Bio-Design and Manufacturing (2021) 4:689–716
https://doi.org/10.1007/s42242-021-00150-7

REVIEW

Organoids: a novel modality in disease modeling

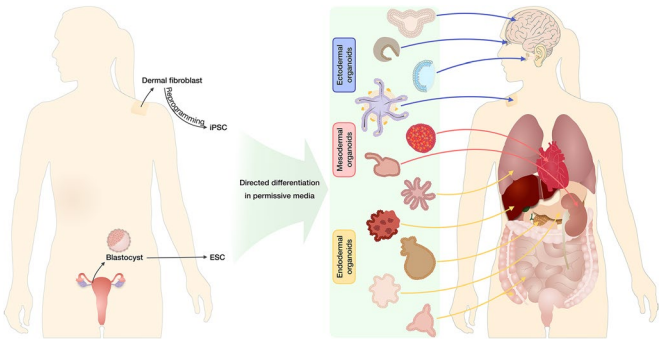
Zahra Heydari[1,2] · Farideh Moeinvaziri[1,2] · Tarun Agarwal[3] · Paria Pooyan[1] · Anastasia Shpichka[4,5,6] · Tapas K. Maiti[3] · Peter Timashev[4,5,6,7] · Hossein Baharvand[1,2] · Massoud Vosough[1,8]

Received: 12 November 2020 / Accepted: 12 June 2021 / Published online: 9 August 2021

Abstract

Limitations of monolayer culture conditions have motivated scientists to explore new models that can recapitulate the architecture and function of human organs more accurately. Recent advances in the improvement of protocols have resulted in establishing three-dimensional (3D) organ-like architectures called 'organoids' that can display the characteristics of their corresponding real organs, including morphological features, functional activities, and personalized responses to specific pathogens. We discuss different organoid-based 3D models herein, which are classified based on their original germinal layer. Studies of organoids simulating the complexity of real tissues could provide novel platforms and opportunities for generating practical knowledge along with preclinical studies, including drug screening, toxicology, and molecular pathophysiology of diseases. This paper also outlines the key challenges, advantages, and prospects of current organoid systems.

Graphic abstract



Keywords Organoid · Germ layer · Disease modeling · Drug screening

Zahra Heydari and Farideh Moeinvaziri have contributed equally to this work as first authors.

✉ Peter Timashev
timashev_p_s@staff.sechenov.ru

✉ Hossein Baharvand
baharvand@Royaninstitute.org

✉ Massoud Vosough
masvos@Royaninstitute.org

1 Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran 14155-4364, Iran

2 Department of Developmental Biology, University of Science and Culture, Tehran 14155-4364, Iran

3 Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal 721302, India

4 World-Class Research Center "Digital Biodesign and Personalized Healthcare", Sechenov First Moscow State Medical University, 19991 Moscow, Russia

5 Institute for Regenerative Medicine, Sechenov University, 119991 Moscow, Russia

6 Chemistry Department, Lomonosov Moscow State University, 119991 Moscow, Russia

7 Department of Polymers and Composites, N.N. Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, 119991 Moscow, Russia

8 Department of Regenerative Medicine, Cell Science Research Centre, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran 14155-4364, Iran

Introduction

Shedding light on the cellular and molecular pathophysiology mechanisms of diseases is a principal goal for scientists. Over the years, existing knowledge of the human system and its dynamics have developed from the examination of post-mortem and pathological specimens to animal models [1]. The latter add significant value while presenting many challenges, including high time and monetary costs, ethical concerns, and most importantly, they fail to accurately predict the clinical efficacy of therapeutics due to different pharmacokinetics, pharmacodynamics, and interspecific genetic or metabolic variations [1, 2] (Fig. 1). In addition, few human tissues are accessible for investigation and analysis. As a result, many gaps need to be addressed for a detailed knowledge of human development and disease-related events.

A major recent advancement in 3D culture was the generation of 3D structures called 'organoids' [3]. The term 'organoid' was first used in the literature in 1946 when Smith and Cochrane described a cystic teratoma [4]. Nowadays, organoids are defined as systems originating from either pluripotent stem cells (ESCs or iPSCs) or neonatal/adult stem/progenitor cells, both with the potential to self-organize into in vivo-like organ complexes and the ability to recapitulate essential organ functions (Fig. 2) [1, 3, 5]. In the process of organoid generation, extracellular matrix (ECM) and medium signaling are essential for facilitating cell–cell interactions—these are integral to self-assembly and cellular

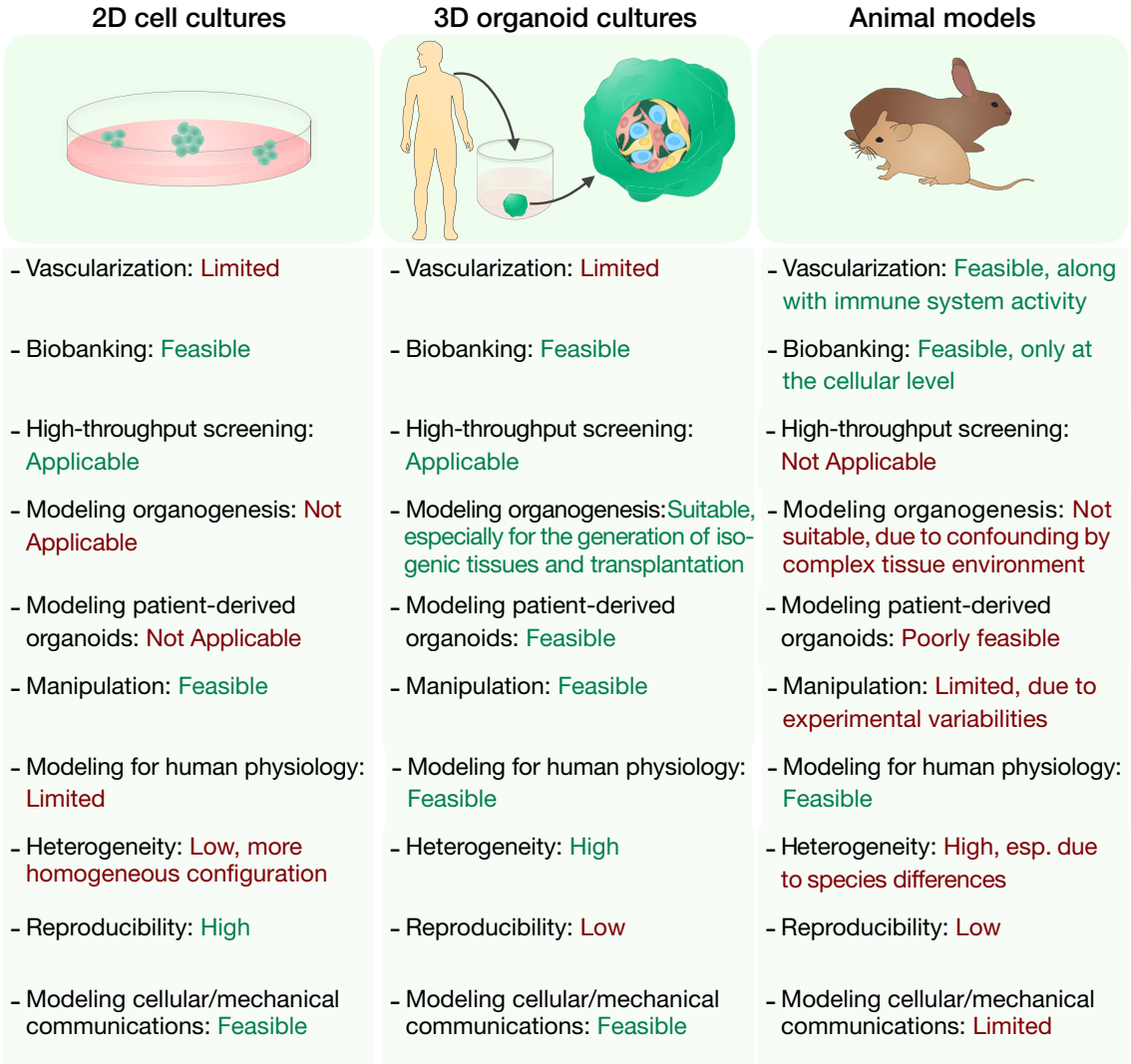


Fig. 1 Advantages and limitations of 2D cell culture systems, 3D organoid cultures, and the establishing of organoids in animal models. Organoids have enormous advantages compared to 2D cultures and animal models, which make them a practical platform for different experiments, modeling diseases, and high-throughput screening

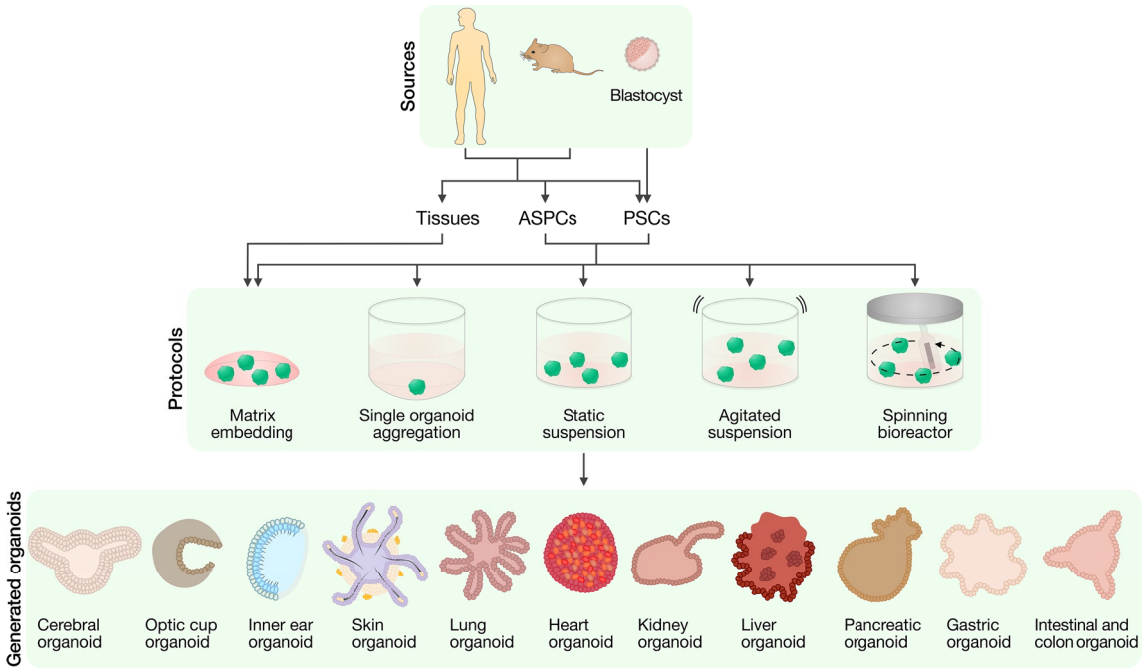


Fig. 2 Different protocols to generate tissue-specific and pluripotent stem-cell-derived organoids. Organoids can be generated from tissue-specific sample biopsies from a variety of organs, or from pluripotent stem cells. The figure illustrates different protocols to induce the maturation and formation of organoids, such as matrix embedding, suspension, using U bottom cell culture plates, and so on. Abbreviations: ASPC, adult stem or progenitor cell; PSC, pluripotent stem cell

differentiation [1, 6]. Consequently, by acting as accurate human disease models, organoids have paved the way for discoveries in human biology and have advanced medical care by providing a platform for drug screening (Fig. 3). In drug discovery processes, in order to obtain a single approved drug, tens of thousands of the new compounds need to pass several screening stages before being considered as Investigational New Drugs (INDs) for further evaluation and subsequent clinical trials. This process is lengthy and expensive, though most (about 80%) approved agents still fail during clinical trials [7]. Thus, organoids can significantly facilitate drug testing. To date, organoid models of microcephaly, cystic fibrosis, ulcerative colitis, and Crohn's disease have already helped develop new pharmaceuticals for these diseases [8, 9]. Besides, organoids can also act as cancer models; however, it is important to note that primary cancer-derived organoids face unique challenges, including the lack of tissue structure and extensive heterogeneity with no tumor microenvironment, and consequently the inability to model localized hypoxia and the input from surrounding immune cells [10] (Box 1).

This review highlights diseases of different germ layers modeled using distinct organoid structures, including the neuronal, hepatic, and renal tissue (Fig. 3). In addition, due to the current COVID-19 pandemic, we have also included descriptions of organoid models that simulate tissue-specific responses to COVID-19 (Box 2).

Ectodermal-derived disease model organoids

Organoids of brain disease models

The in vitro modeling of human brain development was performed using embryoid body (EB) cultures. In 2001, human ESCs were used to generate EBs that could form 2D rosette-like structures after an initial phase of 3D culture [45]. This revealed that EBs could generate clusters of neuroepithelial cells that self-organized into neural tube-like structures [46]. The rosette formations were generated by extending the time spent in the initial 3D culture phase before plating on precoated dishes; this technique generated complex stratified structures reminiscent of the developing cerebral cortex [46–52]. Building on these findings, an in vitro system for the generation of brain-like organoids was developed in 2013 to generate a broader brain region identity termed 'cerebral organoids' [53]. Subsequently, various brain organoid protocols have been introduced over the past few years, all describing different techniques to generate brain organoids that closely mimic the brain as an organ.

Currently, brain organoid models open up the prospect of answering many questions regarding different brain diseases and developmental disorders (Table 1). A study reported using iPSC-derived organoids from patient with microcephaly, a genetic condition caused by a mutation in a gene

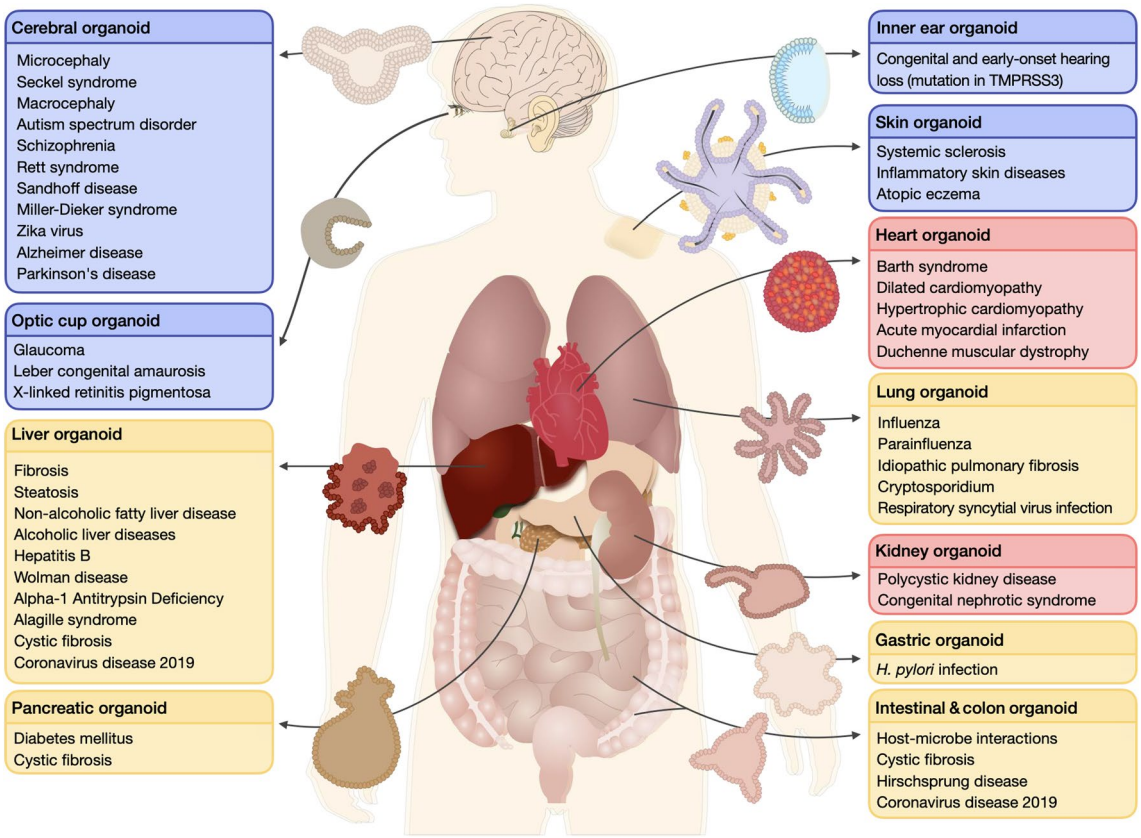


Fig. 3 Summary of generated human organoid disease models

Box 1 Cancer organoid models

Human organoids are crucial players in the pursuit of advancement in cancer biology studies, to improve preclinical studies and to facilitate their translation into effective therapies. Previously, human cancer cell lines, mouse cancer models or patient-derived xenografts (PDXs) consisted the main platform for cancer research and drug discovery [11, 12]. Nowadays, with the advent of adult stem cell-derived organoid technology, cancer tissues have been cultured in vitro as cancer organoids, also known as tumouroids or canceroids. As such, cancer organoids can be derived directly from the tumor tissue of any individual, providing an attractive ex vivo platform to study human cancer biology [11–13]. Cancer organoid models are valuable for recapitulating the human tumor biology, as well as the interactions between neoplastic cells with extracellular matrix, immune cells and tumor vasculature. Additionally, cancer cells can generally grow independently of the niche factors when compared with non-cancerous organoids [11]. Patient samples from colon [14–18], esophageal [19], stomach [20–22], brain [23, 24], prostate [13], pancreas [25–27], liver [28], breast [29], bladder [30], endometrial [31] and lung [32, 33] cancers have been cultured in cancer organoid models. In a recent analysis of drug responses in patients, organoid cultures were matched with the patient response up to 90% [34]. Remarkably, Hans Clevers and colleagues recently established tumor organoid cultures from colorectal cancer and called them 'living organoid biobanks' [14]. These living biobanks of canceroids from gastrointestinal cancers recapitulated the patient response to anti-cancer agents in clinical trials [34, 35]. Altogether, an increasing number of patient-derived canceroids have provided unprecedented access to cancer medicine. In this regard, patient-derived organoid models of the future may provide an in vitro screening platform to predict the best therapeutic option for each individual, to model genetic cancer progression in a better physiological platform, and to study a tumor niche that represents the different grades of tumors

encoding the *CDK5* regulatory subunit-associated protein 2 (CDK5RAP2). Microcephalic patients have a reduced brain size, and this condition is challenging to model using mice [53]. Another study described the use of human Seckel iPSC-derived organoids developed to model Seckel syndrome, which is caused by a mutation in the centrosomal P4.1-associated protein (CPAP) leading to a reduction in the human brain size as well as reduced pre- and postnatal body size. Organoids derived from patient iPSCs with mutated CPAP failed to provide a scaffold for the cilium disassembly complex (CDC) and thus led to premature cellular differentiation in a study, which demonstrated many unknown features of neural progenitor cell differentiation that are important in the mechanism of microcephaly development [54]. To

Box 2 Current organoid models for COVID-19

Due to the inadequate knowledge of the biology of SARS-coronavirus 2 (SARS-CoV-2) that causes the current COVID-19 pandemic, the therapeutic options are limited. Therefore, it is of great importance to construct new disease models to study the biology of SARS-CoV-2 and provide a practical platform for drug screening studies. In one study, human recombinant soluble angiotensin-converting enzyme 2 (hrsACE2) reduced the growth rate of SARS-CoV-2. It was also shown that SARS-CoV-2 can directly infect bioengineered human blood vessel organoids and human kidney organoids, which can be inhibited by hrsACE2. These results demonstrated that hrsACE2 can significantly block the early stages of SARS-CoV-2 infection [36–38] and highlighted its potentially enormous clinical application. Researchers established a human liver ductal organoid to model hepatic infection by SARS-CoV-2, and thus validated viral-induced damage of cholangiocytes ex vivo at the cellular and molecular levels. It was demonstrated that viral infection damages the barrier and bile acid transporting functions of cholangiocytes due to dysregulation of the genes directly involved in tight junction formation and development, as well as bile acid transportation. Thus, liver damage in patients with COVID-19 might be a direct result of cholangiocyte injury and bile acid accumulation [39]. Respiratory symptoms are typical in COVID-19 patients, however, 25% of such patients present gastrointestinal signs/symptoms including anorexia, diarrhea, vomiting, and abdominal pain [40]. ACE2 acts as a SARS-CoV-2 receptor for the viral S protein, which is expressed in a variety of human tissues, including the testis, kidneys, gut, and lungs [41, 42]. It is mainly expressed in the alveolar epithelium type II cells and ciliated cells of lungs, but also has high expression within the brush border of intestinal enterocytes [43]. Recently, a colon model was generated using human pluripotent stem cell-derived colonic organoids (hPSC-COs) to explore the permissiveness of colonic cell types to SARS-CoV-2 infection. Results indicated that, although ACE2 is expressed in all of the hESC-derived colonic cell types, its expression is significantly higher in enterocytes. The COs have also been used in high-throughput drug screening studies of 1280 FDA-approved drugs in viral infection treatments. Remarkably, results indicated that two drugs (mycophenolic acid and quinacrine dihydrochloride) were able to block viral infection in the gut [40]. Finally, human small intestinal organoids have also been generated and shown to support SARS-CoV-2 replication within infected enterocytes [44]

this end, Li et al. reported that cortical organoids generated from patient-specific iPSCs with the dysfunction of *Aspm* gene, a further genetic cause of primary microcephaly in humans, were unable to form normal cortical lamination [55]. Finally, the authors of another study demonstrated that the CRISPR/Cas9 deletion of *PTEN*, a gene associated with human macrocephaly, resulted in the proliferation of neuronal stem cells, and cerebral organoids were created that were more folded and had larger overall mass than normal cerebral organoids [56].

Human brain organoid models of early brain development are invaluable for the study of psychiatric conditions. In 2015, iPSC lines from four patients with severe autism spectrum disorder (ASD) were produced, and the subsequently grown patient-derived organoids exhibited more inhibitory GABAergic neurons due to an upregulation of the forkhead box G1 (*FoxG1*) gene [57]. Furthermore, chromodomain helicase DNA-binding protein 8 (*CHD8*) is a commonly mutated gene in ASD; $CHD8^{+/-}$ cerebral organoids were generated by Wang et al. using CRISPR-Cas9 technology. The authors established that *CHD8* regulates the expression of other genes implicated in ASD, including the downstream signaling molecule associated with DLX6-AS1, which is a long non-coding antisense RNA. DLX6-AS1 critically regulates GABAergic interneuron development and is likely to prove important in therapeutics approaches [58]. The 'disrupted-in-schizophrenia 1' gene (DISC1) interacts with other proteins to influence human brain development, and its mutations are related to numerous psychiatric disorders, such as schizophrenia, bipolar disorder, and autism spectrum disorders. Disrupting the formation of DISC1/Ndel1 complex resulted in cell-cycle deficits in radial glial cells within human forebrain organoids, and the same disruption was observed in organoids derived from iPSCs of schizophrenic patients [59]. Rett syndrome (RTT), another neurodevelopmental disorder, is caused by mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene, which is a gene that influences miRNA expression. The MECP2 gene has also been linked to neuropsychiatric disorders including ASD. Patient-derived cerebral organoids from Rett patients demonstrate the effects of MeCP2 deficiency on human neurogenesis and neuronal differentiation and were used to validate the involvement of a novel miRNA-mediated pathway in ASD [60]. Sandhoff disease, a lysosomal storage disorder, which is also known as GM2 gangliosidosis, was modeled in cerebral-specific organoids using iPSCs derived from an infant carrier of this mutation. The diseased organoids had enlarged size with increased cellular proliferation and displayed accumulated GM2 ganglioside, supporting the idea that GM2 ganglioside accumulation alters early neurodevelopmental processes with downstream postnatal effects [61].

Brain organoid technology has led to the discovery of many human brain-specific phenotypes. Two papers have used brain organoids to model a severe genetic condition named Miller–Dieker syndrome (MDS) associated with lissencephaly ('smooth brain'). Mice are naturally lissencephalic and cannot be used to model this defect [62, 63]; therefore, human organoids have enormous potential in elucidating the pathophysiology of this condition.

Zika virus (ZIKV) infection is yet another cause of congenital microcephaly. ZIKV-infected human brain organoids demonstrate delay in the cell cycle progression of neural progenitor cells, leading to cell death and the smaller size of infected organoids, thus mimicking microcephaly [64–66]. The innate immune receptor 'Toll-like receptor 3' (TLR3) was found to be upregulated after ZIKV infection, which

Table 1 Ectoderm-derived organoids for modeling disease

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
Brain	Microcephaly	iPSC	The first cerebral organoid that could recapitulate the development of the brain. Microcephaly was modeled and brain size reduction was shown	Matrigel embedding	[53]
			The model failed to provide a scaffold for the cilium disassembly complex, leading to premature differentiation. Results demonstrated the crucial role of cilia in the pathogenesis of microcephaly and control in brain size	Matrigel embedding	[54]
			Organoids did not form normal cortical lamination, proposing a new approach to study developmental diseases of the CNS	None	[55]
	Macrocephaly	ESC	Cerebral organoids were more folded than normal. New insights were provided in terms of mechanisms regulating the organization of the human cortex	Matrigel embedding	[56]
	Autism spectrum disorder	iPSC	A shift was proposed toward GABAergic neurons caused by FOXG1 as a developmental precursor of Autism spectrum disorder. This opened a new direction in the diagnosis and future therapeutics of autism spectrum disorder	None	[57]
			DLX6-AS1 critically regulated the development of GABAergic interneurons and are likely important in therapeutics approaches	None	[58]
	Schizophrenia	iPSC	Cell-cycle deficits in radial glial cells were observed, which has the implication that genetic insults contributed to psychiatric disorders	Matrigel embedding	[59]
	Rett syndrome	iPSC	The effect of MeCP2 deficiency was observed on neurogenesis and neuronal differentiation. The involvement of a novel miRNA-mediated pathway was proposed	Matrigel embedding	[60]
	Sandhoff disease	iPSC	Enlarged organoids with increased cell proliferation and accumulated GM2 ganglioside were observed, which alters early neurodevelopmental processes with downstream postnatal effects	None	[61]

Table 1 (continued)

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
	Miller–Dieker syndrome	iPSC	The genetic condition of lissencephaly was modeled, which helped elucidate the pathophysiology of the disease	Matrigel embedding [62] Geltrex [63]	[62, 63]
	Zika virus infection	iPSC	Delay in the cell cycle progression of NPC, cell death, and the smaller size of infected organoids were demonstrated (mimicking microcephaly)	Laminin [64] Matrigel embedding [65] Matrigel embedding [66]	[64–66]
		ESC	The inhibition of TLR3-improved pathological phenotype of ZIKV-infected organoids was demonstrated, and a link was indicated between ZIKV-mediated TLR3 activation and a reduction in organoid size	Matrigel embedding	[67]
			ZIKV infection may alter the DNA methylation pattern in the entire genome of neuronal cells. The result showed that these changes might affect the human brain	Matrigel-coated	[68]
		iPSC	Hippeastrine hydrobromide improved ZIKV-infected organoids, making it a drug candidate for ZIKV infection and related neurological complications in fetal and adult patients	None	[69]
		PSC	Additional receptors were identified for ZIKV entry into neural progenitors that could facilitate drug discovery	None	[70]
		iPSC	Brain organoids were infected with ZIKV to test sofosbuvir. Data evidenced the usefulness of sofosbuvir as a potential drug for blocking ZIKV replication	None	[71]
			A compound screening approach was designed and two classes of effective compounds (antiviral and neuroprotective) were identified to protect human NPCs and astrocytes from ZIKV-induced cell death	Matrigel embedding	[72]
		ESC	Cholesterol-25-hydroxylase suppressed ZIKV infection and reduced tissue damage in cortical organoids, and was proposed as a natural antiviral agent for ZIKV infection	None	[73]

Table 1 (continued)

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
	Alzheimer's disease	iPSC	Reduction in amyloid formation and tau pathology was observed in organoid models after treatment with β- and γ-secretase inhibitors. This model might greatly increase the pre-clinical drug discovery phase in Alzheimer's disease	Matrigel suspension	[79]
			The role of p25/Cdk5 was validated in tauopathy. Additional efforts to develop inhibitors of p25-mediated Cdk5 dysregulation could benefit patients	Matrigel suspension	[80]
			Aβ accumulation, tau aggregates, and cellular apoptosis reported as hallmark features of AD were observed in the generated model, which offers a new platform for drugs screening for therapeutic intervention	None	[81]
		Neurons, astrocytes, microglia	A 3D human AD model was introduced by using neurons, astrocytes, and microglia in a microfluidic system. A more precise human brain model was presented to understand the neural–glial interactions and for drug discovery	Matrigel embedding	[82]
	Parkinson's disease	iPSC	An organoid-based model recapitulating the pathological hallmarks of LRRK2-associated with sporadic PD was presented, which aids the advance of therapeutic discovery	Matrigel embedding	[83]
			Reduction in dopaminergic neurons and increase of FOXA2 was observed in patient-derived organoids, which contained midbrain dopaminergic neurons for investigating the pathogenesis of the disease	None	[84]
			Differences in the expression levels of LIM homeobox transcription factor-alpha and tyrosine hydroxylase markers between organoids from diseased patients and healthy individuals was observed. This provides a model for studying the cellular interactions within the human brain	Matrigel suspension	[85]

Table 1 (continued)

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
		ESC	Massive midbrain astrocyte cell death was shown upon treatment with astrocyte-mediated dopaminergic neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6- tetrahydropyridine, providing an advanced patient-specific platform for in vitro disease modeling	Matrigel embedding	[86]
Inner ear	Congenital and early-onset hearing loss (TMPRSS3 mutation				
	mESC	Tmprss3 mutations induced hair cell apoptosis and degeneration in inner ear organoids	Matrigel suspension		
Retina	Leber Congenital Amaurosis	iPSC	LCA-retinal organoid made an optic cup structure but exhibited decreased ciliation and reduced cilium length	Matrigel suspension	
	X-linked retinitis pigmentosa	iPSC	Significant defects in the photoreceptors were observed. This approach could validate the important defects in photoreceptors and cilia	None	
	Retinitis Pigmentosa 2 (RP2) nonsense mutation		The loss of Kif7 was observed at the cilia tips of optic cup photoreceptors in organoids. Translational read-through inducing drugs, such as PTC124, were able to restore Kif7 levels, which provided evidence that this drug could be beneficial for treatment	Geltrex	
	Late-onset retinitis pigmentosa		The first late-onset retinitis pigmentosa model with a consistent phenotype was generated. This offers an insight into the PDE6B-related mechanism of RP	Matrigel suspension	
Skin	Systemic sclerosis	iPSC	The skin organoid represented the characteristics of systemic sclerosis and exhibited the classic characteristics of skin cells disease	–	
	Atopic eczema		The mechanisms in which *FLG* haploinsufficiency leads to atopic skin inflammation were elucidated, which might help understand the filaggrin-deficient phenotype and the underlying molecular mechanisms related to atopic skin inflammation	None	

CNS, Central nervous system; NPC, Neural progenitor cells; AD, Alzheimer's disease; PD, Parkinson's disease; RP, Retinitis pigmentosa

led to alterations in gene expression patterns and the disruption of physiological neurogenesis. Interestingly, the inhibition of TLR3 improved the pathological phenotype of ZIKV-infected human brain organoids [67]. Research using a model of the developing brain based on embryonic stem cell-derived brain organoids indicates that prenatal ZIKV infection may alter the DNA methylation pattern in the entire genome of neuronal cells, resulting in brain diseases later in life [68]. Zhou et al. indicated that hippeastrine hydrobromide (HH) improved cellular growth and differentiation in ZIKV-infected human fetal-like forebrain organoids [69]. To model the effects of ZIKV, Watanabe et al. described brain organoids that were able to develop cortical and basal ganglia structures similar to the human fetal brain and identified new potential receptors for ZIKV entrance to neural progenitors [70]. Sacramento et al. showed the potential of sofosbuvir, an anti-hepatitis C virus (HCV) drug, to block ZIKV replication in hepatoma cells, neuroblastoma cells, neural stem cells, and brain organoids [71]. By performing a drug repurposing screening of about 6000 compounds, Xu et al. identified two classes of effective compounds (neuroprotective and antiviral) that could be used in combination to protect human neural progenitors and astrocytes from ZIKV-induced cell death [72]. Li et al. highlighted the role of cholesterol-25-hydroxylase (CH25H) as a natural antiviral agent during ZIKV infection, in that it reduced ZIKV-mediated tissue damage in human cortical organoids [73]. Overall, these Zika-infected brain organoid models were applied successfully to test the efficacy of different treatment strategies for Zika virus infection. However, the apparent contradiction in findings suggests that organoid models require further development and should be used in combination with other models to improve disease modeling and drug discovery [74–78].

Due to the immaturity of neurons derived in vitro, it remains unclear how much knowledge on neurodegenerative diseases may be acquired from human iPSC-derived organoid models. The first study that used human brain organoids to investigate Alzheimer's disease (AD), published in 2016, developed a scaffold-free culture method to generate brain organoids derived from multiple patients with familial AD (FAD). These FAD-generated organoids reproduced numerous AD-like pathologies, such as amyloid aggregation, hyperphosphorylated tau protein accumulation, and endosome abnormalities. Furthermore, they showed that the patient-derived organoids treated with β- and γ-secretase inhibitors exhibited significantly reduced amyloid formation and tau pathology [79]. To validate the role of p25/Cdk5 in tauopathy, a cerebral organoid model was developed from isogenic iPSCs and was used to demonstrate that the blockade of p25 mediates tau-associated pathology and the increased expression of synaptophysin [80]. A sophisticated AD model was generated from human iPSCs derived from AD-affected patients with Down syndrome. Cells within this model exhibited Aβ accumulation, tau aggregates, and cellular apoptosis, all hallmark features of AD [81]. Park et al. presented a new 3D human AD tri-culture of neurons, astrocytes, and microglia within a microfluidic system, which was able to model important features of AD, including Aβ aggregation, phosphorylated tau accumulation and neuroinflammatory activity, as well as axonal cleavage resulting from neurotoxic activities. This study has facilitated the understanding of neural-glial interactions and AD drug discovery [82].

Parkinson's disease (PD) is another common neurological disorder; in one study, midbrain organoids were developed from iPSCs exhibiting the Parkinson's disease-associated LRRK2 G2019S mutation. These organoids recapitulated the pathological hallmarks of LRRK2-associated sporadic PD and offered great potential for screening targeted therapies for patients with this disease [83]. In a separate study, midbrain organoids derived from PD patients carrying the *LRRK2*-G2019S mutation exhibited a reduced number and complexity of midbrain dopaminergic neurons and increased expression of FOXA2 (required for midbrain dopaminergic neuron generation), when compared to the control organoids [84]. Recently, an organoid model of idiopathic PD was developed from the iPSCs of patients with idiopathic PD. Compared with their healthy counterparts, the created mature and multicellular organoid-like structures displayed significant differences in the expression levels of neuronal markers, including LIM homeobox transcription factor-alpha and tyrosine hydroxylase expression [85]. Mature midbrain-like organoids (MOs) with a homogeneous distribution of neurons have also been generated. These MOs contain multiple neuronal subtypes, including midbrain dopaminergic neurons. Interestingly, midbrain astrocytes undergo massive cell death in the generated organoids upon treatment with astrocyte-mediated dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an agent known to cause PD [86].

The results of the above studies show that human brain organoids might provide an efficient drug discovery platform for brain-related diseases.

Organoids of inner ear disease models

During embryonic development, the inner ear arises from a thickening of the surface ectoderm adjacent to the hindbrain. The simple thickened epithelium deepens to form an otic vesicle. Otic vesicles develop into the mature inner ear structure in response to specific morphogenetic events and inductive interactions with surrounding tissues [87, 88]. The first inner ear organoid was generated in 2013 [89], and the first report of an inner ear disease organoid model was published in 2019 [90] (Table 1). The type II transmembrane

protease 3 (TMPRSS3) is required for proper mammalian hearing, and mutations in TMPRSS3 cause congenital and early-onset hearing loss in humans. The exact mechanism of this process is unknown; however, mouse stem cell-derived inner ear organoids recently used to investigate the underlying mechanisms of TMPRSS3-related hearing loss or deafness revealed that TMPRSS3 is a crucial component for hair cell homeostasis, and its mutations induce hair cell apoptosis and degeneration [90].

Organoids of retinal disease models

Retinal organoids can recapitulate the spatiotemporal differentiation of the retina and have proven as effective models for understanding retinal development and diseases, as well as drug screening tools [91]. Retinal tissue 3D organoids were first generated in their entirety from mouse cells in 2011 and from human embryonic stem cells in 2012 [92, 93] (Table 1). Several such organoids have been described to date. Leber Congenital Amaurosis (LCA) is a disease of the retina that leads to hereditary blindness. Parfitt et al. generated LCA-retinal organoid models from iPSCs exhibiting a common mutation in the cilia-related gene CEP290. The grown organoid cells were able to form an optic cup structure but exhibited decreased ciliation and reduced cilium length. Normal cilium length and ciliary trafficking returned after treatment to restore the expression of full-length CEP290 [94]. X-linked retinitis pigmentosa (XLRP) is a rare genetic eye disease caused by mutations to the Retinitis Pigmentosa GTPase Regulator (*RPGR*). In one study, significant defects in the photoreceptor were observed in patient-specific organoid models generated from three retinitis pigmentosa patients harboring *RPGR* mutations. However, the CRISPR-Cas9 correction of the RPGR mutation rescued both photoreceptor structure and electrophysiological properties [95]. In a different study, retinal organoids generated from a patient carrying the Retinitis Pigmentosa 2 (RP2) nonsense mutation revealed that the loss of Kif7 at the cilia tips in iPSC-derived 3D optic cup photoreceptors was rescued by translational read-through inducing drugs, such as PTC124 [96]. Late-onset RP is more challenging to model than early-onset RP, while one study successfully developed retinal organoids from late-onset RP proband-derived iPSCs harboring the *PDE6B* mutation. These organoids were able to recapitulate the late-onset disease phenotype and thus provided new insights into the PDE6B-related mechanism of RP [97].

Organoids of skin disease models

The human skin has a surface area of almost 2 m^2 in adults and acts as an important physical barrier protecting the internal environment from outside pathogens. In vitro human skin organoid models are expected to be useful for studying the mechanisms of hair follicle induction, inhibition, and modeling (Table 1). One study generated an organoid model from systemic sclerosis (SSc)-derived iPSCs by inducing their differentiation into keratinocytes and fibroblasts. SSc is a rare autoimmune disease characterized by skin fibrosis, featuring the excessive production and accumulation of collagen in the skin. Patient-derived skin organoids exhibit the classic characteristics of SSc skin cells, which highlights their importance as drug screening tools [98]. Atopic eczema (also known as 'atopic dermatitis' or 'eczema') is an itchy inflammatory skin disorder involving mutations to the gene encoding filaggrin (*FLG*). Recently, organoids derived from primary human keratinocytes with and without siRNA-mediated knockdown of FLG have been used to elucidate the mechanisms by which *FLG* haplo-insufficiency leads to atopic inflammation [99].

Mesodermal-derived disease model organoids

Organoids of cardiac disease models

Drug development for cardiovascular diseases is challenged by a limited supply of tissue samples and appropriate in vitro culture conditions. Meanwhile, 3D cardiac organoids can help create new physiological models of cardiovascular diseases (Table 2).

Barth syndrome (BTHS) is a mitochondrial disorder caused by the mutation of Tafazzin (TAZ). Patient-derived iPSCs and a microchip were used to create BTHS organoid models that replicated the pathophysiology of BTHS cardiomyopathy and helped derive a new therapeutic strategy for BTHS [100]. Dilated cardiomyopathy (DCM) is a major cause of heart failure and premature death and is characterized by progressive left ventricular dilation and systolic dysfunction. The most common genetic causes of DCM are mutations resulting in the truncation of the sarcomere protein titin (TTNtv). Cardiac organoids derived from titin-mutated iPSCs have highlighted how the inadequate generation of sarcomeres impaired tissue responses to mechanical and β-adrenergic stress, likely causing DCM [101]. Hypertrophic cardiomyopathy (HCM) leads to sudden cardiac death. The first 3D human engineered cardiac tissue model for HCM was created using cells from a patient with cardio-facio-cutaneous syndrome (CFCS), a syndrome that is characterized by genetic mutations to any of MEK1 or MEK2 or BRAF genes. A 3D in vitro disease model was constructed from BRAF-mutant cells using a combination of tissue engineering and iPSC technology, which was able to mimic the key features of HCM associated with CFCS. This new in vitro model has the

Table 2 Mesoderm-derived organoids for modeling various diseases

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
Cardiac	Barth syndrome	iPSC	Organoids well replicated the pathophysiology of Barth syndrome cardiomyopathy. The model offered insights into the pathogenesis of Barth syndrome and opened up a new treatment strategy	None	[100]
	Dilated cardiomyopathy		The engineered model showed sarcomere insufficiency and impaired responses to mechanical and β-adrenergic stress. This specifies that titin mutations through disrupting the linkages between sarcomerogenesis and adaptive remodeling cause dilated cardiomyopathy	Collagen solution	[113]
	Hypertrophic cardiomyopathy		The organoid was able to mimic key features of hypertrophic cardiomyopathy associated with cardio-facio-cutaneous syndrome. This 3D microtissue proposes a model for studying intrinsic mechanisms and for therapeutic screening	Collagen-Matrigel mixture	[102]
			The model represented the AMP-activated protein kinase function and the mechanism of PRKAG2 cardiomyopathy. The results offered connections between metabolic sensing, myocyte survival, and TGFβ signaling	Collagen-Matrigel solution	[103]
	Acute myocardial infarction	ESC	After triggering cryoinjury with dry ice, cardiac organoids displayed tissue regeneration over a period of 2 weeks. This study proposed the regenerative ability of immature human heart tissue as an intrinsic capacity	Collagen solution	[104]
	Duchenne muscular dystrophy	iPSC	Restoring proper contractile function was observed in Duchenne muscular dystrophy-engineered heart muscle organoids. By including genome editing tools, this model offered a powerful tool for eliminating genetic etiology and correcting muscular abnormalities	Collagen solution	[105]
Kidney	Polycystic kidney disease	iPSC	The model recapitulated the glomerulopathies involved in the podocyte epithelium injury, and proved as a reproducible and versatile model in regenerative medicine	Matrigel sandwich	[106]
			The model highlighted the importance of podocalyxin in microvillus assembly, and provided a powerful tool for studying human kidney regeneration	GelTrex	[114]
		PSCs	A human cellular system was established by combining polycystic kidney disease organoids with physical components. This approach provided a model of polycystic kidney disease	Matrigel embedding	[108]

Table 2 (continued)

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
	Autosomal recessive polycystic kidney disease	iPSC	Patient-derived hiPSC-mutant organoids showed cystic phenotype, which could be effectively prevented by gene correction or drug treatment. This finding offered new avenues for studying kidney development and drug discovery	None	[110]
	Congenital nephrotic syndrome		This organoid glomeruli model exhibited lower expression of PODOCIN and NEPHRIN, and established an accessible approach for screening the podocyte toxicity	None	[109]
			The nephrotic syndrome was reconstructed using iPSC-derived kidney organoids. This new approach revealed the initial phase of podocyte abnormalities	None	[111]
	Nephronophthisis-related ciliopathy (NPHP-RC)		The tubular epithelia of proband-derived organoids featured shortened and club-shaped primary cilia. This model established the common pathogenic mechanisms for this rare heterogenetic disease	Matrigel suspension	[112]
	Coronavirus disease 2019	hESC	Clinical grade hrsACE2 reduced SARS-CoV-2 recovery of Vero cells by a factor of 1000–5000; an equivalent mouse rsACE2 had no effect	Vitronectin	[115]

potential to screen for new therapeutic approaches to treat this and related heart conditions [102]. Missense mutations in the regulatory subunit PRKAG2 activate AMP-activated protein kinase (AMPK), and this process mimics some features of HCM. To model this type of cardiomyopathy, myocytes differentiated from patient-derived iPSCs were combined into 3D cardiac micro-tissues. The model highlighted essential connections between metabolism and transcript regulation with a better outlook for understanding the AMPK function and the mechanism of PRKAG2 cardiomyopathy [103]. In acute myocardial infarction (AMI), cardiomyocytes die due to ischemia. The process of AMI was modeled using human cardiac-derived organoids exposed to local tissue damage using cryoinjury by dry ice. Interestingly, the organoids displayed tissue regeneration over a period of 2 weeks after acute injury [104]. Duchenne muscular dystrophy (DMD) is associated with the lethal degeneration of cardiac and skeletal muscle. To explore the therapeutic value of gene editing to bypass the metabolic abnormalities in DMD cardiac contractility, Long et al. generated 3D DMD-engineered heart muscles (DMD-EHM) from human iPSCs. The authors used the gene editing of heart muscle cells to repair the contractile dysfunction in DMD-EHM organoids [105].

These achievements highlight the value of organoid technologies and the means by which developments in human cardiac disease organoid models may facilitate cardiac drug discovery.

Organoids of kidney disease models

Recent work has incorporated genome editing tools into patient iPSC-derived kidney organoids, thus providing a unique opportunity to study human kidney diseases with a precise control of the genetic background (Table 2). Freedman et al. described a human model for polycystic kidney disease (PKD) where the knockout of polycystic kidney disease-related genes PKD1 or PKD2 by CRISPR/Cas9 led to cysts forming in kidney tubules. The authors further showed that podocalyxin gene (PODXL)-defective kidney organoids have junctional organization defects in podocyte-like cells, and hiPSC-derived podocyte cells might have the potential to recapitulate the glomerulopathies involved in the podocyte epithelium injury [106]. In a follow-up study, loss-of-function mutations in the human PODXL gene (PODXL knockout hiPSC-derived podocytes) were shown to cause defects in microvillus assembly, leading to increased space between podocytes and in turn the formation of porous junctions, highlighting the importance of podocalyxin in microvillus assembly [107]. By culturing mutant kidney organoids in suspension culture, Freedman and colleagues established an efficient model of PKD cystogenesis, in which the cysts of

PKD patients were recapitulated. The PKD2−/− organoids were defective in expressing of the polycystin-1 protein, and the knockdown of polycystin-2 protein also decreased polycystin-1 protein expression. These mutations altered organoid ECM remodeling and thus emphasized the importance of the ECM in maintaining tubular architecture and adhesion forces for cystogenesis [108]. Hale et al. grew organoid glomeruli from a congenital nephrotic syndrome patient (organoid-derived glomeruli, OrgGloms), which exhibited lower expression levels of PODOCIN and NEPHRIN. These proteins are uniquely expressed in the kidney and are involved in the structure of filtration slits of podocytes and renal filtration barrier function, respectively. Thus, this organoid model represents an accessible approach for modeling human podocytopathies, screening podocyte toxicity, and exploring drug efficacy [109]. Low et al. established an in vitro model from autosomal recessive PKD patient-derived hiPSCs. Notably, mutant organoids showed a cystic phenotype, a characteristic PKD feature, which could be prevented by gene correction or drug treatment [110]. Mutations in the NPHS1 gene causes congenital nephrotic syndrome resulting in an impaired slit diaphragm (SD) formation. The kidney organoids generated from a patient with an NPHS1 missense mutation were used to reveal the initial phase of podocyte abnormalities [111]. Reprogramming and gene editing protocols were used to derive both proband-derived iPSCs and isogenic gene-corrected iPSCs for generating kidney organoids. The organoid generated from proband-derived iPSCs presented a nephronophthisis-related ciliopathy (NPHP-RC), and interestingly, the tubular epithelium of proband organoids were shortened, club-shaped primary cilia [112].

Although the kidney has a complex structure and intricate functions, new methods to model kidney disease using organoids and in turn facilitate our understanding of pathobiology and drug discovery are emerging.

Endoderm-derived disease model organoids

Organoids of lung disease models

The lung epithelium derived from the endodermal germ layer undergoes a complex series of endoderm-mesoderm-mediated signaling events to develop into the final arborized network of conducting airways (bronchi, bronchioles) and gas-exchanging units (alveoli). The alveolar surfaces are lined by alveolar type 1 (AT1) and alveolar type 2 (AT2) epithelial cells. The covering epithelium of airways and alveoli faces several inflammatory conditions and progressive diseases, including idiopathic pulmonary fibrosis (IPF), bronchial asthma, chronic obstructive pulmonary disease, and respiratory infections such as those by coronavirus and influenza [116] (Table 3).

The first successful organoid model for lung epithelial cells was developed by McQualter et al. using stem/progenitor cells in the presence of fractionated primary mouse lung stromal cells [117]. Wilkinson et al. generated an IPF model using human iPSC-derived mesenchymal cell organoids, which were treated with TGF-β to induce fibrosis [118]. Strikoudis et al. developed ESC-derived organoids using CRISPR/Cas9 to introduce frameshift mutations in Hermansky-Pudlak syndrome (HPS) genes. The clinical features of this syndrome were similar to IPF. Genome-wide expression analysis revealed the upregulation of interleukin-11 (IL-11) in the epithelial cells of HPS mutant fibrotic organoids. Notably, IL-11 functioned as a fibrosis inducer in wild-type (WT) organoids, while the IL-11 deletion prevented fibrosis in HPS4−/−. These data suggest that IL-11 is a therapeutic target and a promising molecule to facilitate fibrosis-associated lung disease modeling [119]. Using 3D lung organoids from patients with IPF, Surolia et al. demonstrated that the inhibition of vimentin intermediate filaments (VimIFs) decreased the invasiveness of IPF fibroblasts and conferred protection against fibrosis in a murine model of experimental lung injury [120].

Viral infections of the distal lung parenchyma can progress from pneumonia to acute respiratory distress syndrome. Recent developments in multicellular and physiologically active organoid cultures derived from PSCs, as well as stem cells isolated from biopsies, have opened up horizons to better understand microbial pathogenesis and host-microbe interactions [121]. Cryptosporidium, a protozoan parasite, is a primary cause of diarrhea and a major culprit of child mortality worldwide, though no relevant experimental culture system to facilitate drug development has yet been constructed. In a recent study, epithelial organoids were established derived from the human small intestine and lungs, and were infected with Cryptosporidium. The results indicated that Cryptosporidium could replicate and complete its entire life cycle within these organoids [122]. Infections with a different pathogen, the influenza virus, represent a major global public health threat. Zhou et al. developed long-term expanding human airway organoids (AO) composed of four types of airway epithelial cells: ciliated, goblet, club, and basal cells. These AOs exhibited physiological phenotypes including beating cilia and elevated serine protease levels crucial for the productive infection of human influenza viruses [123]. One study established human stem cell-derived airway organoids with similar morphological characteristics to human airways, including mucus-secreting goblet cells, club cells, and ciliated epithelial cells with active ciliary beating and mucus movement over the epithelial surface. These organoids served as a platform to compare the viral replication capability, tissue tropism, as well as cytokine and chemokine induction of avian influenza A viruses isolated from humans (types Sh2/H7N9, H5N1/483,

Table 3 Endoderm-derived organoids for modeling diseases

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
Lung	Idiopathic pulmonary fibrosis	iPSC-MSC	The model recapitulated IPF for drug screening	Alginate beads	[118]
		IPF patient-derived primary cells	The inhibition of VimIFs resulted in decreased invasiveness of IPF fibroblasts and conferred protection against fibrosis in a murine model of experimental lung injury	HEMA-coated	[120]
	Cryptosporidium	Bronchial airway tissue resection	Cryptosporidium could replicate and complete its entire life cycle within the organoids	Matrigel embedding	[122]
	Influenza	Lung biopsies	Physiological phenotypes were exhibited including beating cilia and elevated serine protease levels, which are crucial for productive infection of human influenza viruses	Matrigel embedding	[123]
		PSCs	The organoid served as a platform to compare viral replication capability, tissue tropism, as well as cytokine and chemokine induction of avian influenza A virus	Matrigel embedding	[124]
	Parainfluenza		The organoid simulated viral evolution and pathogenesis, as well as important features of human viral infections	Matrigel embedding	[125]
	Respiratory syncytial virus infection		The branching structures were similar to those observed in the second trimester of human gestation	Matrigel embedding	[126]
Liver	Fibrosis	Primary HSC and the HepaRG cell line	This organoid displayed fibrotic features including HSC activation, collagen secretion, and ECM deposition after exposure to the pro-fibrotic compounds	None	[132]
		iPSCs-HSCs and HepaRG cell line	In this organoid, in vivo human HSCs functions were recapitulated at the transcriptional, cellular, and functional levels, and the gene expression profile was intermediate between the quiescent and activated HSCs	None	[133]
	NAFLD	Adult liver stem cells from mouse, human, dog, and cat	The analysis of genes involved in fat accumulation, including *SREBF1*, *CPT1A*, and *PPARG,* revealed that liver organoids from dog and human cells exhibited distinct regulation of lipid metabolism	Matrigel embedding	[135]
		PSC-HLC, stellate-like cells, Kupffer-like cells	The secretion of IL-6, TNF-α, and IL-8 were increased compared to untreated organoids after treatment with free fatty acids	Matrigel embedding	[136]
		HepG2 cell line and LX-2 cell line	The physiological phenotype was restored by incubating the organoids with anti-steatotic and anti-fibrotic drugs, including liraglutide and elafibranor	None	[147]

Table 3 (continued)

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
	ALD	ESCs and MSC	Treatment with ethanol induced numerous ALD-associated pathophysiological changes, including oxidative stress, steatosis, inflammatory mediators release, and fibrosis	Matrigel embedding	[138]
	Hepatitis B	PHHs converted into HepLCs	This model expressed NTCP. Molecular data revealed the HBV transcriptome in host cells upon transfection, corroborating the use of this organoid model for exploring HPV infection	None	[142]
		iPSC-HLC, MSCs and HUVEC	The model featured high-level expression of NTCP and showed well-maintained long-term HBV propagation, which was associated with hepatic dysfunction	None	[143]
	Coronavirus disease 2019	Cholangiocytes	Viral infection damaged the bile acid transport in cholangiocytes due to dysregulation of genes involved in tight junction development and bile acid transportation	Matrigel embedding	[39]
	Wolman disease	Patient-derived iPSCs	Treatment with FGF19 increased the survival rate in the culture system and reduced lipid accumulation, ROS production, and stiffening	Matrigel embedding	[136]
	A1AT deficiency	Patient-derived Lgr5 + liver stem cells	A1AT accumulation was similar to the original biopsies from patients, and the aggregation of A1AT protein blocked elastase activity in A1AT organoids	Matrigel embedding	[145]
	Alagille syndrome		Structural duct defects in the biliary tree were simulated	Matrigel embedding	[145]
		iPSC-derived cholangiocytes and hepatocytes	The *JAG1* mutation in Alagille syndrome was studied using this organoid model	Matrigel embedding	[146]
	Cystic fibrosis	PSC-derived cholangiocytes	Epithelial functions, including rhodamine efflux and CFTR-mediated fluid secretion, were detected. Data showed that functionally impaired hPSC-derived cholangiocytes from cystic fibrosis patients are rescued by CFTR correctors	Collagen/Matrigel mixture	[147]
Pancreas	Diabetes mellitus	PSCs	The hPSC-ECs were demonstrated to express pancreatic endocrine hormones and EC clusters started insulin secretion in response to glucose stimulus and potassium channel inhibition in vitro	None	[150]
	Cystic fibrosis		Acinar and ductal progeny were modeled	Matrigel embedding	[152]

Table 3 (continued)

Tissue/organ	Disease	Cell source	Outcomes and achievements	Matrix	Ref
Gaster	H. pylori infection	iPSC	*H. pylori* infection resulted in the rapid association of the virulence factor CagA with the c-Met receptor, activation of signaling, and induction of epithelial proliferation	Matrigel embedding	[155]
Colon and intestine	Host-microbe interaction		After exposing organoids to *Salmonella enterica* serovar Typhimurium by microinjection into the organoid lumen, the cytokine expression pattern was changed and *S. e.* Typhimurium attacked the epithelial barrier	Matrigel embedding	[169]
		ESCs	The iHIOs with the addition of human neutrophils can model *E. coli* intestinal infection and innate cellular responses	Matrigel embedding	[170]
	Cryptosporidium	Duodenal biopsies	Cryptosporidium could replicate and complete its entire life cycle within the organoids	Matrigel embedding	[122]
	Coronavirus disease 2019	PSC-COs	The expression of ACE2 was significantly higher in enterocytes. High-throughput drug screening was investigated for 1,280 FDA-approved drugs in viral infections. The result showed that mycophenolic acid and quinacrine dihydrochloride were able to block viral infection in the gut	Matrigel embedding	[40]
		Biopsy-human small intestinal cells	The intestinal epithelium supported virus replication	Matrigel embedding	[44]
	Cystic fibrosis	Patient-derived primary intestinal stem cells	CRISPR/Cas9-mediated gene editing repaired the CFTR mutation and restored the functional phenotypes	Matrigel embedding	[173]
		PSC-HIO	Spironolactone reversed fibrosis caused by TGF-β induction	Matrigel embedding	[174]
	Hirschsprung disease	PSCs	The model studied the cellular and molecular pathogenesis of Hirschsprung disease with a mutation in the *PHOX2B* gene	Matrigel embedding	[166]

H5N6/39715, and H1N1pdm/415742). They were comparable to human ex vivo bronchus cultures and could serve as an alternative experimental model for studying virus tropism and replication competence to assess the pandemic threats of novel influenza viruses [134]. Porotto et al. demonstrated the successful spread of human parainfluenza virus 3 (HPIV3) in lung organoids derived from hPSCs by infecting AT2 cells in the organoids. Using this model, viral evolution and pathogenesis could be simulated, and essential features of human viral infection could be mimicked in these organoids [125]. Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children younger than 1 year of age in the United States [121]. Lung bud organoids (LBO) generated from hPSCs were used to model lung parenchyma in the second trimester of gestation in human life. The LBOs were composed of mesoderm and pulmonary endoderm cells that developed into branching airways and early alveolar structures upon xenotransplantation or in a Matrigel 3D culture. The analysis of expression and structural features showed that the branching structures were similar to those observed in the second trimester of human gestation. Two days after the infection of LBOs with RSV, infected cells began to swell, detach, and shed into the organoid lumen [126].

In summary, lung organoids represent state-of-the-art platforms to model lung diseases by recapitulating the interactions between host pulmonary cells, immune cells, the ECM, and invading pathogens. Therefore, they consist powerful tools for modeling human lung diseases, drug screening, and toxicity assays.

Organoids of liver disease models

Liver diseases are among the most common pathologies of the digestive tract, occurring at increasing frequencies due to alcohol abuse, obesity, viral infections, and the lack of physical activity [127]. Fatty liver and viral hepatitis are two common liver diseases leading to cirrhosis and ultimately liver cancer. The liver is a complex metabolic and detoxifying organ with a sophisticated inner microenvironment; therefore, generating accurate models of the diseased liver for exploring treatment options has been challenging [128–130] (Table 3).

Liver fibrosis is characterized by excessive ECM deposition, which leads to scarred tissue and eventually organ failure. Hepatic stellate cells (HSCs), the major collagen-producing cells of the liver, are the main cell types involved in liver fibrosis following injury from metabolic, cholestatic, viral, or toxic causes. When hepatocytes are damaged, a cascade of events is triggered activating quiescent HSCs into a myofibroblastic (activated) HSC state [131]. Leite et al. developed a 3D co-culture of primary stellate cells and the HepaRG cell line. Following repeated exposure to the pro-fibrotic compounds for 14 days, including allyl alcohol and methotrexate, hepatic organoids displayed fibrotic features, such as HSC activation, collagen secretion, and ECM deposition [132]. Another research consortium developed a liver fibrosis model using highly reproducible iPSCs-derived HSCs that remained quiescent when maintained as 3D spheroids with HepaRG, but became activated to secrete pro-collagen in response to hepatocyte toxicity, thus recapitulating in vivo human HSCs function [133].

Non-alcoholic fatty liver disease (NAFLD), a complex condition characterized by steatosis, is multifactorial in etiology with many identified environmental and genetic risk factors. NAFLD patients may develop a more severe non-alcoholic steatohepatitis (NASH) disease that can further progress to liver fibrosis and cirrhosis [134]. Kruitwagen et al. established a liver steatosis organoid model using adult liver stem cells from mouse, human, dog, and cat [135]. When fatty acids were added to the culture media, the liver stem cells absorbed the fat, leading to lipid accumulation. The analysis of genes central to fat accumulation, including SREBF1, CPT1A and PPARG, revealed that liver organoids from dog and human cells exhibited the distinct regulation of lipid metabolic pathways [135]. However, pro-fibrotic or inflammatory cell types are absent in culture conditions, limiting the ability to fully model liver diseases in vitro. Ouch et al. used 11 pluripotent stem cell lines from healthy and sick individuals to differentiate into multicellular human liver organoids composed of hepatocyte-like cells, hepatic stellate-like cells, and Kupffer-like cells. The organoids were incubated with free fatty acids to induce steatosis and fibrosis [136], which led to the increased secretion of IL-6, TNF-α, and IL-8 compared to untreated organoids. Moreover, the authors used patient-derived iPSCs to create organoid models of Wolman disease, an inherited disorder, and established that the organoids were capable of stipulating many of the clinical disease features. These patient-derived organoids were treated with FGF19, which increased the survival rate of the cultured system and reduced lipid accumulation, ROS production, and stiffening [136]. A recent organoid model was developed by Pingitore et al., which is based on cells homozygous for the PNPLA3 I148M sequence variant, the most vital genetic determinant of NAFLD. Hepatocyte (HepG2) and hepatic stellate (LX-2) cell lines were incubated with free fatty acids, which resulted in fat accumulation (steatosis) and collagen secretion (fibrosis). A physiological phenotype was restored by incubating the organoids with anti-steatotic and anti-fibrotic drugs, including liraglutide and elafibranor [137].

Alcoholic liver diseases (ALDs) are among the major causes of chronic liver illnesses affecting many people worldwide, and leading to irreversible fibrosis and cirrhosis. Wang et al. developed liver organoids from hESCs co-cultured with MSC, and treatment of the organoids with

ethanol successfully induced numerous ALD-associated pathophysiological changes, including oxidative stress, steatosis, release of inflammatory mediators, and fibrosis [138].

Chronic viral hepatitis has a high incidence in the general population; approximately 2 billion people are infected with HBV worldwide, leading to significant morbidity and mortality [139]. Several models have been developed for HBV infection, but none display individualized genetic backgrounds. Primary human hepatocytes (PHHs) are a valuable model for HBV infection studies due to the high expressions levels of NTCP, a receptor used by HBV to enter hepatocytes [140]; PHHs, however, have limited supply and are of variable quality [141]. A recent study revealed that PHHs could be efficiently converted into hepatic progenitor-like cells (HepLCs) by exploiting relevant developmental signals, such as the NAD+-dependent deacetylase SIRT1 signaling. Organoids formed using HepLCs were found to express NTCP, and molecular data suggested the presence of HBV transcriptome in host cells upon transfection, corroborating the use of this organoid model for exploring HPV infection [142]. Liver organoids have also been generated from a co-culture of iPSC-derived hepatocytes, mesenchymal stem cells (MSCs), and human umbilical vein endothelial cells (HUVEC). Interestingly, these liver organoids expressed high levels of NTCP and maintained long-term HBV propagation, which was associated with hepatic dysfunction [143].

Inherited liver diseases are a group of metabolic and genetic defects that occur due to the failure of enzyme/transport proteins involved in specific metabolic pathways in the liver [144]. Alpha-1 antitrypsin (A1AT) deficiency is a hereditary condition that leads to chronic obstructive pulmonary disease and chronic liver disease; the most common related disorders are Alagille syndrome and cystic fibrosis (CF). Alagille syndrome is caused by mutations in the Notch-signaling pathway, which results in partial to complete biliary atresia.

Several studies developed patient-derived hepatocytes and cholangiocytes to investigate and model genetic liver diseases [136]. Huch et al. employed Lgr5 + liver stem cells to construct organoid-based inherited disease models using biopsies from patients with A1AT deficiency or Alagille syndrome. A1AT accumulated in these organoids similarly to the original biopsies from patients. The results also confirmed that the aggregation of A1AT protein blocks elastase activity in A1AT organoids. Organoids derived from an Alagille syndrome patient reproduced the structural duct defects in the biliary tree of such patients, and this study was the first model of human 3D system generated to study the syndrome. Homologous recombination by CRISPR/Cas9 was able to successfully repair the associated genetic defect [145]. Guan et al. also developed an in vitro model system to study the JAG1 mutation in Alagille syndrome using iPSCs differentiated into 3D human hepatic organoids. The generated organoids were composed of hepatocytes and cholangiocytes organized into epithelial cells surrounding the lumina of bile duct-like structures [146]. Ogawa et al. recently developed a cystic fibrosis model and corrected the CFTR (cystic fibrosis transmembrane conductance regulator) misfolding and translocation in cell membranes in patient-derived cholangiocyte organoids (hPSC-derived cholangiocytes) by using inhibitors to decrease misfolding and promote protein stabilization [147].

In conclusion, although the current differentiation protocols in the development of hepatic organoids are not promising, it is essential to continue developing in vitro models to study disease pathophysiology and to conduct preclinical pharmaco-toxicological studies. This may be achieved through the adaptation of media composition and the application of ECM in organoid culture systems.

Organoids of pancreatic disease models

In general, the pancreas is a composite gland containing an exocrine compartment of acinar and ductal cells and an endocrine compartment of alpha, beta, gamma, epsilon, and PP (pancreatic polypeptide) cells organized within 'Langerhans islets' [148]. Numerous diseases arising from defects in the different compartments affect the pancreas, including diabetes mellitus (DM), CF, and pancreatic adenocarcinoma (Table 3). DM is the most frequent endocrine disease, which is characterized by hyperglycemia, and is classically categorized in 2 types: type 1 is an autoimmune disease in which pancreatic β cells suffer damage, and type 2 is caused by peripheral insulin resistance with insufficient insulin production by pancreatic β cells [149]. Kim et al. developed pancreatic islet-like clusters in vitro from hESCs and iPSCs and demonstrated that cell cultures had glucose-responsive insulin secretion. The hPSC-derived endocrine cells (ECs) were shown to express pancreatic endocrine hormones (insulin, somatostatin, and pancreatic polypeptide) and EC clusters (ECCs) started insulin secretion in response to glucose stimulus and potassium channel inhibition in vitro [150].

Cystic fibrosis is an inherited disorder that affects multiple organs, including the pancreas, which is typically one of the first affected organs; current knowledge about the pathophysiological changes in the pancreas during CF is limited [151]. Hohwieler et al. designed an approach to generate pancreatic organoids from hPSCs that resembled acinar and

ductal progeny. Following the same approach, iPSC-derived organoids from CF patients were also generated, which exhibited pancreatic cell specification and recapitulated the key developmental events in vitro. The CFTR mutated pancreatic organoids proved to be a promising reliable platform to reflect the patient phenotype in the functional swelling assay, and thus are potential disease models for individualized drug testing in pancreatic tissue [152].

Organoids of gastric disease models

Gastric diseases, including digestive ulcer disease and gastric cancer, affect 10% of the world's population. Most of them are caused by chronic Helicobacter pylori infection [153]. Gastric organoids (GO) can be derived from iPSCs as well as adult stem cells from the corpus and antropyloric epithelium [154] (Table 3). McKracken et al. established hiPSC-GO cultures to identify the unique signaling mechanisms that regulate early endoderm patterning and gastric endocrine cell differentiation upstream to NEUROG3. Moreover, they modeled the pathophysiological response of the gastric epithelium to H. pylori and showed that H. pylori infection caused the rapid association of virulence factor CagA with the c-Met receptor, leading to the activation of signaling and stimulation of epithelial proliferation [155]. Bartfeld et al. also generated GOs from surgical samples of human gastric corpus and provided sufficient evidence to demonstrate that stem cells existed in adult human gastric tissue. The organoids contained four different cells of the stomach: pit mucous cells, gland mucous cells, chief cells, and enteroendocrine cells. The GOs were used to analyze the primary response of the human epithelium to H. pylori and revealed that the infection induced robust NF-kB activation [156]. Numerous studies have reported on the pathogenesis of H. pylori infection using organoid models for validating the established hypothesis, and discovering new mechanisms [157–160].

Organoids of intestinal and colon disease models

In the intestine, several signaling pathways, including Wnt, Notch, fibroblast growth factor (FGF)/epidermal growth factor (EGF), and bone morphogenetic protein (BMP)/Nodal signaling, are essential during tissue development, adult homeostasis, and repair following injury [161, 162]. The luminal surface of the mammalian intestine consists of a single layer of self-assembling epithelial cells. This epithelium is divided into two regions: villus and crypt. The latter contains intestinal stem cells with high expression of LGR5, a protein involved in WNT signaling. Thus, LGR5 + cells represent a reliable source to generate organoids [163]. Intestinal organoids (IOs) have been developed from different sources, including postnatal and adult epithelial cells [164], adult intestinal stem cells (known as 'enteroids') [165], and PSC-derived organoids [166] (Table 3).

The apical part of the epithelial surface of the lumen is the main attachment site for many intestinal pathogens. The generation of IOs offers a tool for studying host-microbe interactions [167]. The mechanisms of secretory diarrhea caused by *Vibrio cholerae* have been studied using organoid models from human duodenal and jejunal biopsies [168]. HiPSC-derived organoids have also been used to explore the interactions between human intestinal epithelium and enteric pathogens. Forbester et al. developed human intestinal organoids (HIOs) from hiPSCs to investigate the interaction of *Salmonella enterica* serovar Typhimurium with the human intestinal epithelium; the authors demonstrated that, after exposing organoids to the bacteria by microinjection into the organoid lumen, the cytokine expression pattern was changed and *S. e.* Typhimurium attacked the epithelial barrier [169]. These results show that HIOs can recapitulate human tissue responses to bacterial pathogens, including the internalization of bacteria, upregulated secretion of mucus, epithelial barrier interruption, and prompted pro-inflammatory cytokine expression [170–172].

In other diseases such as CF, IOs generated from patient-derived primary intestinal stem cells accurately recapitulated the CF phenotype, and CRISPR/Cas9-mediated gene editing was able to repair the CFTR mutation and restore the functional phenotypes [173]. Studies on intestinal fibrosis modeling have been used with respect to Crohn's disease (CD). Stem cell-derived HIOs have been developed as a model of fibrosis in CD using TGF-β to induce fibrosis. Treatment with spironolactone, an anti-fibrotic drug, was able to reverse fibrosis caused by TGF-β induction [174].

The enteric nervous system (ENS) is the intrinsic nervous system of the bowel that regulates many gut functions, such as motility, secretion, and epithelial permeability. The congenital absence of this system within the bowel results in Hirschsprung disease [175]. HIOs have been generated to model this disorder; these HIOs comprised functional ENS derived from human PSCs differentiated to form neural crest cells. ENS-containing HIOs were able to form neuroglial structures and had an electromechanical coupling that regulated waves of propagating contraction. This model was used to study the cellular and molecular pathogenesis of Hirschsprung's disease with a mutation in the *PHOX2B* gene [166].

Box 3 Vascularized organoids

Given the incredible successes achieved so far, the organoid field faces many limitations hindering its broader application in disease modeling. For example, the lack of supportive vasculature for increased perfusion is highly critical. Vasculature not only plays an important role in many physiological regulations, but blood vessels are also known to promote signal transduction between cell–cell and cell–matrix, with their main function of transporting nutrients, oxygen, and remove wastes [179]. By incorporating vasculature into organoids, the size and duration organoid culture performance could be significantly increased. This might be crucial for organoids to mimic real organs [179]. Recently, studies have progressively focused on developing vascularized organoids with different approaches ranging from co-culture with endothelial cells, to co-differentiation with mesodermal progenitors and in vivo transplantation, etc. In this regard, successful experiments were able to establish brain, liver, and kidney organoids [176, 179–182]. Among them, sufficient perfusion was achieved in organoids through in vivo transplantation because of the appropriate microenvironment and niche. Currently, scientists' attempts continue to find a better way to vascularize organoids, which will help to construct more accurate disease models. Eventually, as an improved technology, organoid technology combined with high-tech approaches such as microfluidic engineering or bio-printing techniques could lead to fully in vitro vascularized organoids, acting as more reliable platforms to further expand our understanding of diseases and facilitate drug discovery through advanced modeling

On the whole, current technologies with multiple co-cultured cells and strategies for rebuilding culture microenvironments need to be improved. More advanced culture media and improved ECM can provide the possibility to gain deeper insights into interactions between GI epithelial cells and microbiota, which constitutes a high-priority research field.

Challenges and future prospects

Organoids are powerful tools to overcome many of the failures inherent to current in vitro or animal systems, and have the potential to significantly expand our understanding of the pathogenic mechanism of numerous diseases. They have the capacity to self-organize and form complex structures making them ideal in vitro platforms for disease modeling and drug screening; however, many obstacles remain in the technology that need to be addressed in future work. Specifically, the size of all organoids developed to date is limited by the diffusion distance restriction due to the lack of vascular structures (i.e., blood vessels). Strategies such as vascularization by co-culturing with endothelial cells or engineering approaches may help to overcome this obstacle [176] (Box 3). Some organoid models are insufficient and slow-maturing, thus limiting the modeling of later developmental disease stages, with this issue more typical of retinal and cerebral organoids and thicker tissues such as the brain that may undergo necrosis in the organoid interior. Growing the organoids by transplantation may be a solution to this hurdle. In multi-organ individuals like humans, each organ is involved in synchronous and multiple interactions with all other organ systems. Thus, modeling the interactions between ecto-, meso-, and endoderm tissues during development may be a promising approach to better recapitulate the organ system interactions typical of in vivo organisms. Combining organoids into assembloids can be used to model similar aspects of cell–cell interactions and connectivity between regions to those between human body tissues. Koike et al. generated a multi-organ system that structurally and functionally integrates hepato-biliary-pancreatic organ domains, and that was developed at the foregut-midgut boundary [177]. Interactions with other cells and ECM components are critical aspects of organoid models. Tissue engineering is an ever-evolving approach to using ECM and other synthetic cell-instructive matrices in composite organoids [178] (Fig. 4). However, we are far from the exact models of real organs, which necessitates considerable improvement in this field. The ultimate goal of organoid technologies will be to harness the control of amazing self-organizing abilities of cells for our own benefit and apply them for cell replacement, whole-organ transplantation, and drug screening approaches.

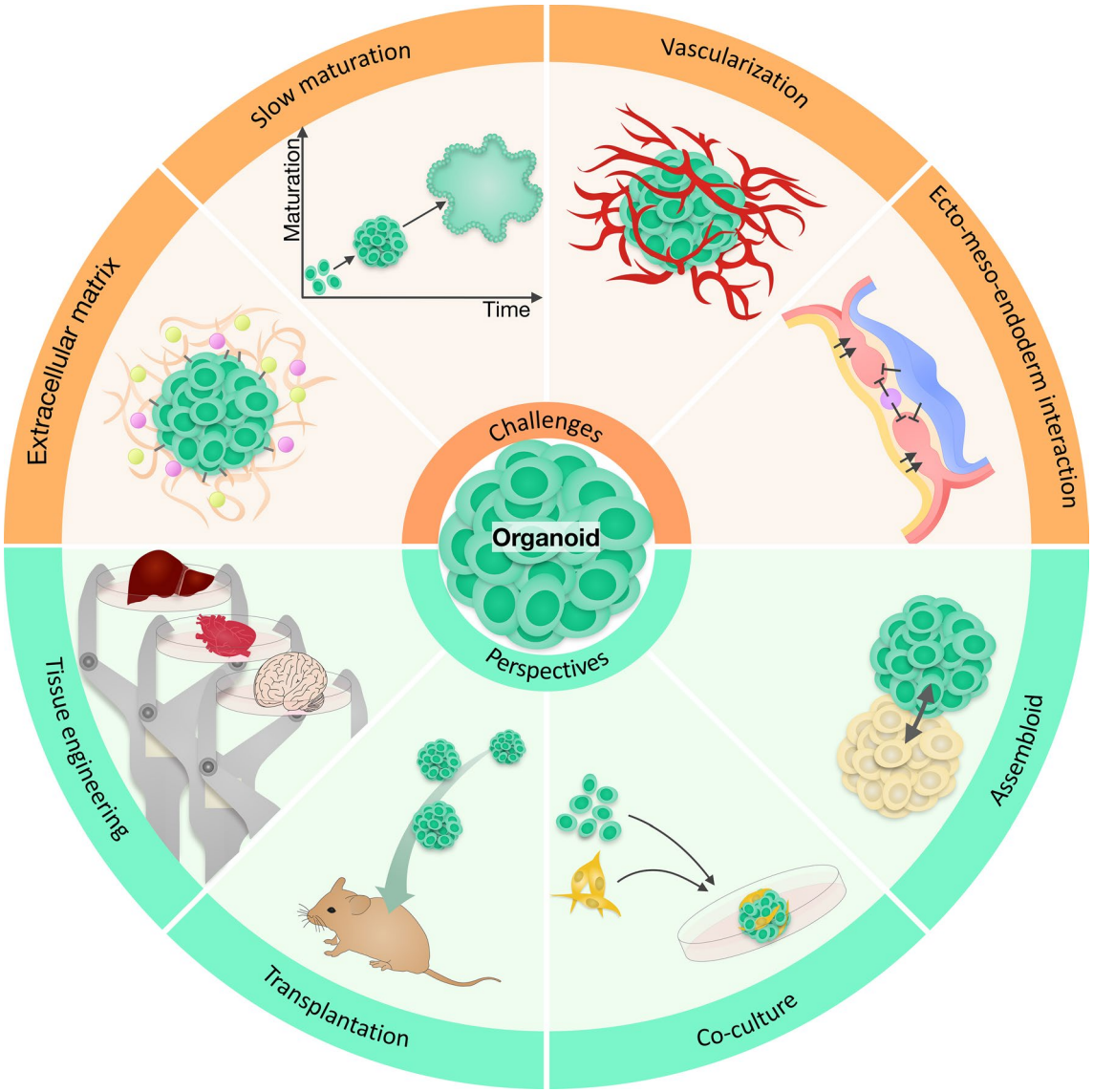


Fig. 4 View of organoid challenges and perspectives. The schematic illustration represents organoid technology challenges including slow maturation, lack of ECM, vascularization, and ecto-meso-endoderm interactions with a foreseeable solution to overcome these weaknesses by transplantation, tissue engineering, co-culture, and assembloids

Acknowledgements This study was financially supported by the National Cancer Control Charity Foundation (Registration Number 41476, Grant Number, 235), Iran National Science Foundation, INSF (Grant Number, 97014445), and by the Ministry of Science and Higher Education of the Russian Federation within the framework of state support for the creation and development of World-Class Research Centers "Digital biodesign and personalized healthcare" (No. 075-15-2020-926). Authors appreciate supportive colleagues who helped them in different research centers to complete this work.

Author contributions ZH and FM were involved in writing original draft and review. TA, PP, AS, and TKM helped in writing, editing and visualization. PT, HB, and MV were involved in conceptualization, helped in writing, funding, and supervision. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Huch M, Knoblich JA, Lutolf MP et al (2017) The hope and the hype of organoid research. Development (Cambridge, England) 144(6):938–941. https://doi.org/10.1242/dev.150201

2. Heydari Z, Najimi M, Mirzaei H et al (2020) Tissue engineering in liver regenerative medicine: insights into novel translational technologies. Cells 9(2):304. https://doi.org/10.3390/cells9020304
3. Lehmann R, Lee CM, Shugart EC et al (2019) Human organoids: a new dimension in cell biology. Mol Biol Cell 30(10):1129–1137. https://doi.org/10.1091/mbc.E19-03-0135
4. Smith E, Cochrane WJ (1946) Cystic organoid teratoma: (report of a case). Can Med Assoc J 55(2):151–152
5. Clevers H (2016) Modeling development and disease with organoids. Cell 165(7):1586–1597. https://doi.org/10.1016/j.cell.2016.05.082
6. Nobakht Lahrood F, Saheli M, Farzaneh Z et al (2020) Generation of transplantable three-dimensional hepatic-patch to improve the functionality of hepatic cells in vitro and in vivo. Stem Cells Dev 29(5):301–313. https://doi.org/10.1089/scd.2019.0130
7. Kondo J, Inoue M (2019) Application of cancer organoid model for drug screening and personalized therapy. Cells 8(5):470. https://doi.org/10.3390/cells8050470
8. Dutta D, Heo I, Clevers H (2017) Disease modeling in stem cell-derived 3D organoid systems. Trends Mol Med 23(5):393–410. https://doi.org/10.1016/j.molmed.2017.02.007
9. Lancaster MA, Huch M (2019) Disease modelling in human organoids. Dis Model Mech 12(7):39347. https://doi.org/10.1016/j.molmed.2017.02.007
10. Es HA, Montazeri L, Aref AR et al (2018) Personalized cancer medicine: an organoid approach. Trends Biotechnol 36(4):358–371. https://doi.org/10.1016/j.tibtech.2017.12.005
11. Kim J, Koo B-K, Knoblich JA (2020) Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol 21(10):571–584. https://doi.org/10.1038/s41580-020-0259-3
12. Cañadas I, Barbie DA (2017) Organoid culture: applications in development and cancer. Ex vivo engineering of the tumor microenvironment. Springer, Berlin, pp 41–54. https://doi.org/10.1016/j.cell.2014.08.016
13. Gao D, Vela I, Sboner A et al (2014) Organoid cultures derived from patients with advanced prostate cancer. Cell 159(1):176–187. https://doi.org/10.1016/j.cell.2014.08.016
14. van de Wetering M, Francies HE, Francis JM et al (2015) Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161(4):933–945. https://doi.org/10.1016/j.cell.2015.03.053
15. Fujii M, Shimokawa M, Date S et al (2016) A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. Cell Stem Cell 18(6):827–838. https://doi.org/10.1016/j.stem.2016.04.003
16. Weeber F, van de Wetering M, Hoogstraat M et al (2015) Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. Proc Natl Acad Sci USA 112(43):13308–13311. https://doi.org/10.1073/pnas.1516689112
17. Engel RM, Chan WH, Nickless D et al (2020) Patient-derived colorectal cancer organoids upregulate revival stem cell marker genes following chemotherapeutic treatment. J Clin Med 9(1):128. https://doi.org/10.3390/jcm9010128
18. Ooft SN, Weeber F, Dijkstra KK et al (2019) Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci Transl Med 11(513):eaay2574. https://doi.org/10.1126/scitranslmed.aay2574
19. Li X, Francies HE, Secrier M et al (2018) Organoid cultures recapitulate esophageal adenocarcinoma heterogeneity providing a model for clonality studies and precision therapeutics. Nat Commun 9(1):1–13. https://doi.org/10.1038/s41467-018-05190-9
20. Seidlitz T, Merker SR, Rothe A et al (2019) Human gastric cancer modelling using organoids. Gut 68(2):207–217. https://doi.org/10.1136/gutjnl-2017-314549
21. Yan HH, Siu HC, Law S et al (2018) A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. Cell Stem Cell 23(6):882–897. https://doi.org/10.1016/j.stem.2018.09.016
22. Nanki K, Toshimitsu K, Takano A et al (2018) Divergent routes toward Wnt and R-spondin niche independency during human gastric carcinogenesis. Cell 174(4):856–869. https://doi.org/10.1016/j.cell.2018.07.027
23. Jacob F, Salinas RD, Zhang DY et al (2020) A patient-derived glioblastoma organoid model and biobank recapitulates inter-and intra-tumoral heterogeneity. Cell 180(1):188–204. https://doi.org/10.1016/j.cell.2019.11.036
24. Fusco P, Parisatto B, Rampazzo E et al (2019) Patient-derived organoids (PDOs) as a novel in vitro model for neuroblastoma tumours. BMC Cancer 19(1):1–11. https://doi.org/10.1186/s12885-019-6149-4
25. Boj SF, Hwang C-I, Baker LA et al (2015) Organoid models of human and mouse ductal pancreatic cancer. Cell 160(1–2):324–338. https://doi.org/10.1016/j.cell.2014.12.021
26. Driehuis E, van Hoeck A, Moore K et al (2019) Pancreatic cancer organoids recapitulate disease and allow personalized drug screening. Proc Natl Acad Sci USA 116(52):26580–26590. https://doi.org/10.1073/pnas.1911273116
27. Seino T, Kawasaki S, Shimokawa M et al (2018) Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. Cell Stem Cell 22(3):454–467. https://doi.org/10.1016/j.stem.2017.12.009
28. Broutier L, Mastrogiovanni G, Verstegen MM et al (2017) Human primary liver cancer–derived organoid cultures for disease modeling and drug screening. Nat Med 23(12):1424. https://doi.org/10.1038/nm.4438
29. Sachs N, de Ligt J, Kopper O et al (2018) A living biobank of breast cancer organoids captures disease heterogeneity. Cell 172(1–2):373–386. https://doi.org/10.1016/j.cell.2017.11.010
30. Lee SH, Hu W, Matulay JT et al (2018) Tumor evolution and drug response in patient-derived organoid models of bladder cancer. Cell 173(2):515–528. https://doi.org/10.1016/j.cell.2018.03.017
31. Boretto M, Maenhoudt N, Luo X et al (2019) Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. Nat Cell Biol 21(8):1041–1051. https://doi.org/10.1038/s41556-019-0360-z
32. Kim M, Mun H, Sung CO et al (2019) Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. Nat Commun 10(1):1–15. https://doi.org/10.1038/s41467-019-11867-6
33. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD et al (2019) Long-term expanding human airway organoids for disease modeling. EMBO J 38(4):e100300. https://doi.org/10.15252/embj.2018100300
34. Vlachogiannis G, Hedayat S, Vatsiou A et al (2018) Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science 359(6378):920–926. https://doi.org/10.1126/science.aao2774
35. Li M, Izpisua Belmonte JC (2019) Organoids—preclinical models of human disease. N Engl J Med 380(6):569–579. https://doi.org/10.1056/NEJMra1806175
36. Shpichka A, Bikmulina P, Peshkova M et al (2020) Engineering a model to study viral infections: bioprinting, microfluidics, and organoids to defeat Coronavirus Disease 2019 (COVID-19). Int J Bioprint 6(4):302. https://doi.org/10.18063/ijb.v6i4.302
37. Hossein-Khannazer N, Shokoohian B, Shpichka A et al (2021) An update to "novel therapeutic approaches for treatment of COVID-19." J Mol Med 99(2):303–310. https://doi.org/10.1007/s00109-020-02027-1

38. Monteil V, Kwon H, Prado P et al (2020) Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell 181(4):905–913. https://doi.org/10.1016/j.cell.2020.04.004
39. Zhao B, Ni C, Gao R et al (2020) Recapitulation of SARS-CoV-2 infection and cholangiocyte damage with human liver ductal organoids. Protein Cell 11:1–5. https://doi.org/10.1007/s13238-020-00718-6
40. Duan X, Han Y, Yang L et al (2020) Identification of drugs blocking SARS-CoV-2 infection using human pluripotent stem cell-derived colonic organoids. bioRxiv. https://doi.org/10.1101/2020.05.02.073320
41. Qi F, Qian S, Zhang S et al (2020) Communications br. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. Biochem Biophys Res Commun 526(1):135–140. https://doi.org/10.1016/j.bbrc.2020.03.044
42. Hossein-Khannazer N, Shokoohian B, Shpichka A et al (2020) Novel therapeutic approaches for treatment of COVID-19. J Mol Med (Berl) 98(6):789–803. https://doi.org/10.1007/s00109-020-01927-6
43. Zhao Y, Zhao Z, Wang Y et al (2020) Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. Am J Respir Crit Care Med 202(5):756–759. https://doi.org/10.1164/rccm.202001-0179LE
44. Lamers MM, Beumer J, van der Vaart J et al (2020) SARS-CoV-2 productively infects human gut enterocytes. Science 369:50–54. https://doi.org/10.1126/science.abc1669
45. Zhang SC, Wernig M, Duncan ID et al (2001) In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol 19(12):1129–1133. https://doi.org/10.1038/nbt1201-1129
46. Baharvand H, Mehrjardi NZ, Hatami M et al (2007) Neural differentiation from human embryonic stem cells in a defined adherent culture condition. Int J Dev Biol 51(5):371–378. https://doi.org/10.1387/ijdb.72280hb
47. Eiraku M, Watanabe K, Matsuo-Takasaki M et al (2008) Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 3(5):519–532. https://doi.org/10.1016/j.stem.2008.09.002
48. Mariani J, Simonini MV, Palejev D et al (2012) Modeling human cortical development in vitro using induced pluripotent stem cells. Proc Natl Acad Sci 109(31):12770–12775. https://doi.org/10.1073/pnas.1202944109
49. Edri R, Yaffe Y, Ziller MJ et al (2015) Analysing human neural stem cell ontogeny by consecutive isolation of Notch active neural progenitors. Nat Commun 6:6500. https://doi.org/10.1038/ncomms7500
50. Shi Y, Kirwan P, Smith J et al (2012) Human cerebral cortex development from pluripotent stem cells to functional excitatory synapses. Nat Neurosci 15(3):477. https://doi.org/10.1038/nn.3041
51. Hatami M, Mehrjardi NZ, Kiani S et al (2009) Human embryonic stem cell-derived neural precursor transplants in collagen scaffolds promote recovery in injured rat spinal cord. Cytotherapy 11(5):618–630. https://doi.org/10.1080/14653240903005802
52. Zare-Mehrjardi N, Khorasani MT, Hemmesi K et al (2011) Differentiation of embryonic stem cells into neural cells on 3D poly (D, L-lactic acid) scaffolds versus 2D cultures. Int J Artif Organs 34(10):1012–1023. https://doi.org/10.5301/ijao.5000002
53. Lancaster MA, Renner M, Martin C-A et al (2013) Cerebral organoids model human brain development and microcephaly. Nature 501(7467):373. https://doi.org/10.1038/nature12517
54. Gabriel E, Wason A, Ramani A et al (2016) CPAP promotes timely cilium disassembly to maintain neural progenitor pool. EMBO J 35(8):803–819. https://doi.org/10.15252/embj.201593679
55. Li R, Sun L, Fang A et al (2017) Recapitulating cortical development with organoid culture in vitro and modeling abnormal spindle-like (ASPM related primary) microcephaly disease. Protein Cell 8(11):823–833. https://doi.org/10.1007/s13238-017-0479-2
56. Li Y, Muffat J, Omer A et al (2017) Induction of expansion and folding in human cerebral organoids. Cell Stem Cell 20(3):385–396. https://doi.org/10.1016/j.stem.2016.11.017
57. Mariani J, Coppola G, Zhang P et al (2015) FOXG1-dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders. Cell 162(2):375–390. https://doi.org/10.1016/j.cell.2015.06.034
58. Wang P, Mokhtari R, Pedrosa E et al (2017) CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells. Mol Autism 8(1):11. https://doi.org/10.1186/s13229-017-0124-1
59. Ye F, Kang E, Yu C et al (2017) DISC1 regulates neurogenesis via modulating kinetochore attachment of Ndel1/Nde1 during mitosis. Neuron 96(5):1041–1054. https://doi.org/10.1016/j.neuron.2017.10.010
60. Mellios N, Feldman DA, Sheridan SD et al (2018) MeCP2-regulated miRNAs control early human neurogenesis through differential effects on ERK and AKT signaling. Mol Psychiatry 23(4):1051–1065. https://doi.org/10.1038/mp.2017.86
61. Allende ML, Cook EK, Larman BC et al (2018) Cerebral organoids derived from Sandhoff disease-induced pluripotent stem cells exhibit impaired neurodifferentiation. J Lipid Res 59(3):550–563. https://doi.org/10.1194/jlr.M081323
62. Bershteyn M, Nowakowski TJ, Pollen AA et al (2017) Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 20(4):435–449. https://doi.org/10.1016/j.stem.2016.12.007
63. Iefremova V, Manikakis G, Krefft O et al (2017) An organoid-based model of cortical development identifies non-cell-autonomous defects in Wnt signaling contributing to Miller-Dieker syndrome. Cell Rep 19(1):50–59. https://doi.org/10.1016/j.celrep.2017.03.047
64. Cugola FR, Fernandes IR, Russo FB et al (2016) The Brazilian Zika virus strain causes birth defects in experimental models. Nature 534(7606):267–271. https://doi.org/10.1038/nature18296
65. Garcez PP, Loiola EC, da Costa RM et al (2016) Zika virus impairs growth in human neurospheres and brain organoids. Science 352(6287):816–818. https://doi.org/10.1126/science.aaf6116
66. Qian X, Nguyen HN, Song MM et al (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell 165(5):1238–1254. https://doi.org/10.1016/j.cell.2016.04.032
67. Dang J, Tiwari SK, Lichinchi G et al (2016) Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. Cell Stem Cell 19(2):258–265. https://doi.org/10.1016/j.stem.2016.04.014
68. Janssens S, Schotsaert M, Karnik R et al (2018) Zika virus alters DNA methylation of neural genes in an organoid model of the developing human brain. MSystems 3(1):e00219. https://doi.org/10.1128/mSystems.00219-17
69. Zhou T, Tan L, Cederquist GY et al (2017) High-content screening in hPSC-neural progenitors identifies drug candidates that inhibit Zika virus infection in fetal-like organoids and adult brain. Cell Stem Cell 21(2):274–283. https://doi.org/10.1016/j.stem.2017.06.017
70. Watanabe M, Buth JE, Vishlaghi N et al (2017) Self-organized cerebral organoids with human-specific features predict effective

drugs to combat Zika virus infection. Cell Rep 21(2):517–532. https://doi.org/10.1016/j.celrep.2017.09.047
71. Sacramento CQ, De Melo GR, De Freitas CS et al (2017) The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. Sci Rep 7:40920. https://doi.org/10.1038/srep40920
72. Xu M, Lee EM, Wen Z et al (2016) Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat Med 22(10):1101–1107. https://doi.org/10.1038/nm.4184
73. Li C, Deng Y-Q, Wang S et al (2017) 25-Hydroxycholesterol protects host against Zika virus infection and its associated microcephaly in a mouse model. Immunity 46(3):446–456. https://doi.org/10.1016/j.immuni.2017.02.012
74. Retallack H, Di Lullo E, Arias C et al (2016) Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proc Natl Acad Sci 113(50):14408–14413. https://doi.org/10.1073/pnas.1618029113
75. Nowakowski TJ, Pollen AA, Di Lullo E et al (2016) Expression analysis highlights AXL as a candidate Zika virus entry receptor in neural stem cells. Cell Stem Cell 18(5):591–596. https://doi.org/10.1016/j.stem.2016.03.012
76. Wells MF, Salick MR, Wiskow O et al (2016) Genetic ablation of AXL does not protect human neural progenitor cells and cerebral organoids from Zika virus infection. Cell Stem Cell 19(6):703–708. https://doi.org/10.1016/j.stem.2016.11.011
77. Meertens L, Labeau A, Dejarnac O et al (2017) Axl mediates ZIKA virus entry in human glial cells and modulates innate immune responses. Cell Rep 18(2):324–333. https://doi.org/10.1016/j.celrep.2016.12.045
78. Di Lullo E, Kriegstein AR (2017) The use of brain organoids to investigate neural development and disease. Nat Rev Neurosci 18(10):573. https://doi.org/10.1038/nrn.2017.107
79. Raja WK, Mungenast AE, Lin Y-T et al (2016) Self-organizing 3D human neural tissue derived from induced pluripotent stem cells recapitulate Alzheimer's disease phenotypes. PLoS ONE 11(9):e0161969. https://doi.org/10.1371/journal.pone.0161969
80. Seo J, Kritskiy O, Watson LA et al (2017) Inhibition of p25/Cdk5 attenuates tauopathy in mouse and iPSC models of frontotemporal dementia. J Neurosci 37(41):9917–9924. https://doi.org/10.1523/JNEUROSCI.0621-17.2017
81. Gonzalez C, Armijo E, Bravo-Alegria J et al (2018) Modeling amyloid beta and tau pathology in human cerebral organoids. Mol Psychiatry 23(12):2363–2374. https://doi.org/10.1038/s41380-018-0229-8
82. Park J, Wetzel I, Marriott I et al (2018) A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat Neurosci 21(7):941–951. https://doi.org/10.1038/s41593-018-0175-4
83. Kim H, Park HJ, Choi H et al (2019) Modeling G2019S-LRRK2 sporadic Parkinson's disease in 3D midbrain organoids. Stem Cell Rep 12(3):518–531. https://doi.org/10.1016/j.stemcr.2019.01.020
84. Smits LM, Reinhardt L, Reinhardt P et al (2019) Modeling Parkinson's disease in midbrain-like organoids. NPJ Parkinson's Dis 5(1):1–8. https://doi.org/10.1038/s41531-019-0078-4
85. Chlebanowska P, Tejchman A, Sułkowski M et al (2020) Use of 3D organoids as a model to study idiopathic form of Parkinson's disease. Int J Mol Sci 21(3):694. https://doi.org/10.3390/ijms21030694
86. Kwak TH, Kang JH, Hali S et al (2020) Generation of homogeneous midbrain organoids with in vivo-like cellular composition facilitates neurotoxin-based Parkinson's disease modeling. Stem Cells 38(6):727–740. https://doi.org/10.1002/stem.3163
87. Lim R, Brichta AM (2016) Anatomical and physiological development of the human inner ear. Hear Res 338:9–21. https://doi.org/10.1016/j.heares.2016.02.004
88. Wu DK, Kelley MW (2012) Molecular mechanisms of inner ear development. Cold Spring Harbor Perspect Biol 4(8):a008409. https://doi.org/10.1101/cshperspect.a008409
89. Koehler KR, Mikosz AM, Molosh AI et al (2013) Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture. Nature 500(7461):217–221. https://doi.org/10.1038/nature12298
90. Tang PC, Alex AL, Nie J et al (2019) Defective Tmprss3-Associated hair cell degeneration in inner ear organoids. Stem Cell Rep 13(1):147–162. https://doi.org/10.1016/j.stemcr.2019.05.014
91. Fligor CM, Langer KB, Sridhar A et al (2018) Three-dimensional retinal organoids facilitate the investigation of retinal ganglion cell development, organization and neurite outgrowth from human pluripotent stem cells. Sci Rep 8(1):1–14. https://doi.org/10.1038/s41598-018-32871-8
92. Eiraku M, Takata N, Ishibashi H et al (2011) Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472(7341):51–56. https://doi.org/10.1038/nature09941
93. Nakano T, Ando S, Takata N et al (2012) Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell 10(6):771–785. https://doi.org/10.1016/j.stem.2012.05.009
94. Parfitt DA, Lane A, Ramsden CM et al (2016) Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. Cell Stem Cell 18(6):769–781. https://doi.org/10.1016/j.stem.2016.03.021
95. Deng W-L, Gao M-L, Lei X-L et al (2018) Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. Stem Cell Rep 10(4):1267–1281. https://doi.org/10.1016/j.stemcr.2018.02.003
96. Schwarz N, Lane A, Jovanovic K et al (2017) Arl3 and RP2 regulate the trafficking of ciliary tip kinesins. Hum Mol Genet 26(13):2480–2492. https://doi.org/10.1093/hmg/ddx143
97. Gao M-L, Lei X-L, Han F et al (2020) Patient-specific retinal organoids recapitulate disease features of late-onset retinitis pigmentosa. Front Cell Dev Biol 8:128. https://doi.org/10.3389/fcell.2020.00128
98. Kim J-W, Kim Y, Kim J et al (2018) AB0189 3d skin organoid mimicking systemic sclerosis generated by patient-derived induced pluripotent stem cells: 'disease in a dish' and development of animal model. BMJ Publishing Group Ltd. https://doi.org/10.1136/annrheumdis-2018-eular.4502
99. Elias MS, Wright SC, Nicholson WV et al (2019) Functional and proteomic analysis of a full thickness filaggrin-deficient skin organoid model. Wellcome Open Res 4:134. https://doi.org/10.12688/wellcomeopenres.15405.2
100. Wang G, McCain ML, Yang L et al (2014) Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat Med 20(6):616–623. https://doi.org/10.1038/nm.3545
101. Hinson JT, Chopra A, Nafissi N et al (2015) HEART DISEASE. Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science (New York, NY) 349(6251):982–986. https://doi.org/10.1126/science.aaa5458
102. Cashman TJ, Josowitz R, Johnson BV et al (2016) Human engineered cardiac tissues created using induced pluripotent stem cells reveal functional characteristics of BRAF-mediated hypertrophic cardiomyopathy. PLoS ONE 11(1):e0146697. https://doi.org/10.1371/journal.pone.0146697
103. Hinson JT, Chopra A, Lowe A et al (2016) Integrative analysis of PRKAG2 cardiomyopathy iPS and microtissue models identifies AMPK as a regulator of metabolism, survival, and fibrosis. Cell Rep 17(12):3292–3304. https://doi.org/10.1016/j.celrep.2016.11.066
104. Voges HK, Mills RJ, Elliott DA et al (2017) Development of a human cardiac organoid injury model reveals innate regenerative

potential. Development 144(6):1118–1127. https://doi.org/10.1242/dev.143966
105. Long C, Li H, Tiburcy M et al (2018) Correction of diverse muscular dystrophy mutations in human engineered heart muscle by single-site genome editing. Sci Adv 4(1):eaap9004. https://doi.org/10.1126/sciadv.aap9004
106. Freedman BS, Brooks CR, Lam AQ et al (2015) Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nat Commun 6:8715. https://doi.org/10.1038/ncomms9715
107. Kim YK, Refaeli I, Brooks CR et al (2017) Gene-edited human kidney organoids reveal mechanisms of disease in podocyte development. Stem cells (Dayton, Ohio) 35(12):2366–2378. https://doi.org/10.1002/stem.2707
108. Cruz NM, Song X, Czerniecki SM et al (2017) Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. Nat Mater 16(11):1112–1119. https://doi.org/10.1038/nmat4994
109. Hale LJ, Howden SE, Phipson B et al (2018) 3D organoid-derived human glomeruli for personalised podocyte disease modelling and drug screening. Nat Commun 9(1):1–17. https://doi.org/10.1038/s41467-018-07594-z
110. Low JH, Li P, Chew EGY et al (2019) Generation of human PSC-derived kidney organoids with patterned nephron segments and a de novo vascular network. Cell Stem Cell 25(3):373–387. https://doi.org/10.1016/j.stem.2019.06.009
111. Tanigawa S, Islam M, Sharmin S et al (2018) Organoids from nephrotic disease-derived iPSCs identify impaired NEPHRIN localization and slit diaphragm formation in kidney podocytes. Stem Cell Rep 11(3):727–740. https://doi.org/10.1016/j.stemcr.2018.08.003
112. Forbes TA, Howden SE, Lawlor K et al (2018) Patient-iPSC-derived kidney organoids show functional validation of a ciliopathic renal phenotype and reveal underlying pathogenetic mechanisms. Am J Hum Genet 102(5):816–831. https://doi.org/10.1016/j.ajhg.2018.03.014
113. Hinson JT, Chopra A, Nafissi N et al (2015) Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science 349(6251):982–986. https://doi.org/10.1126/science.aaa5458
114. Kim YK, Refaeli I, Brooks CR et al (2017) Gene-edited human kidney organoids reveal mechanisms of disease in podocyte development. Stem Cells 35(12):2366–2378. https://doi.org/10.1002/stem.2707
115. Monteil V, Kwon H, Prado P et al (2020) Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell 181:905–913. https://doi.org/10.1016/j.cell.2020.04.004
116. Miller AJ, Dye BR, Ferrer-Torres D et al (2019) Generation of lung organoids from human pluripotent stem cells in vitro. Nat Protoc 14(2):518–540. https://doi.org/10.1038/s41596-018-0104-8
117. McQualter JL, Yuen K, Williams B et al (2010) Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. Proc Natl Acad Sci USA 107(4):1414–1419. https://doi.org/10.1073/pnas.0909207107
118. Wilkinson DC, Alva-Ornelas JA, Sucre JM et al (2017) Development of a three-dimensional bioengineering technology to generate lung tissue for personalized disease modelling. Stem Cells Transl Med 6(2):622–633. https://doi.org/10.5966/sctm.2016-0192
119. Strikoudis A, Cieślak A, Loffredo L et al (2019) Modeling of fibrotic lung disease using 3D organoids derived from human pluripotent stem cells. Cell Rep 27(12):3709–3723. https://doi.org/10.1016/j.celrep.2019.05.077
120. Surolia R, Li FJ, Wang Z et al (2019) Vimentin intermediate filament assembly regulates fibroblast invasion in fibrogenic lung injury. JCI Insight 4(7):e123253. https://doi.org/10.1172/jci.insight.123253
121. Ramani S, Crawford SE, Blutt SE et al (2018) Human organoid cultures: transformative new tools for human virus studies. Curr Opin Virol 29:79–86. https://doi.org/10.1016/j.coviro.2018.04.001
122. Heo I, Dutta D, Schaefer DA et al (2018) Modelling Cryptosporidium infection in human small intestinal and lung organoids. Nat Microbiol 3(7):814–823. https://doi.org/10.1038/s41564-018-0177-8
123. Zhou J, Li C, Sachs N et al (2018) Differentiated human airway organoids to assess infectivity of emerging influenza virus. Proc Natl Acad Sci USA 115(26):6822–6827. https://doi.org/10.1073/pnas.1806308115
124. Hui KP, Ching RH, Chan SK et al (2018) Tropism, replication competence, and innate immune responses of influenza virus: an analysis of human airway organoids and ex-vivo bronchus cultures. Lancet Respir Med 6(11):846–854. https://doi.org/10.1016/S2213-2600(18)30236-4
125. Porotto M, Ferren M, Chen Y-W et al (2019) Authentic Modeling of human respiratory virus infection in human pluripotent stem cell-derived lung Organoids. MBio 10(3):e00723-e819. https://doi.org/10.1128/mBio.00723-19
126. Chen Y-W, Huang SX, De Carvalho ALRT et al (2017) A three-dimensional model of human lung development and disease from pluripotent stem cells. Nat Cell Biol 19(5):542–549. https://doi.org/10.1038/ncb3510
127. Agarwal T, Subramanian B, Maiti TK (2019) Liver tissue engineering: challenges and opportunities. ACS Biomater Sci Eng 5(9):4167–4182. https://doi.org/10.1021/acsbiomaterials.9b00745
128. Wu L-J, Chen Z-Y, Wang Y et al (2019) Organoids of liver diseases: From bench to bedside. World J Gastroenterol 25(16):1913. https://doi.org/10.3748/wjg.v25.i16.1913
129. Heydari Z, Vosough M (2017) New platforms for drug screening and toxicology: necessity or need? Mod Med Lab J 2(1):107–109. https://doi.org/10.30699/mmlj17.1.3.107
130. Vosough M, Omidinia E, Kadivar M et al (2013) Generation of functional hepatocyte-like cells from human pluripotent stem cells in a scalable suspension culture. Stem Cells Dev 22(20):2693–2705. https://doi.org/10.1089/scd.2013.0088
131. Sacchi M, Bansal R, Rouwkema J (2020) Bioengineered 3D models to recapitulate tissue fibrosis. Trends Biotechnol 38(6):623–636. https://doi.org/10.1016/j.tibtech.2019.12.010
132. Leite SB, Roosens T, El Taghdouini A et al (2016) Novel human hepatic organoid model enables testing of drug-induced liver fibrosis in vitro. Biomaterials 78:1–10. https://doi.org/10.1016/j.biomaterials.2015.11.026
133. Coll M, Perea L, Boon R et al (2018) Generation of hepatic stellate cells from human pluripotent stem cells enables in vitro modeling of liver fibrosis. Cell Stem Cell 23(1):101–113. https://doi.org/10.1016/j.stem.2018.05.027
134. Maepa SW, Ndlovu HJ (2020) Advances in generating liver cells from pluripotent stem cells as a tool for modelling liver diseases. Stem Cells 38:606–612. https://doi.org/10.1002/stem.3154
135. Kruitwagen HS, Oosterhoff LA, Vernooij IG et al (2017) Long-term adult feline liver organoid cultures for disease modeling of hepatic steatosis. Stem Cell Rep 8(4):822–830. https://doi.org/10.1016/j.stemcr.2017.02.015
136. Ouchi R, Togo S, Kimura M et al (2019) Modeling steatohepatitis in humans with pluripotent stem cell-derived organoids. Cell Metab 30(2):374–384. https://doi.org/10.1016/j.cmet.2019.05.007

137. Pingitore P, Sasidharan K, Ekstrand M et al (2019) Human multilineage 3D spheroids as a model of liver steatosis and fibrosis. Int J Mol Sci 20(7):1629. https://doi.org/10.3390/ijms20071629
138. Wang S, Wang X, Tan Z et al (2019) Human ESC-derived expandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury. Cell Res 29(12):1009–1026. https://doi.org/10.1038/s41422-019-0242-8
139. Jefferies M, Rauff B, Rashid H et al (2018) Update on global epidemiology of viral hepatitis and preventive strategies. World J Clin Cases 6(13):589–599. https://doi.org/10.12998/wjcc.v6.i13.589
140. Galle PR, Hagelstein J, Kommerell B et al (1994) In vitro experimental infection of primary human hepatocytes with hepatitis B virus. Gastroenterology 106(3):664–673. https://doi.org/10.1016/0016-5085(94)90700-5
141. Ruoß M, Vosough M, Königsrainer A et al (2020) Towards improved hepatocyte cultures: progress and limitations. Food Chem Toxicol 138:111188. https://doi.org/10.1016/j.fct.2020.111188
142. Fu G-B, Huang W-J, Zeng M et al (2019) Expansion and differentiation of human hepatocyte-derived liver progenitor-like cells and their use for the study of hepatotropic pathogens. Cell Res 29(1):8–22. https://doi.org/10.1038/s41422-018-0103-x
143. Nie Y-Z, Zheng Y-W, Miyakawa K et al (2018) Recapitulation of hepatitis B virus–host interactions in liver organoids from human induced pluripotent stem cells. EBioMedicine 35:114–123. https://doi.org/10.1016/j.ebiom.2018.08.014
144. Scorza M, Elce A, Zarrilli F et al (2014) Genetic diseases that predispose to early liver cirrhosis. Int J Hepatol 2014:713754. https://doi.org/10.1155/2014/713754
145. Huch M, Gehart H, Van Boxtel R et al (2015) Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 160(1–2):299–312. https://doi.org/10.1016/j.cell.2014.11.050
146. Guan Y, Xu D, Garfin PM et al (2017) Human hepatic organoids for the analysis of human genetic diseases. JCI Insight 2(17):e94954. https://doi.org/10.1172/jci.insight.94954
147. Ogawa M, Ogawa S, Bear CE et al (2015) Directed differentiation of cholangiocytes from human pluripotent stem cells. Nat Biotechnol 33(8):853. https://doi.org/10.1038/nbt.3294
148. Hohwieler M, Müller M, Frappart P-O et al (2019) Pancreatic progenitors and organoids as a prerequisite to model pancreatic diseases and cancer. Stem Cells Int 2019:9301382. https://doi.org/10.1155/2019/9301382
149. Chen L, Magliano DJ, Zimmet PZ (2012) The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol 8(4):228–236. https://doi.org/10.1038/nrendo.2011.183
150. Kim Y, Kim H, Ko UH et al (2016) Islet-like organoids derived from human pluripotent stem cells efficiently function in the glucose responsiveness in vitro and in vivo. Sci Rep 6:35145. https://doi.org/10.1038/srep35145
151. Cutting GR (2015) Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet 16(1):45–56. https://doi.org/10.1038/nrg3849
152. Hohwieler M, Illing A, Hermann PC et al (2017) Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling. Gut 66(3):473–486. https://doi.org/10.1136/gutjnl-2016-312423
153. Wen S, Moss SF (2009) Helicobacter pylori virulence factors in gastric carcinogenesis. Cancer Lett 282(1):1–8. https://doi.org/10.1016/j.canlet.2008.11.016
154. Barker N, Huch M, Kujala P et al (2010) Lgr5+ ve stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 6(1):25–36. https://doi.org/10.1016/j.stem.2009.11.013
155. McCracken KW, Catá EM, Crawford CM et al (2014) Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature 516(7531):400–404. https://doi.org/10.1038/nature13863
156. Bartfeld S, Bayram T, van de Wetering M et al (2015) In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology 148(1):126–136. https://doi.org/10.1053/j.gastro.2014.09.042
157. Bertaux-Skeirik N, Feng R, Schumacher MA et al (2015) CD44 plays a functional role in Helicobacter pylori-induced epithelial cell proliferation. PLoS Pathog 11(2):e1004663. https://doi.org/10.1371/journal.ppat.1004663
158. Wroblewski LE, Piazuelo MB, Chaturvedi R et al (2015) Helicobacter pylori targets cancer-associated apical-junctional constituents in gastroids and gastric epithelial cells. Gut 64(5):720–730. https://doi.org/10.1136/gutjnl-2014-307650
159. Holokai L, Chakrabarti J, Broda T et al (2019) Increased programmed death-ligand 1 is an early epithelial cell response to Helicobacter pylori infection. PLoS Pathog 15(1):e1007468. https://doi.org/10.1371/journal.ppat.1007468
160. Sebrell TA, Hashimi M, Sidar B et al (2019) A novel gastric spheroid co-culture model reveals chemokine-dependent recruitment of human dendritic cells to the gastric epithelium. Cell Mol Gastroenterol Hepatol 8(1):157–171. https://doi.org/10.1016/j.jcmgh.2019.02.010
161. Lancaster MA, Huch MJ (2019) Disease modelling in human organoids. Dis Model Mech 12(7):dmm039347. https://doi.org/10.1242/dmm.039347
162. Agarwal T, Onesto V, Lamboni L et al (2021) Engineering biomimetic intestinal topological features in 3D tissue models: retrospects and prospects. Bio-Design Manuf 4:1–28. https://doi.org/10.1007/s42242-020-00120-5
163. Van Der Flier LG, Clevers HJ (2009) Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol 71:241–260. https://doi.org/10.1146/annurev.physiol.010908.163145
164. Ootani A, Li X, Sangiorgi E et al (2009) Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat Med 15(6):701–706. https://doi.org/10.1038/nm.1951
165. Sato T, Vries RG, Snippert HJ et al (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459(7244):262–265. https://doi.org/10.1038/nature07935
166. Workman MJ, Mahe MM, Trisno S et al (2017) Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nat Med 23(1):49–59. https://doi.org/10.1038/nm.4233
167. Almeqdadi M, Mana MD, Roper J et al (2019) Gut organoids: mini-tissues in culture to study intestinal physiology and disease. Am J Physiol Cell Physiol 317(3):C405–C419. https://doi.org/10.1152/ajpcell.00300.2017
168. Foulke-Abel J, In J, Kovbasnjuk O et al (2014) Human enteroids as an ex-vivo model of host–pathogen interactions in the gastrointestinal tract. Exp Biol Med (Maywood) 239(9):1124–1134. https://doi.org/10.1177/1535370214529398
169. Forbester JL, Goulding D, Vallier L et al (2015) Interaction of Salmonella enterica serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells. Infect Immun 83(7):2926–2934. https://doi.org/10.1128/IAI.00161-15
170. Karve SS, Pradhan S, Ward DV et al (2017) Intestinal organoids model human responses to infection by commensal and Shiga toxin producing *Escherichia coli*. PLoS ONE 12(6):e0178966. https://doi.org/10.1371/journal.pone.0178966

171. Leslie JL, Huang S, Opp JS et al (2015) Persistence and toxin production by Clostridium difficile within human intestinal organoids result in disruption of epithelial paracellular barrier function. Infect Immun 83(1):138–145. https://doi.org/10.1128/IAI.02561-14
172. Engevik MA, Yacyshyn MB, Engevik KA et al (2015) Human Clostridium difficile infection: altered mucus production and composition. Am J Physiol Gastrointest Liver Physiol 308(6):G510–G524. https://doi.org/10.1152/ajpgi.00091.2014
173. Schwank G, Koo B-K, Sasselli V et al (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13(6):653–658. https://doi.org/10.1016/j.stem.2013.11.002
174. Rodansky ES, Johnson LA, Huang S et al (2015) Intestinal organoids: a model of intestinal fibrosis for evaluating anti-fibrotic drugs. Exp Mol Pathol 98(3):346–351. https://doi.org/10.1016/j.yexmp.2015.03.033
175. Heuckeroth RO (2018) Hirschsprung disease—integrating basic science and clinical medicine to improve outcomes. Nat Rev Gastroenterol Hepatol 15(3):152. https://doi.org/10.1038/nrgastro.2017.149
176. Takebe T, Sekine K, Enomura M et al (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 499(7459):481–484. https://doi.org/10.1038/nature12271
177. Koike H, Iwasawa K, Ouchi R et al (2019) Modelling human hepato-biliary-pancreatic organogenesis from the foregut–midgut boundary. Nature 574(7776):112–116. https://doi.org/10.1038/s41586-019-1598-0
178. Agarwal T, Celikkin N, Costantini M et al (2021) Recent advances in chemically defined and tunable hydrogel platforms for organoid culture. Manufacturing, pp 1–34. https://doi.org/10.1007/s42242-021-00126-7
179. Zhang S, Wan Z, Kamm RD (2021) Vascularized organoids on a chip: strategies for engineering organoids with functional vasculature. Lab Chip 21(3):473–488. https://doi.org/10.1039/d0lc01186j
180. Yu JJ (2020) Vascularized organoids: a more complete model. Int J Stem Cells 14:127–137. https://doi.org/10.15283/ijsc20143
181. Homan KA, Gupta N, Kroll KT et al (2019) Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat Methods 16(3):255–262. https://doi.org/10.1038/s41592-019-0325-y
182. Cakir B, Xiang Y, Tanaka Y et al (2019) Engineering of human brain organoids with a functional vascular-like system. Nat Methods 16(11):1169–1175. https://doi.org/10.1038/s41592-019-0586-5