders and cholangiopathies, offering a platform for *in vitro* drug validation for conditions like polycystic liver disease and cystic fibrosis cholangiopathy [142].

In the past decade, hepatocytes and liver organoids derived from iPSCs have played a significant role in the *in vitro* study of liver physiology and pathology. However, challenges related to limitations, safety, and stability still exist. Efforts are ongoing to enable their clinical application and help address various disease-related issues as soon as possible.

Intestine organoids

Hans Clevers' laboratory has initiated a new chapter in the development of intestinal organoids, utilizing single mouse LGR5 intestinal stem cells to self-organize into an intestinal crypt-villus structure *in vitro*. In the past, biological model systems such as the mouse model were employed to study enteroendocrine cells. Additionally, certain cell lines have been used as research models, such as the mouse GLUTag cell line, which serves as a model for regulating GLP-1 secretion [143]. There are also BON cell lines for endometrial cancer, which can be used to model serotonin production [144]. While these models have advanced the study of the enteroendocrine system to some extent, they still possess certain limitations, including the inability to simulate the

in vivo environment and a lack of cell-to-cell interactions. Over the past decade, organoid models derived from AdSCs have been developed to investigate interactions between enteroendocrine cells, other epithelial cell types, and the enteric nervous system. However, the iPSC method can construct a more ideal model suitable for disease research.

For colonic diseases, studies have been conducted to create colonic organoids. Colon organoids were generated from iPSCs derived from patients with familial adenomatous polyposis (FAP). PSCs were induced into directed endoderm using CHIR and activin A. In the presence of B27, CHIR, and FGF4 were utilized to induce hindgut endoderm (HE). Starting from day 8, HE cells were treated with a medium containing CHIR, LDN, and EGF for 12 days to produce colonic epithelial cells, which were subsequently embedded in Matrigel to gradually form organoids [145]. Compounds that have been reported to be effective against colon cancer, such as rapamycin [146] and XAV 939 [147], can be considered as potential drugs to treat FAP. The results have shown that these two compounds reduced cell proliferation not only in colon cancer but also in normal colon tissue. However, this potential drawback, which could harm healthy colonic crypts, may limit their therapeutic use. In contrast, geneticin reduces the over-activation of WNT signaling, thereby decreasing the excessive proliferation of colonic epithelial cells, which is strongly associated with colorectal cancer. One of the advantages of disease modeling is the ability to screen for drugs that can reverse the pathogenic phenotype [145].

The main symptoms of COVID-19 primarily affect the respiratory tract, but ACE2 expression is highest in the microvilli of intestinal epithelial cells in the human body. Relevant studies have suggested that the gastrointestinal tract may serve as a potential entry route for SARS-CoV-2 [148]. Numerous experiments conducted with intestinal organoids have demonstrated that iPSC-derived intestinal organoids can be employed as a research model to replicate COVID-19 infection. For instance, remdesivir and EK1, two viral fusion inhibitors, have been discovered to inhibit coronavirus infection and restore the morphology of intestinal organoids [149]. Certain studies have utilized intestinal organoids induced by coronavirus infection and subsequently employed TEM imaging and gene set analysis to unveil the presence of viral particles and the upregulation of apoptosis-related genes.

The study of these intestinal models can further our understanding of the physiology, developmental biology, regenerative medicine, and pathophysiology of the intestine. Moreover, they can be employed to investigate host-pathogen interactions, digestion and absorption processes, transplantation, and more. For example, intestinal

organoids can be used to explore intestinal barrier integrity and metabolism [150], iPSC-derived intestinal organoids that contain the enteric nervous system can help investigate intestinal motility disorders, and they can even serve as models for toxicology assessments [151].

Traditional organoids are primarily derived from homeostatic stem cells. However, when the body is damaged, homeostatic stem cells may not be sufficient to compensate for tissue loss. Previous studies have reported that constructing a proliferative intestinal organoid system can better reflect the regenerative response of the intestinal epithelium to injury *in vivo*, and have identified VPA and EPZ 6438 as important regulators of intestinal organoid proliferation [152]. This innovative organoid system may play a more critical role in exploring intestinal diseases and regeneration in the future. Nonetheless, iPSC-derived organoids have smaller cell populations than normal tissue and tend to decline over time [153]. The controlled formation of intestinal organoids remains a major challenge for future research [154].

Lung organoids

Previous studies have provided a strategy for deriving lung organoids from iPSCs. Initially, iPSCs are differentiated into endoderm in a medium containing activin A. Subsequently, the definitive endoderm is cultured in a medium containing NOG, FGF4, CHIR99021, and SB431542 to encourage differentiation into foregut spheroids. Finally, these foregut spheroids are embedded in Matrigel for culture, with the addition of FGF10 to the medium.

Since the development of lung organoids, extensive basic research has been conducted. For instance, NKX2.1 in lung epithelial progenitor cells can be used to screen for markers that enhance expression, allowing for more efficient and targeted differentiation towards specific fates [155]. Some experiments have revealed that the culture process of iPSC-derived lung organoids shares common features with fetal lung development. Under conditions that stimulate FGF signaling, lung organoids preferentially generate NKX2.1, SOX2, and SOX9 epithelial cells, resembling early lung progenitors [156]. When lung organoids are placed in media that activate BMP, FGF, and WNT signaling pathways, lung epithelial cells undergo morphological branching, akin to the processes during lung development [157].

Furthermore, the advent of lung organoids has facilitated the modeling and study of various diseases, including pulmonary fibrosis, congenital diseases, and neonatal respiratory distress syndrome. In the wake of the global COVID-19 pandemic, iPSC-derived lung organoids have proven valuable for modeling the disease, and candidate drugs for treatment have been tested in relevant studies [150, 158]. Another possibility is the induction of human lung lobes from hPSCs. Although the coverage of human lobes is not as extensive as in the rat model, this *in vitro* lung engineering approach may address the issue of the shortage of lung donors for transplantation [159].

Numerous efforts have been made to address idiopathic pulmonary fibrosis (IPF), but the pathophysiological mechanism of the disease remains poorly understood. The primary reason is the lack of models for studying pathological mechanisms and drug screening. While animal models and 2D cell cultures have contributed to progress in exploring the mechanisms of IPF, they also come with their own limitations. IPSC-derived organoids, including lung bud organoids and alveolar epithelial organoids, offer a more accurate simulation of the *in vivo* environment, making them a powerful model for studying respiratory diseases [160]. Nevertheless, the lung is a highly complex organ, and IPF involves the interaction of various cell types, making it a significant challenge to decipher the pathology. The development of human fetal-derived mesenchymal organoids for IPF modeling [161] and fibrotic lung organoids created through the CRISPR/Cas9 gene editing system [162] have been instrumental in exploring the pathological mechanisms of IPF.

It is undeniable that iPSC-derived lung organoids hold great potential for studying specific aspects of human development and disease. Lung organoid systems provide a valuable platform for basic research, toxicity assessment, drug screening, and personalized disease modeling.

Retinal organoids

The retina originates from the neuroectoderm and functions as a light-sensitive region of the eye. During embryonic development, the optic vesicle invaginates to form the optic cup divided into two layers, with the outermost layer forming the retinal pigment epithelium and the inner layer highly specialized to constitute the retinal neuroepithelial layer composed of retinal neurons and glial cells. The neural retina then continues to stratify, with the development of layers of photoreceptor and ancillary cell types, such as horizontal cells, bipolar cells, and so on.

Retinal morphogenesis depends on an intrinsic self-organizing program, and pluripotent stem cells can differentiate into retinal organoids by regulation of endogenous factors. Optic nerve cup-like organoids derived from mESCs can aggregate and develop in serum-free medium containing low levels of growth factors to form stratified neural retinal tissues, similar to the developmental trajectory

in vivo [25]. When transplantation of ESC or iPSC-derived retinal cells into a model of advanced retinal degeneration without the outer nuclear layer, the transplanted tissue could establish synaptic connections with host cells and restore light response. The studies provided proof of concept for transplantation therapy of retinal cells [163, 164]. Three-dimensional optic nerve cup organoids of hiPSC origin can spatiotemporally recapitulate the major steps of retinal development, and differentiate into mature photoreceptors, giving rise to possibilities for disease modeling and future therapies for retinal dysfunction [165]. Recently, a human retinoblastoma model was successfully established in mice. hiPSCs from retinoblastoma patients were cultured and differentiated into retinal organoids and then transplanted into the vitreous of mouse eyes to support tumor growth, which was molecularly, cellularly and genomically identical to the tumor patients [166]. This human cancer model facilitates the study of pathogenesis and specific treatments for patients with particular germline cancers.

Inner ear organoids

The inner ear develops from the otic placode of the ectoderm. The inner ear contains a large population of specialised sensory cells, known as 'hair cells', which are able to convert mechanical stimuli into electrochemical signals [167]. The development of the inner ear is modulated by signaling pathways such as BMP, Wnt and Sox2, and regulating the inputs of these signaling factors *in vitro* induces the directed differentiation of PSCs towards sensory hair cells in the inner ear [168]. In 2013, Koehler and his colleagues partially recapitulated the developmental process of inner ear *in vitro* by temporally controlling the signaling pathway with precise timing and differentiating mouse ESCs into functionally mature sensory hair cells in a 3D culture system [169]. Subsequently, by a similar approach, Koehler induced inner ear organoids innervated by sensory neurons from human ESCs [170]. Recently, multiple immunostaining and single-cell RNA sequencing techniques were used to compare the characteristics of early human embryonic otocyst and fetal sensory organs with the human inner ear organoids, together with an assessment of the expression and localization of key markers at the same stage, providing evidence that the inner ear organoids can be further used as a developmental and disease models [171].

Since the first technical breakthrough of intestinal organoids in 2009, more and more organoids have been cultured and applied to various organs, including brain, liver, lung, pancreas, kidney, etc., which can reduce the complexity of experimental operation and better optimize the experimental protocol. They have important applications in drug screening, transplantation, disease modeling, gene editing, personalized treatment, and toxicology research. In terms of disease modeling, even complex and fine brain organs can be constructed to construct structures such as cerebellum and cortical layers for the study of various diseases. The kidney and liver organoids, which are the most important metabolic functions, also provide a platform for toxicity prediction. In addition to iPSCs and ESCs, organoids derived from primary tumors such as colon, breast, and pancreas can also be used for drug screening and provide substrates for personalized therapy. In summary, more studies are needed to evaluate the efficacy and safety of these methods to guide the clinical application of organoids (Table 1).

Organoids derived from tumor

Cancer is one of the significant threats to human health worldwide. In current preclinical research on tumors, there are several types of tumor models: tumor cell line models (CCL), patient-derived tumor xenograft models (PDTX), and patient-derived organoids (PDO).

CCL, originating from immortalized cancer cell lines derived from patient tumors, are traditional models widely employed in tumor research. In 1951, the initial human tumor cell line was isolated from an American cancer patient, which is widely known as HeLa cells [172]. CCLs span a variety of human tumors such as breast, central nervous system, colon, kidney, leukemia, lung, melanoma, ovary, and prostate [173]. Despite their diverse origins, these CCLs continue to play a crucial role in modern cancer research. PDTX is a valuable model which small fragments of tumors are surgically removed from cancer patients and then transplanted directly into immunodeficient mice [174]. In 1969, Ragaard and Povlsen successfully implanted human colonic tumor tissue into nude mice [175]. This achievement might be the first PDTX model. PDTXs are highly favored by researchers because they largely preserve the cellular and histopathological structures of the original tumors. In numerous preclinical cancer research studies, the presence of PDTXs is quite common. For example, in recently published studies in colorectal cancer, breast cancer [176], CC [177] and HCC [178]. Tumor organoids serve as a pivotal model system in the realm of precision medicine, owing to their unique capability to faithfully preserve the fundamental characteristics of the primary tumor [179]. They have emerged as a highly promising model for precision medicine, primarily due to their aptitude for predicting drug responses tailored to the unique profiles of individual patients. Tumor organoids can be effectively cultured from a

diverse array of cancer types, encompassing brain, lung, liver, pancreatic, and breast cancer, among others [180]. The envisioned process involves procuring a patient's tumor specimen, generating and sustaining organoids, exposing these patient-derived tumor organoids to a wide range of pharmaceutical agents, and subsequently administering the most optimal drug or drug combination. These diverse tumor models contribute to our understanding of cancer biology and aid in the development of potential treatments and they each have their respective advantages and disadvantages (Table 3).

Brain cancer

In a 2018 article published in the journal *Nature Methods*, Bian and their colleagues conducted a study to explore the use of genetically modified cerebral organoids as a reliable model for investigating the mechanisms involved in the formation of brain tumors [181]. The goal of this research was to enhance the utility of cerebral organoids by genetically modifying them to mimic the development of brain tumors. They introduced genetic mutations commonly associated with brain cancers, such as TP53 and PTEN alterations. This research is significant in the fields of cancer biology and neuro-oncology, providing a valuable platform for studying the molecular and cellular processes involved in brain tumor development. Glioblastomas are highly aggressive and deadly brain cancers known for their complexity and the presence of areas with low oxygen levels (hypoxic regions). In 2016, Hubert and their team conducted

Table 3: The advantages and disadvantages of CCL, PDTX and PDO.

Tumor models	Advantages	Disadvantages
CCL	Lower cost; Easy to maintain; High-throughput screening, Convenient manipulation	Loss original tumor complexity; Lack tumor microenvironment; Lack genetic diversity
PDTX	Maintain tumor heterogeneity; Mimics *in vivo* microenvironment; More physiological environment; From human tumor	Technically challenging; Expensive; Tumor restrictions; Long research time
PDO	Maintain tumor heterogeneity; Mimics *in vivo* microenvironment; Personalized medicine; Platform of drug discovery	Longer culturing; Technically challenging; Expensive

CCL, tumor cell line models; PDTX, patient-derived tumor xenograft models; PDO, patient-derived organoids.

a study aimed at creating a 3D organoid culture system using human glioblastoma samples [182]. The main goal was to develop an *in vitro* model that accurately reproduces the hypoxic gradients and the diversity of cancer stem cells found in glioblastoma tumors in real-life conditions. The study successfully generated a 3D organoid culture system derived from human glioblastoma tissue. This organoid culture system shows promise as a valuable tool for studying glioblastomas and potentially other types of cancer.

Lung cancer

Lung cancer is a significant global cause of death. Lung cancer can be broadly classified into two main types: non-small cell lung cancer (NSCLC), and small cell lung cancer (SCLC). Within NSCLC, the most common subtype is lung adenocarcinoma (LUAD). It is a complex disease with diverse characteristics, both in terms of physical features and genetic makeup. This complexity makes it challenging to create animal models for research [183]. Researchers are increasingly using lung cancer organoids (LCOs) because they can faithfully replicate the structure and function of lung tissue found in the body.

LCOs are vital tools for researching the mechanisms of lung cancer. Zhang et al. demonstrated that the sustained expression and activation of KRAS, along with the loss of Lkb1, can transform normal lung organoids into malignant ones, promoting the transition to lung squamous cell carcinoma (LUSC) [184]. Subsequently, Dost and their colleagues used organoid systems derived from human iPSCs and mouse lung epithelial cells to simulate the development of LUAD. In both systems, the expression of KRAS in lung alveolar epithelial progenitor cells led to changes in the transcriptional program, resulting in the downregulation of genes associated with differentiation and maturation [185]. These two studies provided groundbreaking insights into the mechanisms of lung tumor formation. Kim et al. established various subtypes of LCOs, such as adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, and small cell carcinoma [186]. Li et al. [187] and Chen et al. [188] also successfully established biobanks of LCOs of different subtypes. These organoids exhibit a high degree of consistency with the original lung cancer tissue, offering new directions for personalized and precision therapies for lung cancer. Additionally, LCOs can be used for drug sensitivity testing. One study used 212 LCOs generated from 107 patients for drug sensitivity testing (LCO-DST). LCO-DST accurately predicted the clinical responses of some lung cancer patients to treatment [189]. Beyond drug sensitivity

testing, LCOs can be applied in drug development by conducting high-throughput screening of numerous compounds to identify potential anti-cancer drugs [190].

Liver cancer

PLC is the second leading cause of cancer-related deaths worldwide. PLC encompasses various types, including HCC, intrahepatic cholangiocarcinoma (iCCA), and other less common tumors [191]. In cancer research, it is crucial to create accurate models to understand the disease better. Traditional 2D models have limitations, but 3D systems offer a more faithful representation of what happens in the body.

Hepatitis B virus (HBV) is a significant factor in the development of liver cancer. One study investigated liver cancer using PDO created from non-tumor cirrhotic liver tissues of liver transplant patients. Analysis of the gene expression patterns in these organoids revealed unusual early cancer-related characteristics. These findings have the potential to serve as new biological markers for understanding how HBV infection contributes to the development of liver cancer [192]. Naruse and colleagues established a novel *in vitro* model for inducing carcinogenesis through chemical treatments, using liver organoids. They used liver organoids derived from mouse tissues and exposed them to a chemical called diethylnitrosamine (DEN). This model helped explain the patterns of chemical carcinogenesis and how it can lead to liver cancer [193]. Sun et al. took a different approach by using reprogrammed human liver cells (hiHeps) with deactivated p53 and RB genes to create liver organoids. These organoids were then genetically modified to simulate the development of human liver cancer [194]. Li and their team highlighted the potential of tumor organoids in screening drugs for liver cancer treatment. They established 27 different liver cancer cell lines and tested them with 129 different cancer drugs. This research revealed significant variations in how tumors respond to these drugs, offering new insights into personalized treatment strategies for liver cancer [195]. These innovative approaches and organoid models are helping researchers better understand the complexities of liver cancer, providing new avenues for studying its development and potential treatments.

Pancreatic cancer

Pancreatic cancer is an extremely aggressive tumor with a very low 5-year survival rate. It ranks as the seventh most common cancer globally [196]. Pancreatic cancer organoids retain the physiological characteristics and functions of a patient's original tumor cells. These organoids are valuable for personalized drug screening and drug sensitivity testing, offering promise for early diagnosis and personalized treatment of pancreatic cancer.

Researchers, such as those in the Clevers and Tuveson labs, have used surgically removed pancreatic cancer tissues to create pancreatic cancer organ models. They found that transplanting these organoids can mimic the full range of tumor development, offering insights into the entire cancer progression process [43]. One study discovered that the expression of the PDAC-related cancer gene GNASR201C induced cystic growth more effectively in pancreatic ducts compared to pancreatic acinar organoids. On the other hand, $KRAS^{G12D}$ was more effective at simulating cancer when expressed in acinar organoids as opposed to ductal organoids. The researchers developed a renewable source of pancreatic ductal and acinar organoids, providing a tool to study the lineage plasticity and human pancreatic cancer gene actions [197]. A team of researchers from Harvard Medical School, as published in *Nature Medicine*, employed 3D cell culture techniques to amplify and cultivate primary cancer cell organoids from pancreatic cancer patients' tissues. These tumor organoids maintain the characteristics of the original tumor and preserve the specific physiological changes unique to the patients. These pancreatic tumor organoids can be used to simulate pancreatic cancer and conduct drug screening for precision treatments.

Breast cancer

Breast cancer is one of the most common malignancies affecting women globally and stands as the second leading cause of cancer-related deaths in women. The primary research models for breast cancer have traditionally been tumor CCL and PDTX. However, both of these models have their limitations. In 2018, Sachs and their team made a significant breakthrough by creating breast cancer organoids from pathological tissues of breast cancer patients. Furthermore, the team established a biobank comprising over 100 breast cancer organoid samples [198]. Building upon research conducted by Hans Clevers' team, Mazzucchelli and others isolated breast cancer cells from surgical or biopsy samples and then cultivated them into organoids [199]. Li and colleagues successfully cultured organoids from large papillary breast cancer. These organoids continued to grow for over 6 months. These proliferating breast cancer organoids exhibited consistent pathology, hormone receptor expression, and the proliferation marker Ki-67 with post-surgery pathological results [200]. In another study, Nayak and their team cultured primary breast cancer cells from different patients in specific culture conditions,

generating breast cancer organoids. They then exposed these organoids to chemotherapy drugs, doxorubicin, and mitoxantrone. The results highlighted significant differences in the response of organoids from different patients to these two chemotherapy drugs [201]. This underscores the capability of organoid models to replicate the tumor's heterogeneity *in vitro*, offering a basis for personalized treatment approaches.

In summary, organoids derived from AdSCs, pluripotent stem cells, and tumor tissues have their own roles in human disease modeling. They can well simulate the characteristics of *in vivo* tissue biology, and capture some of the multicellular structure, anatomy, and even functional characteristics similar to real organs, providing an important platform for the study of disease modeling in different fields.

Conclusions and perspectives

Ever since Rheinwald and Green first reconstituted 3D tissue structures from cultured human stem cells [202–204], organoids are now defined as self-organized 3D structures grown from stem cells, composed of organ-specific cell types, and capable of reconstituting specific organ functions *in vitro* [205, 206]. After decades of development, organoids have been generated from various tissues, including the small intestine, prostate, brain, lung, and liver. Organoid technology has opened up new possibilities for scientific discovery in developmental biology, drug discovery, and personalized therapy [207], and multiple biobanks of organoids have been established for modeling human diseases

Figure 2: Multiple applications of organoid technology (by Figdraw). The schematic summarizes the different applications of organoids in many fields, including developmental biology, gene editing, drug screening, disease modeling, personalized therapy, and regenerative medicine.

and drug discovery, bringing the promise of personalized medicine closer to reality [36, 208] (Figure 2).

The ethical issues of organoids cannot be ignored. The first ethical issue that is often discussed is that researchers should only carry out experiments with the consent of donors. If the research is carried out without the donor's knowledge and completely separated from the donor's personal data, it will weaken the clinical application of organoids. Informed consent from donors in organoid research means that they have control over the conduct of the experiment and the option to opt out. Interviews showed that the patients who participated in the research wanted to be informed about the experimental study of organoids, and wanted to know the related profits and drug pricing involved in the follow-up [209, 210]. Another issue is the ethical challenge of organoids commercialization. Patients usually donate their tissues for nothing, but in addition to the distrust of the research community, they also worry about the experimental data involved in subsequent organoids, profits, and whether the company will be overly profitable. These negative effects may be solved by providing benefits to the donor. It can be financial support or clinical special care such as experiencing new therapies based on organoid research [211]. The pertinent departments ought to enhance discourse regarding the ethics surrounding organoids and ensure patients are informed about organoids in an open and transparent manner. Furthermore, it is imperative to establish appropriate legislation to regulate the advancement of this technology in clinical practice. Improving ethical supervision, reducing public concerns, and improving the understanding of organoids to help organoids continue to develop on the premise of ethics [212].

Organoids are typically derived from a single germ layer through step-wise differentiation, whereas an organ contains cells from multiple germ layers. Multi-tissue organoids [213, 214] or assembloids [215, 216] are established through the co-culture of cells derived from at least two germ layers (Figures 3 and 4). Significant progress has been made in understanding diseases such as cystic fibrosis, pancreatic cancer, colon cancer, Zika virus, and microcephaly with the use of brain organoids [217–219]. Despite these tremendous achievements and rapid development, organoids have a few limitations that hinder their broader application. Organoid cultures exhibit significant heterogeneity and variable complexity in cellular composition, can undergo poorly controlled morphogenesis, and often lack stromal, vascular, and immunological components [220]. Several achievements have been made in vascularized organoids [221], mainly through transplanting organoids into highly vascular tissues in immunodeficient mice [222] or culturing organoids on microfluidic devices that mimic
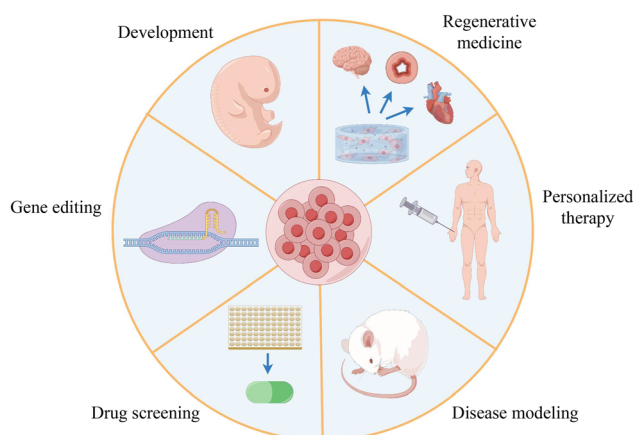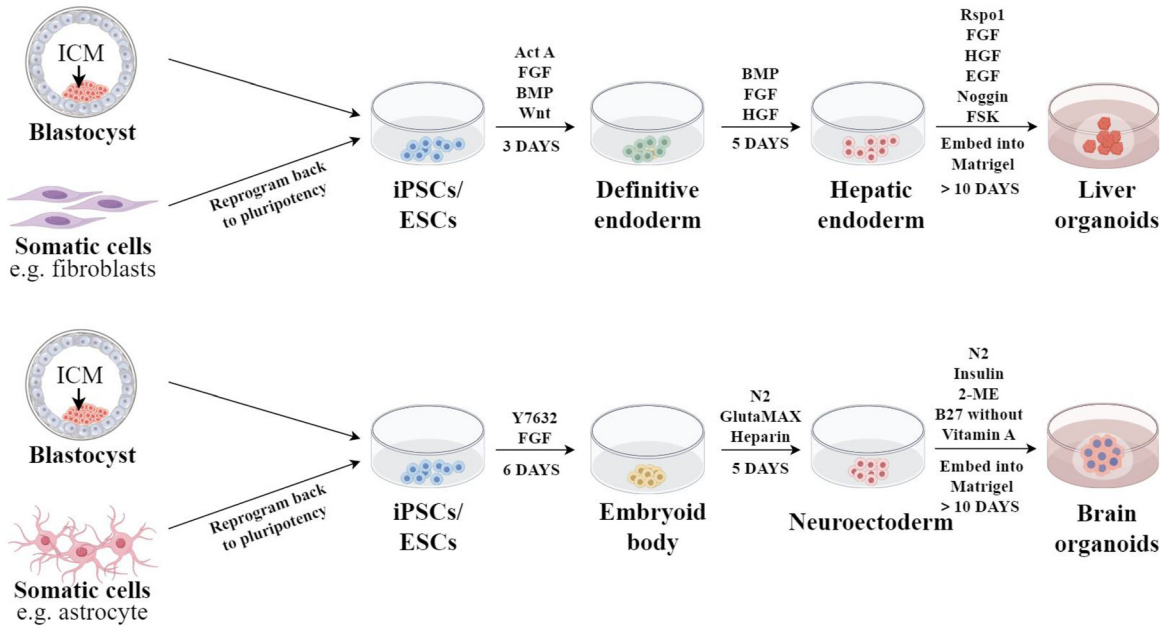
Figure 3: Liver organoids and brain organoids obtained from iPSCs and ESCs (by Figdraw). Induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) respectively from the inner cell mass of blastocysts and somatic cell reprogramming through the cultivation of different numbers of days and cell factors to generate the corresponding organoids.
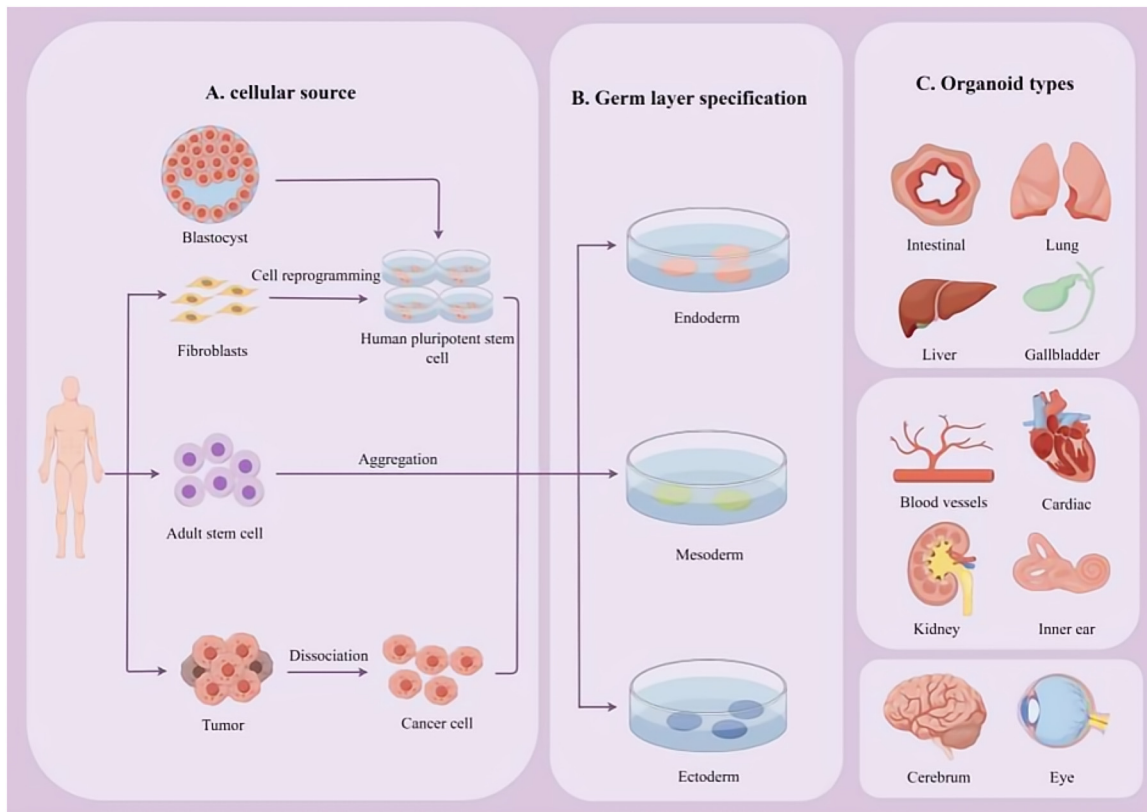

Figure 4: Overview of organoid types (by Figdraw). Starting cell types include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adult stem cells (ASCs), and tumor stem cells, which are aggregated into cell populations by self-organization. The cell population differentiates into three germ layers: endoderm, mesoderm, and ectoderm, which further specializes into various organoid types.

vasculature [223, 224]. The ectopic expression of human ETS variant 2 (ETV2) has also contributed to the formation of a complex vascular-like network in human cortical organoids (hCOs) [78]. However, the formation of specific cell types and the simulation of functional systems are difficult to overcome for organoids, especially the nervous system. The problem is further complicated if the interaction between cells and tissues is also explored. In addition, there is also variability in organoids derived from patients of different ages and genetic backgrounds [20], and the differences in tumor-like tumors can also lead to heterogeneity in tumor results [36]. For the above vulnerabilities, CRISPR-Cas9 technology may be used to engineer organoids with an isogenetic background to reduce variability [225]. Every model has its own advantages and disadvantages, and organoids are not exceptional. Pay attention to the limitations when applying them.

Due to the random configuration of traditional 3D culture, it is difficult to precisely control organoids and their local environment [226, 227]. Organoids, in collaboration with engineers and organ-on-a-chip technology, provide a path to more controllable organoid systems [228–230], which have been used to create the required concentration gradients to regulate tissue patterning and development. Scaffolds made of biological materials can improve the abnormal tissue structure caused by the organoid self-organization [231]. A significant step has been taken in modeling the alveolar–capillary unit of the lung with a biomimetic microsystem that reconstitutes the critical functional alveolar-capillary interface of the human lung [232]. However, simulating the important physiological process of organoids *in vivo* vascularization and innervation is still one of the indispensable keys to understanding the mechanism of tumorigenesis, metastasis, or neurodegenerative disease [233]. In addition, the wide application of Matrigel cannot reflect the dynamic changes of biomechanics in organs. To solve this problem, some biological companies or laboratories have cooperated with engineering laboratories to develop synthetic substrates with both biophysical and biochemical properties [234]. Furthermore, the combination of organoids with other technologies, including CRISPR/Cas9 genome-editing technology [235], mass spectrometry, single-cell RNA sequencing, and cryo-electron microscopy (cryo-EM) [206], has further advanced our understanding of organoid assembly and organ development. In the future, the integration of organoids with other technologies may accelerate the development of organoids, simulating more complex human physiological and pathological situations and improving the accuracy of organoids to reproduce human physiological processes.

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