Organoids: a novel modality in disease modeling

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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

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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

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**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.
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 patients with microcephaly, a genetic condition caused by a mutation in a gene...
encoding the \textit{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].
human macrocephaly, resulted in the proliferation of neurons. The CRISPR/Cas9 deletion of PTEN, a gene associated with PTEN mutated gene in ASD; CHD8+/− cerebral organoids were generated using CRISPR-Cas9 technology. Aspm gene, a further genetic cause of primary microcephaly generated from patient-specific iPSCs with the dysfunction of Aspm gene, 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.

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. 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.

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. 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. 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. Organoids did not form normal cortical lamination, proposing a new approach to study developmental diseases of the CNS. | Matrigel embedding              | [53] |
|              |                           |             |                                                                                                                                                                                                                         |                                 |      |
| Macrocephaly | ESC                       | 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. DLX6-AS1 critically regulated the development of GABAergic interneurons and are likely important in therapeutics approaches. | None                            | [57] |
| 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] |
| 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]  |
|             |                          | iPSC        | 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]  |
|             |                          | ESC         | 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]  |
| Tissue/organ Disease | 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] |
| 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] |

The role of p25/Cdk5 was validated in tauopathy. Additional efforts to develop inhibitors of p25-mediated Cdk5 dysregulation could benefit patients. 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.

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 suspension

None

Matrigel embedding

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

[84]
| 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) | 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 |
| 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, neuroblastsoma 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 neuro-inflammatory 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 m² 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]|
|              | 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]|
| DDM          | 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]|
|              |                    | PSCs        | The model highlighted the importance of podocalyxin in microvillus assembly, and provided a powerful tool for studying human kidney regeneration                                                                                                                                             | GeTrex                  | [114]|
|              |                    |             | 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]|

*PSCs* human pluripotent stem cells; *ESC* embryonic stem cells; *iPSC* induced pluripotent stem cells; *DDM* Duchenne muscular dystrophy; *Barth* Barth syndrome.
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) knockout hiPSC-derived podocytes 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

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**Table 2 (continued)**

| Tissue/organ     | Disease                                      | Cell source | Outcomes and achievements                                                                                     | Matrix          | Ref   |
|------------------|----------------------------------------------|-------------|---------------------------------------------------------------------------------------------------------------|-----------------|-------|
| Autosomal recessive polycystic kidney disease | 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 | 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] |
| Nephronphthisis-related ciliopathy (NPHP-RC) | Nephronphthisis-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 | 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 | Vitronecit | [115] |
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,

Endoderm-derived disease model organoids

Organoids of lung disease models

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.
| 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, CPTIA, 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] |
| 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 | iPSC-derived cholangiocytes and hepatocytes | Structural duct defects in the biliary tree were simulated | Matrigel embedding | [145] |
| Cystic fibrosis | PSC-derived cholangiocytes | The JAG1 mutation in Alagille syndrome was studied using this organoid model | Matrigel embedding | [146] |
| 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] |
| 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 | ESCs        | 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]   |
| Cryptosporidium   |                        | Duodenal biopsies | Cryptosporidium could replicate and complete its entire life cycle within the organoids.                                                                                                                                   | Matrigel embedding      | [122]   |
| Coronavirus disease 2019 | PSC-COs | 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]    |
| Cystic fibrosis   | Patient-derived primary intestinal stem cells | PSC-HIO | CRISPR/Cas9-mediated gene editing repaired the CFTR mutation and restored the functional phenotypes.                                                                                                                   | Matrigel embedding      | [173]   |
|                   |                        |             | Spironolactone reversed fibrosis caused by TGF-β induction.                                                                                                                                                                 |                        |         |
| Hirschsprung disease | PSCs | PSCs | The model studied the cellular and molecular pathogenesis of Hirschsprung disease with a mutation in the *PHOX2B* gene.                                                                                                                                                             | Matrigel embedding      | [166]   |
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].
On the whole, current technologies with multiple co-cultured cells and strategies for rebuilding culture micro-environments 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.

**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.
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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.

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**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.
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