Review

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Biomedical applications of organoids in genetic diseases

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Abstract: Organoid technology has significantly transformed biomedical research by providing exceptional prospects for modeling human tissues and disorders in a laboratory setting. It has significant potential for understanding the intricate relationship between genetic mutations, cellular phenotypes, and disease pathology, especially in the field of genetic diseases. The intersection of organoid technology and genetic research offers great promise for comprehending the pathophysiology of genetic diseases and creating innovative treatment approaches customized for specific patients. This review aimed to present a thorough analysis of the current advancements in organoid technology and its biomedical applications for genetic diseases. We examined techniques for modeling genetic disorders using organoid platforms, analyze the approaches for incorporating genetic disease organoids into clinical practice, and showcase current breakthroughs in preclinical application, individualized healthcare, and transplantation. Through the integration of knowledge from several disciplines, such as genetics, regenerative medicine, and biological engineering, our aim is to enhance our comprehension of the complex connection between genetic variations and organoid models in relation to human health and disease.

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Introduction

Organoid technology has significantly transformed biomedical research by providing exceptional opportunities for modeling human tissues and disorders in a laboratory setting [1]. Organoids, which are small three-dimensional (3D) organs created from stem cells, accurately reproduce the intricate structure and functions of natural tissues [2]. As a result, they serve as a valuable tool for researching the genesis of organs, understanding how diseases work, and testing potential treatments. Organoid technology, the application of organoid systems, has a wide range of biomedical applications in genetic diseases. Simultaneously, the understanding of genetic factors responsible for human diseases has significantly progressed driven by advancements in genomics, gene editing, and functional characterization approaches. The intersection of organoid technology and genetic research has great potential for comprehending the pathophysiology of genetic diseases and creating innovative treatment approaches customized for specific patients [3].

Genetic diseases, disorders resulting from mutations in one or more genes, cover a broad range of ailments that impact almost every organ system in the human body [3]. In the past, understanding the molecular causes of these diseases has been a difficult task because of the intricate nature of human biology and the limitations of traditional model systems. Although animal models are useful for certain elements of disease modeling, they frequently do not accurately replicate human physiology and pathology [4]. Cell lines can be easily expanded in large quantities. However, they do not possess the complex organization and specialized functions seen in tissues, which are essential for investigating disorders unique to certain organs. Furthermore, acquiring, preserving, and cultivating patient-derived primary cells *ex vivo* might be challenging, despite their physiological significance [5].

Organoid technology has effectively overcome several limitations, providing researchers with a potent tool to

accurately simulate human tissues and diseases. They have the remarkable ability to imitate the cellular variety, spatial organization, and functional properties of real organs [6]. Microenvironmental signals encompass the physical, chemical, and biological cues surrounding cells within a tissue. Organoids can undergo differentiation into several cell types and arrange themselves into tissue-like structures by replicating the microenvironmental signals that occur during organ formation [7]. This makes them ideal for modeling diseases, investigating pathology, screening drugs, and applications in regenerative medicine.

Organoids offer significant potential for understanding the intricate relationship between genetic mutations, cellular phenotypes, and disease pathology, especially in the field of genetic diseases. Researchers can adjust the genetic background of organoid models by employing genome editing technologies to introduce patient-specific genetic mutations [8]. This allows them to analyze the impact on cellular function and disease development in a precise manner. This method enables the creation of genetically identical control samples and the comparison of disease-related characteristics in a controlled experimental environment, making it easier to identify the primary molecular mechanisms underlying disease initiation [5].

Moreover, organoids produced from patient cells provide a distinct opportunity to replicate the diversity present in human populations, enabling researchers to explore the differences between individuals in terms of their susceptibility to diseases, the course of those diseases, and their response to therapy [9]. Researchers may systematically investigate the links between genotype and phenotype and develop individualized therapy strategies for individual patients by creating biobanks of patient-derived organoid lines that cover a wide range of genetic backgrounds and disease phenotypes [10].

This review aimed to present a thorough analysis of the current advancements in organoid technology and its biomedical applications for genetic diseases (Figure 1). We examined techniques for modeling genetic disorders using organoid platforms, analyze the approaches for incorporating genetic disease organoids into clinical practice, and showcase current breakthroughs in preclinical application, individualized healthcare, and transplantation. Through the integration of knowledge from several disciplines, such as genetics, regenerative medicine, and biological engineering, our aim is to enhance our understanding of the complex connection between genetic variations and organoid models in relation to human health and disease.

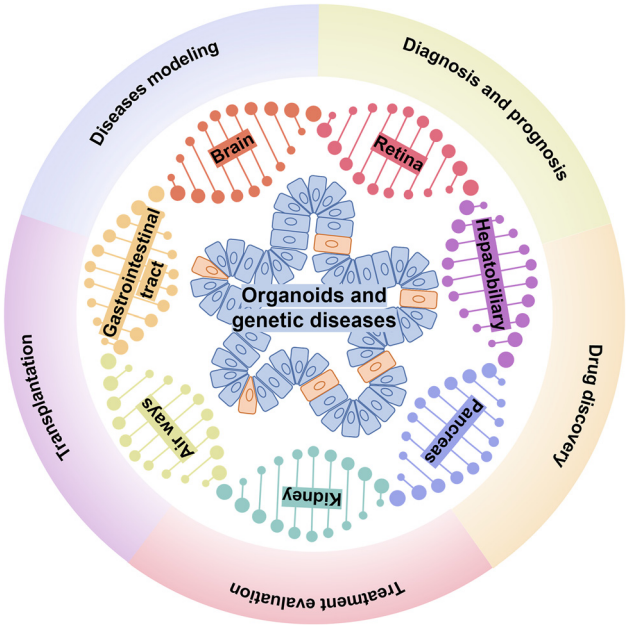

Figure 1: Biomedical applications of genetic disease organoids. Organoids have been employed to simulate hereditary disorders in organs like brain, retina, hepatobiliary, pancreas, kidney, air ways, and gastrointestinal tract. They have a wide range of applications in the field of genetic disease modeling, diagnosis and prognosis, drug discovery, treatment evaluation, transplantation.

Organoid models of genetic diseases

Organoids have the ability to replicate the growth of organs *in vitro*, allowing for the modeling and examination of hereditary diseases specific to those organs [11]. The emergence and rapid advancement of organoid technologies have yielded significant knowledge on the causes, development, and identification of possible therapeutic options for genetic disease.

Organoids generated from people with genetic disorders have been used as patient-specific models to simulate genetic diseases *in vitro*, enabling the study of the pathophysiology of these diseases and the exploration of novel therapy strategies. A benefit of employing patient-derived organoids for disease modeling is their capacity to effectively address the symptomatic diversity of human diseases through precision medicine [12]. Furthermore, the integration of organoid models with gene-editing technologies, such as CRISPR-Cas9, offers enhanced possibilities for investigating intricate genetic diseases that are challenging to replicate in

laboratory settings [11]. This approach also allows for the use of unedited cells as isogenic controls [5].

Organoids have been extensively employed by researchers to simulate several hereditary disorders. Take hereditary liver disease as an example, the A1AT protein accumulated in organoid cells derived from patients with α1-antitrypsin deficiency, which may reflect the clinical and pathophysiological characteristics of this disease [13]. Hepatobiliary organoids (HBOs) have been established [14] and used to study several genetic conditions related to the liver and bile ducts, including Alagille syndrome [15, 16], Wolman disease [17], Von Gierke disease [18], and CF-related bile duct disease [19]. Brain organoids are used as powerful tools to model and investigate the mechanisms of genetic disorders like primary microcephaly [20, 21], congenital central hypoventilation syndrome [22], rett syndrome [23, 24], AUTS2 syndrome [25], tuberous sclerosis complex [26, 27], DiGeorge syndrome [28, 29], Huntington's disease [30], Fragile X syndrome [31], amyotrophic lateral sclerosis [32], Crigler-Najjar Syndrome [33], congenital pituitary hypoplasia [34], spinal muscular atrophy [35], ribosomopathies-related brain diseases [36], POLG-related encephalopathy [37], and Down syndrome [38, 39]. Organoids of inherited retinal diseases, such as nonsyndromic CLN3 disease [40], retinitis pigmentosa (RP) [41–44], autosomal dominant optic atrophy (ADOA) [45, 46], stargardt disease [47], leber congenital amaurosis [47–50], Charcot-Marie-Tooth (CMT) disease type 1A [51], and enhanced S-cone syndrome [52, 53] have been generated. Genetic diseases of organs including gastrointestinal tract [54, 55], air ways [56–58], pancreas [59, 60], and kidney [61–68] have been modeled by organoids as well. To obtain an overview of inherited diseases simulated using organoids, please refer to Table 1. Despite variations in outcomes across different laboratories caused by factors such

Table 1: Organoid models of human genetic diseases.

Organs or tissues	Diseases	Cells/genetic background	References
Brain	Primary microcephaly	Gene-edited iPSCs or ESCs carrying the homozygous c.3704A>T mutation in the *CPAP/CENPJ* gene, or homozygous LOF mutations in *IER3IP1*	[20, 21]
	Congenital central hypoventilation syndrome	Gene-edited iPSCs carrying *PHOX2B-PARM* mutant	[22]
	Rett syndrome	Gene-edited iPSCs carrying *MeCP2*-truncated mutation; patient-derived iPSCs lacking *MECP2* expression	[23, 24]
	AUTS2 syndrome	Patient-derived iPSCs harboring the $AUTS2^{T534P}$ variant	[25]
	Tuberous sclerosis complex	Patient-derived iPSCs harboring heterozygous mutations in the *TSC1* or *TSC2* genes	[26, 27]
	DiGeorge syndrome (22q11.2 deletion syndrome)	Patient-derived iPSCs with a deletion at 22q11.2	[28, 29]
	Huntington's disease	Patient-derived iPSCs with a CAG expansion in the *huntingtin (HTT)* gene	[30]
	Fragile X syndrome	Patient-derived iPSCs with the loss of expression of *FMR1*	[31]
	Amyotrophic lateral sclerosis	Patient-derived iPSCs harboring the C9orf72 with ~800 hexanucleotide repeat expansion [HRE] repeats	[32]
	Crigler-Najjar syndrome	Patient-derived iPSCs harboring the *UGT1A1* mutation	[33]
	Congenital pituitary hypoplasia	Patient-derived iPSCs with a mutation in the *OTX2* gene	[34]
	Spinal muscular atrophy (SMA)	Patient-derived iPSCs with both *SMN1* alleles are deleted	[35]
	Ribosomopathies -related brain diseases	Gene-edited iPSCs carrying *SNORD118*-mutant	[36]
	POLG-related encephalopathy	Patient-derived iPSCs carrying *POLG* mutations: homozygous c.2243 G>C, p.W748S (WS5A), or compound heterozygous c.1399 G>A, p.A467T and c.2243 G>C, p.W748S (CP2A)	[37]
	Down syndrome	Patient-derived iPSCs with trisomy 21	[38, 39]
Retina	Nonsyndromic CLN3 disease	Patient-derived iPSCs carrying the 1 kb-deletion and c.175G>A variants in *CLN3*	[40]
	Retinitis pigmentosa	Patient-derived iPSCs carrying mutations in *RP2* (c.358C>T, p.R120X), *USH2A* (c.8559-2A>G/c.9127_9129delTCC), *RPGR*, or *PDE6B*; gene-edited iPSCs carrying a knockout in *RP2* (RP2 KO)	[41–44]
	Autosomal dominant optic atrophy	Patient-derived iPSC carrying a heterozygous *OPA1* c.2708_2711delTTAG:p.R905[a] variant, or *OPA1* intron 24 c.2496 + 1 G>T mutation	[45, 46]
	Stargardt disease	Patient-derived iPSCs with compound heterozygous for the frequent c.5882G>A (p.Gly1961Glu) missense variant and a c.4947delC (p.Glu1650Argfs[a]12) frameshift variant	[47]
	Leber congenital amaurosis	Patient-derived iPSCs carrying dominant c.G264T (p.K88 N) or c.413delT (p.I138fs48) mutation in *CRX*, a Cys89Arg mutation in *AIPL1*, or mutations in *CEP290*	[47–50]
	Charcot-Marie-Tooth (CMT) 1A	Patient-derived iPSCs with a duplication of the *PMP22* gene	[51]
	Enhanced S-cone syndrome	Patient-derived iPSCs with the *NR2E3* premature mutation (c.119-2-A>C) or mutations in *NRL*	[52, 53]

Table 1: (continued)

Organs or tissues	Diseases	Cells/genetic background	References
Hepatobiliary	α1-Antitrypsin deficiency	Patient-derived iPSCs carrying a *SERPINA1* Glu342Lys mutation	[13]
	Alagille syndrome	Patient-derived iPSCs carrying a heterozygous mutation or a splice site mutation in the *JAG1* gene	[15, 16]
	Wolman disease	Patient-derived iPSCs	[17]
	Glycogen storage disease type Ia (von Gierke's disease)	Patient-derived iPSCs with decreased *G6PC* mRNA expression and G6Pase enzyme activity	[18]
	CF-related bile duct disease	Patient-derived iPSCs carrying a compound heterozygous mutation in the *CFTR* gene	[19]
Gastrointestinal tract	CF-related intestinal diseases	Patient-derived iPSCs carrying mutations in *CFTR* gene	[54, 55]
Air ways	CF-related air way diseases	Patient-derived iPSCs with *CFTR* $^{F508del/F508del}$ mutation	[58]
	Primary ciliary dyskinesia	Patient-derived iPSCs carried mutations in *DNAI2*, *LRRC6*, *DNAH11*, *CCDC65* and *DNAH5* respectively	[56, 57]
Pancreas	Pancreatic dysplasia	Patient-derived iPSCs carrying *GNAS*$^{WT/R201C}$ mutation	[60]
	Congenital hyperinsulinism	Gene-edited hESCs carrying *ABCC8*-deficient mutant	[59]
Kidney	Autosomal dominant polycystic kidney disease (ADPKD)	Gene-edited iPSCs carrying *PKD-/-* mutant	[61, 62]
	Autosomal recessive polycystic kidney disease (ARPKD)	Gene-edited hESCs carrying *PKHD1* mutant	[63]
	Alport syndrome	Patient-derived iPSCs carrying *COL4A5* c.1634 G>A (p.G545D) missense mutation or *COL4A5* c.1652_53 dupTC (p.T552Sfs^{a}6) nonsense mutation	[64]
	Karyomegalic interstitial nephritis (KIN)	Gene-edited hESCs carrying *FAN1*-mutation	[65]
	Nephropathic cystinosis	Patient-derived iPSCs carrying mutations in the *CTNS* gene	[66]
	Autosomal recessive renal tubular dysgenesis (AR-RTD)	Patient-derived iPSCs; gene-edited iPSCs carrying *ACE-/-* and *AGTR1-/-* mutation	[67]
	Mucin 1 kidney disease	Patient-derived iPSCs carrying frameshift mutation in *MUC1* gene	[68]

iPSC, induced pluripotent stem cell; hESC, human embryonic stem cell; CFTR, cystic fibrosis, transmembrane conductance regulator.

as cell lines, procedures, protocols, and experiment operators, there is a notable coherence in the data indicating that organoids derived from patient or mutant cells can effectively replicate the characteristics of diseases [69]. This suggests that they have the potential to serve as a valuable model for investigating pathophysiologic mechanisms.

Combined with tools like multi-omics analysis, organoid models of genetic diseases can provide detailed insights into the metabolic and functional alterations that occur at the molecular, subcellular, and cellular levels throughout disease development. Van Lent et al. used an organoid model to study the disease characteristics of CMT disease type 1A (CMT1A) [51]. Their investigation revealed initial changes in the ultrastructure of myelin, including an increase in the distance between periodic lines and excessive myelin production around tiny axons. In addition, they noted the existence of onion-bulb-shaped structures during a subsequent stage of growth. These markers are not present in the CMT1A-corrected isogenic line or the CMT2A induced pluripotent stem cell (iPSC) line, which provides evidence that these changes are exclusive to CMT1A [51]. In order to study the development of ADOA, Lei et al. created isogenic iPSCs that carried a specific mutation (*OPA1* c.2708_2711delTTAG) [70]. They discovered that this mutant variant resulted in impaired initial and terminal differentiation, as well as abnormal electrophysiological characteristics in retinal ganglion cells derived from organoids. Furthermore, this particular variation hinders the growth of progenitor cells and leads to impaired mitochondrial functioning. Urresti et al. provide evidence that the 16p11.2 CNV mutation affects the balance between neurons and neural progenitors in organoids during the early stages of neurogenesis [71]. They notice an increase in the number of neurons and a decrease in neural progenitors in cases where the deletion occurs. The analysis of transcriptomic and proteomic profiling showed that the 16p11.2 CNV disrupts several biological processes, such as neuron migration, actin cytoskeleton, ion channel activity, synaptic-related activities, and Wnt signaling. These studies indicate that the combination of organoids and gene editing can be a potent technique for accurately identifying disease-related phenotypes and offering important resources for further exploration of disease pathogenesis.

Diagnosis and prognosis application of genetic disease organoids

Organoids of genetic disease can recapitulate the disease-related phenotypes, which makes them useful for disease diagnosis and prognosis. In this section, the cystic fibrosis (CF) organoids in clinical application will be used as an illustration. CF is a potentially fatal genetic disease that occurs when there is a malfunction in the transfer of chloride ions (Cl^-) and bicarbonate ions (HCO_3^-) mediated by the CF transmembrane conductance regulator (CFTR) protein [72]. Two-dimensional (2D) and 3D patient-derived cell models (PDCM) are regarded as the most dependable disease models currently accessible, as they accurately replicate the ion channel pathophysiology observed in living organisms [73].

Organoids in diagnosing cystic fibrosis

Diagnosing CF can be challenging, especially when the sweat chloride concentration (SCC) falls within an intermediate range and less than two CF-causing CFTR mutations are detected [74]. The physiological CFTR assays suggested in the guidelines, namely nasal potential difference and intestinal current measurement, are neither easily accessible nor practical for individuals of all age groups [75]. The study demonstrated that rectal organoid morphology analysis (ROMA) can differentiate between organoids derived from individuals with and without CF based on a clear phenotypic distinction: CF organoids have an irregular form and do not possess a visible lumen, in contrast to non-CF organoids. Within this procedure, two indices were computed, the circularity index and the intensity ratio, to quantify the roundness of organoids and the presence of a central lumen. ROMA is used to complement the diagnosis of CF when the information from SCC and genetics is not enough for accurate categorization. ROMA is a standardized and centralized test that can be used as the primary physiological assay in cases where the diagnosis is ambiguous, with the potential for future inclusion in the diagnostic work-up [76]. In addition, advanced deep-learning technologies like OrgaSegment can be used to analyze and investigate the process of CFTR-based fluid secretion [77]. OrgaSegment is a segmentation model based on MASK-RCNN that enables the precise segmentation of individual intestinal patient-derived organoids from bright-field pictures. This label-free segmentation approach may also assist in studying other epithelial ion transport processes in organoids.

Organoids in predicting cystic fibrosis

The predictive significance of a patient-derived organoid-based biomarker is particularly crucial in clinical settings for individuals with rare CFTR mutations that have uncertain clinical implications, given the extensive range of CFTR variants [78]. The swelling of patient-derived organoids caused by forskolin (FIS) was found to be closely linked to changes in lung function over time. Specifically, there was an estimated difference in the annual fall of FEV1pp of 0.32 % (95 % CI 0.11–0.54 %; p=0.004) for every 1000-point change in AUC. Sensitive methods for identifying and measuring the restoration of CFTR function in response to CFTR-directed therapies include FIS of intestinal organoids and *in vivo* CFTR function biomarkers [74]. Nevertheless, Graeber et al. did not detect any link when examining the low levels of functional improvement resulting from lumacaftor-ivacaftor treatment in F508del-homozygous patients [79]. More research involving large groups of patients with genetic disease will be necessary to ascertain the precise contribution of patient-derived organoids in predicting the clinical outcomes of individual patients.

Organoids for genetic diseases drug discovery and preclinical evaluation

Owing to their impressive ability to replicate disease-related characteristics and molecular processes, organoid models of genetic diseases are an exceptional resource for identifying targets, discovering drugs, and evaluating treatments.

Genetic screens are extensively employed to ascertain regulators in biological processes, and they can be conducted in 3D organoids to assess tissue-specific gene function. Esk et al. integrated CRISPR-Cas9 screening with barcoded cellular lineage tracing to facilitate loss-of-function screening in human cerebral organoid tissue [20]. The investigation of microcephaly candidate genes revealed that the endoplasmic reticulum plays a crucial role in regulating tissue integrity and brain size by controlling the secretion of extracellular matrix proteins. The genetic screen conducted on human brain tissue reveals the involvement of various pathways in microcephaly and offers a method for systematic examination of genes in organoids.

Organoids offer advantages over traditional 2D cell cultures by enabling the evaluation of drug effects across various cell types and the identification of drug-induced phenotype reversion in 3D tissue structures. Furthermore, this method allows for the direct evaluation of compounds that interact with human molecules, which eliminates the

potential differences that can occur when using rodent cells due to molecular interspecies variations [5]. In addition, *in vitro* organoid assays have been employed to confirm the efficacy of drugs that have been identified through alternative methods. For instance, Mucin 1 kidney disease kidney organoids confirmed the effectiveness of BRD4780, a small molecule that binds cargo receptor TMED9 and clears mutant MUC1 retention, after its identification through a drug screening of epithelial cells derived from patients [80]. Organoids also enable the preclinical evaluation of novel pharmaceuticals or combinations that are ideally tailored to individual patients. *Ex vivo* examination of organoids can be used to evaluate CFTR activity and provide guidance for personalized therapy in cases where CF-causing mutations are uncommon and not well understood. Mitropoulou et al. reported the initial evidence of a full recovery of lung function after using organoid-guided treatment with a CFTR modulator in a patient with the unusual 1677delTA/R334W genotype [81].

Besides, the *in vivo* organoid xenografts model also works as an effective tool for identifying new disease mechanisms, confirming potential drugs, and improving the effectiveness of drug delivery in clinical applications. Liu et al. have shown that minoxidil, a powerful inducer of autophagy and a drug approved by the FDA, successfully reduced the formation of cysts *in vivo* using the organoid xenograft model of polycystic kidney disease (PKD), which naturally develops tubular cysts [82]. Hernandez et al. present findings on the effectiveness of small amounts of nanoparticles locally delivered to angiomyolipoma (AML) organoid xenografts derived from TSC2-/- iPSCs [83]. Contrasted with the effects of higher oral doses of rapamycin (0.5 mg/kg) tested on organoid-bearing rats, AML organoid xenografts demonstrated the antitumor properties of locally administered low-dose rapamycin-loaded nanoparticles (ranging from 500 ng to 2 μg).

With patient-derived iPSCs, organoid models offer a platform for treatment testing, including gene editing techniques like CRISPR/Cas9. Deng et al. derived retinal pigment epithelial cells and retinal organoids from three RP patients with RPGR gene mutations [41]. Notable abnormalities were detected in the photoreceptors, and the cilia were found to be shorter than normal. Following the correction of mutations using CRISPR/Cas9 gene editing, there was a partial restoration of photoreceptor structures, electrophysiological properties, ciliopathy, and gene expression. Unlike traditional CRISPR/Cas9-based genome editing, adenine base editing (ABE) does not rely on the creation of double-strand breaks and has potential for therapeutic use. Geurts et al. used SpCas9-ABE and xCas9-ABE to target four specific CF organoid samples obtained from patients [84]. All four cases achieved successful genetic and functional restoration, and whole-genome sequencing (WGS) analysis of the repaired lines from two patients did not reveal any off-target alterations. These data demonstrate the importance of having extensive biobanks of patient-derived organoids that represent genetic diseases.

Organoid transplantation for genetic disease

Advancements in culture and gene editing technologies have the potential to enable the transplantation of laboratory-grown organoids, which would effectively address the problem of limited organ donors. Zhang et al. developed a new technique called “patch grafting” that allows for the transplantation of a significant quantity of stem/progenitor cell organoids or suspensions of adult cells into solid organs without causing emboli or ectopic cell distribution [85]. Patch grafting strategies have proven to be successful in resolving the longstanding challenge of transplanting epithelial cells, particularly epithelial stem/progenitor cells, especially with organoids, into solid organs. Patch grafting involves introducing the cells into the recipient’s organ, where they become fully integrated and can be regulated by a complete range of organ-specific systemic and paracrine signals. The efficacy of patch grafts consisting of organoids in rescuing hosts from genetic-based diseases was demonstrated through the use of biliary tree stem cells/early lineage stage mesenchymal cells (BTSC/ELSMC) organoids grafted onto livers. These grafts successfully rescued NRG/FAH-KO mice from type I tyrosinemia, a disease resulting from the deficiency of fumaryl acetoacetate hydrolase. Translational studies are now needed to explore the potential of patch grafting for cell therapies in solid organs and to facilitate its application in clinical programs.

Organoid transplantation in retinal diseases

Implanting retinal organoids derived from iPSCs into animal models with retinal diseases has shown encouraging outcomes. Additionally, multiple clinical trials have verified the safety of transplanting iPSCs derived retinal pigment epithelial cells [86]. The first clinical trial using iPSCs derived retina organoids for patients with advanced RP was approved in June 2020 [87]. Two patients received a transplant of three iPSC-retina sheets in their eyes. The graft successfully survived for more than 1 year in a highly progressive retinal degenerative environment without any significant negative

effects. The transplanted grafts remained viable for a duration of 2 years, exhibiting stable conditions. Additionally, there was an observed increase in retinal thickness at the site of transplantation, with no significant adverse events occurring in either of the subjects. The progression of visual function was less pronounced in the treated eye compared to the untreated eye during the follow-up period. The study demonstrated the practicality and safety of using retinal organoids derived from pluripotent cells as a regenerative therapy. It was also confirmed that the iPSC-retina sheets had a high survival rate in eyes with advanced retinal degeneration. Nevertheless, every sheet has dimensions of approximately 0.5×1.0 mm, and the graft covers a minuscule area. Although the grafted photoreceptor cells may establish a successful synaptic connection with the host retinal cells, the enhancement in visual function would be minimal. Therefore, the subsequent action would involve employing grafts that encompass a broader surface area, achieved by either increasing the quantity of sheets or managing a larger sheet [87, 88].

Organoid transplantation in liver diseases

The transplantation of cryopreserved primary human hepatocytes has been used in patients suffering from a range of diseases, including factor VII deficiency, acute liver failure, and Crigler-Najjar syndrome [89]. Expanding primary hepatocytes *in vitro* is a vital prerequisite for facilitating cell transplantation as a treatment for liver diseases. Peng et al. discovered that hepatocyte organoids can undergo multiple freeze-thaw cycles while maintaining a high level of viability (>90 % viability), making them suitable for transplantation [90]. Hu et al. conducted a comparison between the engraftment in mice of fetal-derived organoids and primary hepatocytes, as well as organoids obtained from a single pediatric source. The results showed that both primary hepatocytes and organoids outperformed fetal-derived organoids in terms of engraftment and proliferation, indicating that fully developed hepatocytes are more suitable for cell transplantation [91]. Li et al. surgically implanted HBOs into a monkey model using the subhepatic peritoneal and submesenteric transplantation techniques [92]. Upon transplantation, hepatic sinusoidal endothelial cells exhibit increased expression of CTSV, potentially impeding the advancement of hepatic sinusoidal capillarization and hepatic fibrosis. They demonstrated the clinical efficacy and safety of HBO transplantation for the management of severe liver disease [92].

Organoid transplantation in kidney diseases

An appealing potential therapeutic option for kidney genetic disease involves the regeneration of lost nephrons using human kidney organoids derived from iPSCs [80]. When grown in a laboratory setting, kidney organoids do not fully develop, have limited blood vessel formation, and may contain unintended cell types [80]. Transplanting kidney organoids under the kidney capsule enhances their anatomical development and blood vessel formation while decreasing the presence of unintended cells. Recent studies employing kidney organoid xenografts have demonstrated that the nephron-like structures present in the grafts exhibited a higher level of maturity compared to kidney organoids cultured *in vitro* [93]. However, these structures still displayed an immature state when compared to the surrounding mouse kidney tissue. The analysis of transcription profiles revealed that the transplantation of kidney organoids led to long-term maintenance, which in turn caused a significant reprogramming of gene expression. This reprogramming was characterized by the suppression of genes related to the cell cycle and the upregulation of genes involved in the organization of the extracellular matrix. Transplanting kidney organoids derived from iPSCs may be possible, but before they can be used as a therapeutic option in humans, it is necessary to develop strategies to enhance the differentiation and purity of nephrons.

Organoid transplantation in hair diseases

The challenge is also evident in the regenerative medicine field for hair follicle (HF) organoids. This involves extracting cells that are resistant to dihydrotestosterone (DHT) from a patient with androgenetic alopecia (AGA) [94]. Subsequently, a substantial quantity of organoids is generated in a laboratory setting and transplanted into the areas of the patient's scalp that are experiencing hair loss, with the aim of promoting the growth of new HFs. Nevertheless, there are numerous obstacles to implementing this method. The current models of HF organoids exhibit low efficiency in generating hair and do not reach maturity when cultured *in vitro* [95]. Research on the use of photobiomodulation (PBM) for HFs has demonstrated a beneficial impact on hair growth in patients with AGA, as well as in mouse models and laboratory experiments involving dermal papilla (DP) cells, keratinocytes, and HF stem cells (HFSCs) [96]. It has the potential to help address the current difficulties encountered in the large-scale production of HF organoids for clinical purposes.

Challenges and future directions

Organoids have significant promise and will play an increasingly vital role in the functional examination of gene mutations and the management of genetic disorders. While several techniques for manufacturing organoids have been documented, there are significant challenges that need to be surmounted to effectively use organoids in preclinical drug research and therapy trials [97].

Researchers have used patient- or mutant-cell-derived organoids to simulate genetic diseases. However, an important question is how organoids generated in a lab compare to organs developed naturally in terms of tissue, cellular, molecular, and functional characteristics. There are studies showing that cells from organoids have a similar transcriptome to cells found naturally in the body [69]. Researchers have also examined the activities of linked genes and tried potential treatments. Nevertheless, the intricate structure of certain tissues, such as the human brain, is exceptionally intricate and not replicated in organoids. In addition, it is important to note that gene mutations may not be the sole causative cause of certain disorders. To effectively replicate disease characteristics using organoids *in vitro*, it is crucial to take into account factors such as *in vivo* cell communications, the extracellular environment, and other relevant elements.

The complete recapitulation of organ development and maturation necessitates the synchronization of several cell types and tissues. The current technology for creating organoids has not yet been able to produce all the different types of cells in the right arrangement in a well-controlled way. This limitation affects the functioning and long-term stability of organoids. Current endeavors have been directed towards the co-cultivation of organoids with other cell types or the fusion of distinct types of organoids to form "assembloids" [98]. Vascularization is a crucial component. The lack of blood vessel formation in organoids impedes their complete development because of insufficient oxygen supply and tissue death in the central region of the organoid. A recent study has shown the simultaneous growth of vascular networks and the peripheral nervous system in neuro-mesodermal assembloids [99]. The researchers also evaluated the functional maturation of these assembloids and saw the successful formation of blood vessels in organoids. These models are anticipated to be used in the future to examine the influence of vascularization on the functionality of certain cell types. Another crucial aspect is the interaction with adjacent tissues. Consider retinal organoids and retinal pigment epithelium (RPE) cells as an illustration. The RPE has several crucial tasks in maintaining the balance of the retina, acting as a barrier for blood in the retina, and supplying nutrients from the blood to the photoreceptor cells. Research has shown the co-cultivation of RPE cells with retinal organoids, which resulted in the observation of a vigorous phagocytic uptake of outer photoreceptor segments [100]. The co-culturing approach enhances the functioning and long-term preservation of organoids, and is anticipated to be a helpful tool for future investigations into the interactions between organoids and their adjacent tissues.

Another factor to take into account is the quality assurance of organoids produced for genetic disease modeling [5]. Organoid differentiation techniques exhibit significant variation among research groups, leading to the production of organoids with distinct levels of quality and cellular composition. Organoids from the same batch can also exhibit significant variations, ranging from moderate to substantial, in several features such as size, proportions of distinct cell types, and developmental phases. Therefore, in cases where gene mutations lead to quantitative alterations in characteristics, it can be challenging to determine whether the observed phenotype is a consequence of the gene mutation itself or simply due to natural variances across different groups [69]. Additional endeavors are required to improve the application of organoid genetic disease models and establish consensus on standardized processes for protocols, quality controls, and data management [99]. Standardized organoids, characterized by consistent forms and sizes, would enhance consistency in phenotypic assessments and facilitate high-throughput genetic and chemical screenings.

In addition to these challenges, the cultivation of organoids is both costly and time-intensive. Organoids of various tissue types necessitate a significant amount of time to develop since they undergo differentiation and maturation at a similar pace to their *in vivo* counterparts, which often spans several weeks or even months. It is important to use strict dependence on growth factors and signaling gradients to guarantee the determination of cell lineages and maintain a balanced renewal of stem cells, and the high cost needs to be taken into consideration [101].

On the other hand, the potential applications of organoid genetic disease models make the temporal challenges tolerable, and bioengineering approaches have the potential to solve these problems. Bioengineered devices like bioreactors and microfluidics can enhance the consistency and effectiveness of organoid differentiation [102]. Bioreactors are well-suited for large-scale tissue cultivation as they create a biologically active environment with adjustable ambient parameters. The tiny spinning bioreactor unit has the ability to decrease medium consumption and minimize space requirements [103]. This enables efficient comparison of various cultural conditions for the purpose of

optimizing protocols. Microfluidic devices enable precise manipulation at a comparatively lower cost and enables the study of complex structure, functional, and physiological changes in cultured cells [104]. Additional techniques like topographically organized scaffolds, programmable 3D matrices, and bioprinting could also be considered [105]. The ease of use and consistency of organoids will be enhanced upon the combination of organoid cultivation, organ-on-a-chip (OOC) technologies, and synergistic engineering.

Despite advancements in organoid technology, which result in the creation of more complex tissue that resembles *in vivo* conditions, it is crucial to acknowledge that organoids are still an experimental system conducted *in vitro* and have inherent limits. Organoids serve as an *in vitro* model for biomedical research, but they do not diminish the significance of animal models and original tissues [106]. The newly discovered insights into human genetic disease biology obtained via the use of organoid systems still need to be confirmed through *in vivo* experimentation. The development and application of organoids also raise complex ethical issues, including consent, privacy, ownership and equitable access. The generation of organoids from patient samples requires explicit consent and privacy protection, as these structures may retain genetic and phenotypic information about the donor. Organoids derived from sensitive tissues such as the brain may challenge our definitions of consciousness and moral status. The ethical landscape surrounding organoids is still evolving and requires robust scrutiny to guide responsible research and clinical translation.

In conclusion, the integration of various organoid technologies, along with preclinical and clinical research conducted on organoid models, will uncover novel disease mechanisms and make valuable contributions to the development of genetic disease diagnostic procedures and medicines.

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References

1. Corsini NS, Knoblich JA. Human organoids: new strategies and methods for analyzing human development and disease. Cell 2022; 185:2756–69.
2. Silva-Pedrosa R, Salgado AJ, Ferreira PE. Revolutionizing disease modeling: the emergence of organoids in cellular systems. Cell 2023; 12:930.
3. Perez-Lanzon M, Kroemer G, Maiuri MC. Organoids for modeling genetic diseases. Int Rev Cell Mol Biol 2018;337:49–81.
4. Sántha M. Biologia futura: animal testing in drug development-the past, the present and the future. Biologia Futura 2020;71:443–52.
5. Miyoshi T, Hiratsuka K, Saiz EG, Morizane R. Kidney organoids in translational medicine: disease modeling and regenerative medicine. Dev Dyn 2020;249:34–45.
6. Piraino F, Costa M, Meyer M, Cornish G, Ceroni C, Garnier VMVM, et al. Organoid models: the future companions of personalized drug development. Biofabrication 2024;16:032009.
7. Corrò C, Novellasdemunt L, Li VSW. A brief history of organoids. Am J Physiol Cell Physiol 2020;319:C151–65.
8. Chen B, Du C, Wang M, Guo J, Liu X. Organoids as preclinical models of human disease: progress and applications. Med Rev (2021) 2024;4: 129–53.
9. Zheng F, Xiao Y, Liu H, Fan Y, Dao M. Patient-specific organoid and organ-on-a-chip: 3D cell-culture meets 3D printing and numerical simulation. Adv Biol 2021;5:e2000024.
10. Bollinger J, May E, Mathews D, Donowitz M, Sugarman J. Patients' perspectives on the derivation and use of organoids. Stem Cell Rep 2021;16:1874–83.
11. Yang S, Hu H, Kung H, Zou R, Dai Y, Hu Y, et al. Organoids: the current status and biomedical applications. MedComm 2023;4: e274.
12. Bose S, Clevers H, Shen X. Promises and challenges of organoid-guided precision medicine. Med (New York, NY) 2021;2:1011–26.
13. Yao Q, Chen W, Yu Y, Gao F, Zhou J, Wu J, et al. Human placental mesenchymal stem cells relieve primary sclerosing cholangitis via upregulation of TGR5 in Mdr2(-/-) mice and human intrahepatic cholangiocyte organoid models. Research 2023;6:0207.
14. Moon HR, Mun SJ, Kim TH, Kim H, Kang D, Kim S, et al. Guidelines for manufacturing and application of organoids: liver. Int J Stem Cells 2024;17:120–9.
15. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MMA, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299–312.
16. Guan Y, Xu D, Garfin PM, Ehmer U, Hurwitz M, Enns G, et al. Human hepatic organoids for the analysis of human genetic diseases. JCI Insight 2017;2:e94954.
17. Ouchi R, Togo S, Kimura M, Shinozawa T, Koido M, Koike H, et al. Modeling steatohepatitis in humans with pluripotent stem cell-derived organoids. Cell Metab 2019;30:374–84.e6.
18. Mun SJ, Hong YH, Shin Y, Lee J, Cho HS, Kim DS, et al. Efficient and reproducible generation of human induced pluripotent stem cell-derived expandable liver organoids for disease modeling. Sci Rep 2023;13:22935.

19. Verstegen MMA, Roos FJM, Burka K, Gehart H, Jager M, de Wolf M, et al. Human extrahepatic and intrahepatic cholangiocyte organoids show region-specific differentiation potential and model cystic fibrosis-related bile duct disease. Sci Rep 2020;10:21900.
20. Esk C, Lindenhofer D, Haendeler S, Wester RA, Pflug F, Schroeder B, et al. A human tissue screen identifies a regulator of ER secretion as a brain-size determinant. Science (New York, NY) 2020;370: 935–41.
21. An HL, Kuo HC, Tang TK. Modeling human primary microcephaly with hiPSC-derived brain organoids carrying CPAP-e1235V disease-associated mutant protein. Front Cell Dev Biol 2022;10:830432.
22. Lui KNC, Li Z, Lai FPL, Lau ST, Ngan ESW. Organoid models of breathing disorders reveal patterning defect of hindbrain neurons caused by PHOX2B-PARMs. Stem Cell Rep 2023;18:1500–15.
23. Hong H, Yoon S-B, Park JE, Lee JI, Kim HY, Nam HJ, et al. MeCP2 dysfunction prevents proper BMP signaling and neural progenitor expansion in brain organoid. Ann Clin Transl Neurol 2023;10: 1170–85.
24. Samarasinghe RA, Miranda OA, Buth JE, Mitchell S, Ferando I, Watanabe M, et al. Identification of neural oscillations and epileptiform changes in human brain organoids. Nat Neurosc 2021; 24:1488–500.
25. Fair SR, Schwind W, Julian DL, Biel A, Guo G, Rutherford R, et al. Cerebral organoids containing an AUTS2 missense variant model microcephaly. Brain 2023;146:387–404.
26. Dooves S, van Velthoven AJH, Suciati LG, Heine VM. Neuron-glia interactions in tuberous sclerosis complex affect the synaptic balance in 2D and organoid cultures. Cells 2021;10:134.
27. Eichmüller OL, Corsini NS, Vértesy Á, Morassut I, Scholl T, Gruber VE, et al. Amplification of human interneuron progenitors promotes brain tumors and neurological defects. Science (New York, NY) 2022;375: eabf5546.
28. Khan TA, Revah O, Gordon A, Yoon S-J, Krawisz AK, Goold C, et al. Neuronal defects in a human cellular model of 22q11.2 deletion syndrome. Nat Med 2020;26:1888–98.
29. Shin D, Kim CN, Ross J, Hennick KM, Wu SR, Paranjape N, et al. Thalamocortical organoids enable in vitro modeling of 22q11.2 microdeletion associated with neuropsychiatric disorders. Cell Stem Cell 2024;31:421–32.e8.
30. Liu Y, Chen X, Ma Y, Song C, Ma J, Chen C, et al. Endogenous mutant Huntingtin alters the corticogenesis via lowering Golgi recruiting ARF1 in cortical organoid. Mol Psychiatr 2024;29:3024–39.
31. Raj N, McEachin ZT, Harousseau W, Zhou Y, Zhang F, Merritt-Garza ME, et al. Cell-type-specific profiling of human cellular models of fragile X syndrome reveal PI3K-dependent defects in translation and neurogenesis. Cell Rep 2021;35:108991.
32. Gao C, Shi Q, Pan X, Chen J, Zhang Y, Lang J, et al. Neuromuscular organoids model spinal neuromuscular pathologies in C9orf72 amyotrophic lateral sclerosis. Cell Rep 2024;43:113892.
33. Pranty AI, Wruck W, Adjaye J. Free bilirubin induces neuro-inflammation in an induced pluripotent stem cell-derived cortical organoid model of crigler-Najjar syndrome. Cells 2023;12:2277.
34. Matsumoto R, Suga H, Aoi T, Bando H, Fukuoka H, Iguchi G, et al. Congenital pituitary hypoplasia model demonstrates hypothalamic OTX2 regulation of pituitary progenitor cells. J Clin Invest 2020;130: 641–54.
35. Afanasyeva TAV, Athanasiou D, Perdigao PRL, Whiting KR, Duijkers L, Astuti GDN, et al. CRISPR-Cas9 correction of a nonsense mutation in LCA5 rescues lebercilin expression and localization in human retinal organoids. Mol Ther Methods Clin Dev 2023;29:522–31.
36. Zhang W, Zhang M, Ma L, Jariyasakulroj S, Chang Q, Lin Z, et al. Recapitulating and reversing human brain ribosomopathy defects via the maladaptive integrated stress response. Sci Adv 2024;10: eadk1034.
37. Chen A, Yangzom T, Hong Y, Lundberg BC, Sullivan GJ, Tzoulis C, et al. Hallmark molecular and pathological features of POLG disease are recapitulated in cerebral organoids. Adv Sci 2024;11:e2307136.
38. Tang XY, Xu L, Wang J, Hong Y, Wang Y, Zhu Q, et al. DSCAM/PAK1 pathway suppression reverses neurogenesis deficits in iPSC-derived cerebral organoids from patients with Down syndrome. J Clin Invest 2021;131:e135763.
39. Jin M, Xu R, Wang L, Alam MM, Ma Z, Zhu S, et al. Type-I-interferon signaling drives microglial dysfunction and senescence in human iPSC models of Down syndrome and Alzheimer's disease. Cell Stem Cell 2022;29:1135–53.e8.
40. Zhang X, Zhang D, Thompson JA, Chen SC, Huang Z, Jennings L, et al. Gene correction of the CLN3 c.175G>A variant in patient-derived induced pluripotent stem cells prevents pathological changes in retinal organoids. Mol Genet Genomic Med 2021;9:e1601.
41. Deng WL, Gao ML, Lei XL, Lv JN, Zhao H, He KW, et al. Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. Stem Cell Rep 2018;10: 1267–81.
42. Guo Y, Wang P, Ma JH, Cui Z, Yu Q, Liu S, et al. Modeling retinitis pigmentosa: retinal organoids generated from the iPSCs of a patient with the USH2A mutation show early developmental abnormalities. Front Cell Neurosci 2019;13:361.
43. Gao ML, Lei XL, Han F, He KW, Jin SQ, Zhang YY, et al. Patient-specific retinal organoids recapitulate disease features of late-onset retinitis pigmentosa. Front Cell Dev Biol 2020;8:128.
44. Lane A, Jovanovic K, Shortall C, Ottaviani D, Panes AB, Schwarz N, et al. Modeling and rescue of RP2 retinitis pigmentosa using iPSC-derived retinal organoids. Stem Cell Rep 2020;15:67–79.
45. Sladen PE, Jovanovic K, Guarascio R, Ottaviani D, Salsbury G, Novoselova T, et al. Modelling autosomal dominant optic atrophy associated with OPA1 variants in iPSC-derived retinal ganglion cells. Hum Mol Genet 2022;31:3478–93.
46. Chen J, Riazifar H, Guan MX, Huang T. Modeling autosomal dominant optic atrophy using induced pluripotent stem cells and identifying potential therapeutic targets. Stem Cell Res Ther 2016;7:2.
47. Su P-Y, Lee W, Zernant J, Tsang SH, Nagasaki T, Corneo B, et al. Establishment of the iPSC line CUIMCi005-A from a patient with Stargardt disease for retinal organoid culture. Stem Cell Res 2022;65: 102973.
48. Lukovic D, Artero Castro A, Kaya KD, Munezero D, Gieser L, Davó-Martínez C, et al. Retinal organoids derived from hiPSCs of an AIPL1-LCA patient maintain cytoarchitecture despite reduced levels of mutant AIPL1. Sci Rep 2020;10:5426.
49. Parfitt DA, Lane A, Ramsden CM, Carr AF, Munro PM, Jovanovic K, et al. Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. Cell Stem Cell 2016;18: 769–81.
50. Shimada H, Lu Q, Insinna-Kettenhofen C, Nagashima K, English MA, Semler EM, et al. In vitro modeling using ciliopathy-patient-derived cells reveals distinct cilia dysfunctions caused by CEP290 mutations. Cell Rep 2017;20:384–96.
51. Van Lent J, Vendredy L, Adriaenssens E, Da Silva Authier T, Asselbergh B, Kaji M, et al. Downregulation of PMP22 ameliorates myelin defects in iPSC-derived human organoid cultures of CMT1A. Brain 2023;146:2885–96.

52. Bohrer LR, Wiley LA, Burnight ER, Cooke JA, Giacalone JC, Anfinson KR, et al. Correction of NR2E3 associated enhanced S-cone syndrome patient-specific iPSCs using CRISPR-cas9. Genes 2019;10:278.
53. Kallman A, Capowski EE, Wang J, Kaushik AM, Jansen AD, Edwards KL, et al. Investigating cone photoreceptor development using patient-derived NRL null retinal organoids. Commun Biol 2020;3:82.
54. Ramalho AS, Fürstová E, Vonk AM, Ferrante M, Verfaillie C, Dupont L, et al. Correction of CFTR function in intestinal organoids to guide treatment of cystic fibrosis. Eur Respir J 2021;57:1902426.
55. Spelier S, de Poel E, Ithakisiou GN, Suen SWF, Hagemeijer MC, Muilwijk D, et al. High-throughput functional assay in cystic fibrosis patient-derived organoids allows drug repurposing. ERJ Open Res 2023;9:00495–2022.
56. van der Vaart J, Böttinger L, Geurts MH, van de Wetering WJ, Knoops K, Sachs N, et al. Modelling of primary ciliary dyskinesia using patient-derived airway organoids. EMBO Rep 2021;22:e52058.
57. Yang W, Chen L, Guo J, Shi F, Yang Q, Xie L, et al. Multiomics analysis of a DNAH5-mutated PCD organoid model revealed the Key role of the TGF-β/BMP and notch pathways in epithelial differentiation and the immune response in DNAH5-mutated patients. Cells 2022;11:4013.
58. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttinger L, Klay D, et al. Long-term expanding human airway organoids for disease modeling. EMBO J 2019;38:e100300.
59. Guo D, Liu H, Ruzi A, Gao G, Nasir A, Liu Y, et al. Modeling congenital hyperinsulinism with ABCC8-deficient human embryonic stem cells generated by CRISPR/Cas9. Sci Rep 2017;7:3156.
60. Breunig M, Merkle J, Wagner M, Melzer MK, Barth TFE, Engleitner T, et al. Modeling plasticity and dysplasia of pancreatic ductal organoids derived from human pluripotent stem cells. Cell Stem Cell 2021;28: 1105–24.e19.
61. Xu Y, Kuppe C, Perales-Patón J, Hayat S, Kranz J, Abdallah AT, et al. Adult human kidney organoids originate from CD24(+) cells and represent an advanced model for adult polycystic kidney disease. Nat Genet 2022;54:1690–701.
62. Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, Agrawal V, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nat Commun 2015;6:8715.
63. Hiratsuka K, Miyoshi T, Kroll KT, Gupta NR, Valerius MT, Ferrante T, et al. Organoid-on-a-chip model of human ARPKD reveals mechanosensing pathomechanisms for drug discovery. Sci Adv 2022;8:eabq0866.
64. Hirayama R, Toyohara K, Watanabe K, Otsuki T, Araoka T, Mae SI, et al. iPSC-derived type IV collagen α5-expressing kidney organoids model Alport syndrome. Commun Bio 2023;6:854.
65. Lim SW, Na D, Lee H, Fang X, Cui S, Shin YJ, et al. Modeling of FAN1-deficient kidney disease using a human induced pluripotent stem cell-derived kidney organoid system. Cell 2023;12:2319.
66. Hollywood JA, Przepiorski A, D'Souza RF, Sreebhavan S, Wolvetang EJ, Harrison PT, et al. Use of human induced pluripotent stem cells and kidney organoids to develop a cysteamine/mTOR inhibition combination therapy for cystinosis. J Am Soc Nephrol 2020;31:962–82.
67. Pode-Shakked N, Slack M, Sundaram N, Schreiber R, McCracken KW, Dekel B, et al. RAAS-deficient organoids indicate delayed angiogenesis as a possible cause for autosomal recessive renal tubular dysgenesis. Nat Commun 2023;14:8159.
68. Dvela-Levitt M, Kost-Alimova M, Emani M, Kohnert E, Thompson R, Sidhom EH, et al. Small molecule targets TMED9 and promotes lysosomal degradation to reverse proteinopathy. Cell 2019;178: 521–35.e23.
69. Zhang Z, Xu Z, Yuan F, Jin K, Xiang M. Retinal organoid technology: where are we now? Int J Mol Sci 2021;22:10244.
70. Lei Q, Xiang K, Cheng L, Xiang M. Human retinal organoids with an OPA1 mutation are defective in retinal ganglion cell differentiation and function. Stem Cell Rep 2024;19:68–83.
71. Urresti J, Zhang P, Moran-Losada P, Yu NK, Negraes PD, Trujillo CA, et al. Cortical organoids model early brain development disrupted by 16p11.2 copy number variants in autism. Mol Psychiatry 2021;26:7560–80.
72. Conti J, Sorio C, Melotti P. Organoid technology and its role for theratyping applications in cystic fibrosis. Children 2022;10:4.
73. Ikpa PT, Bijvelds MJC, de Jonge HR. Cystic fibrosis: toward personalized therapies. Int J Biochem Cell Biol 2014;52:192–200.
74. Graeber SY, van Mourik P, Vonk AM, Kruisselbrink E, Hirtz S, van der Ent CK, et al. Comparison of organoid swelling and in vivo biomarkers of CFTR function to determine effects of Lumacaftor-Ivacaftor in patients with cystic fibrosis homozygous for the F508del mutation. Am J Respir Crit Care Med 2020;202:1589–92.
75. Cuyx S, Ramalho AS, Corthout N, Fieuws S, Fürstová E, Arnauts K, et al. Rectal organoid morphology analysis (ROMA) as a promising diagnostic tool in cystic fibrosis. Thorax 2021;76:1146–9.
76. Cuyx S, Ramalho AS, Fieuws S, Corthout N, Proesmans M, Boon M, et al. Rectal organoid morphology analysis (ROMA) as a novel physiological assay for diagnostic classification in cystic fibrosis. Thorax 2024;79:834–41.
77. Lefferts JW, Kroes S, Smith MB, Niemöller PJ, Nieuwenhuijze NDA, Sonneveld van Kooten HN, et al. OrgaSegment: deep-learning based organoid segmentation to quantify CFTR dependent fluid secretion. Commun Biol 2024;7:319.
78. Muilwijk D, de Poel E, van Mourik P, Suen SWF, Vonk AM, Brunsveld JE, et al. Forskolin-induced organoid swelling is associated with long-term cystic fibrosis disease progression. Eur Respir J 2022;60:2100508.
79. Kerem E, Cohen-Cymberknoh M, Tsabari R, Wilschanski M, Reiter J, Shoseyov D, et al. Ivacaftor in people with cystic fibrosis and a 3849+10kb C→T or D1152H residual function mutation. Ann Am Thorac Soc 2021;18:433–41.
80. Kuo T-C, Cabrera-Barragan DN, Lopez-Marfil M, Lopez-Cantu DO, Lemos DR. Can kidney organoid xenografts accelerate therapeutic development for genetic kidney disorders? J Am Soc Nephrol 2023;34: 184–90.
81. Mitropoulou G, Brandenberg N, Hoehnel S, Ceroni C, Balmpouzis Z, Blanchon S, et al. Rectal organoid-guided CFTR modulator therapy restores lung function in a cystic fibrosis patient with the rare 1677delTA/R334W genotype. Eur Respir J 2022;60:2201341.
82. Liu M, Zhang C, Gong X, Zhang T, Lian MM, Chew EGY, et al. Kidney organoid models reveal cilium-autophagy metabolic axis as a therapeutic target for PKD both in vitro and in vivo. Cell Stem Cell 2024;31:52–70.e8.
83. Hernandez JOR, Wang X, Vazquez-Segoviano M, Lopez-Marfil M, Sobral-Reyes MF, Moran-Horowich A, et al. A tissue-bioengineering strategy for modeling rare human kidney diseases in vivo. Nat Commun 2021;12:6496.
84. Geurts MH, Poel E, Amatngalim GD, Oka R, Meijers FM, Kruisselbrink E, et al. CRISPR-based adenine editors correct nonsense mutations in a cystic fibrosis organoid biobank. Cell Stem Cell 2020;26:503–10.e7.
85. Zhang W, Wauthier E, Lanzoni G, Hani H, Yi X, Overi D, et al. Patch grafting of organoids of stem/progenitors into solid organs can correct genetic-based disease states. Biomaterials 2022;288:121647.
86. He X-Y, Zhao C-J, Xu H, Chen K, Bian BSJ, Gong Y, et al. Synaptic repair and vision restoration in advanced degenerating eyes by transplantation of retinal progenitor cells. Stem Cell Rep 2021;16: 1805–17.

87. Hirami Y, Mandai M, Sugita S, Maeda A, Maeda T, Yamamoto M, et al. Safety and stable survival of stem-cell-derived retinal organoid for 2 years in patients with retinitis pigmentosa. Cell Stem Cell 2023;30: 1585–96.e6.
88. Mandai M. Pluripotent stem cell-derived retinal organoid/cells for retinal regeneration therapies: a review. Regen Ther 2023;22:59–67.
89. Peng WC, Kraaier LJ, Kluiver TA. Hepatocyte organoids and cell transplantation: what the future holds. Exp Mol Med 2021;53: 1512–28.
90. Peng WC, Logan CY, Fish M, Anbarchian T, Aguisanda F, Álvarez-Varela A, et al. Inflammatory cytokine TNFα promotes the long-term expansion of primary hepatocytes in 3D culture. Cell 2018;175:1607–19.e15.
91. Hu H, Gehart H, Artegiani B, Löpez-Iglesias C, Dekkers F, Basak O, et al. Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell 2018;175:1591–606.e19.
92. Li H, Li J, Wang T, Sun K, Huang G, Cao Y, et al. Hepatobiliary organoids differentiated from hiPSCs relieve cholestasis-induced liver fibrosis in nonhuman primates. Int J Biol Sci 2024;20:1160–79.
93. Nam SA, Seo E, Kim JW, Kim HW, Kim HL, Kim K, et al. Graft immaturity and safety concerns in transplanted human kidney organoids. Exp Mol Med 2019;51:1–13.
94. Roets B. Potential application of PBM use in hair follicle organoid culture for the treatment of androgenic alopecia. Materials Today Bio 2023;23:100851.
95. Ramovs V, Janssen H, Fuentes I, Pitaval A, Rachidi W, Chuva de Sousa Lopes SM, et al. Characterization of the epidermal-dermal junction in hiPSC-derived skin organoids. Stem Cell Rep 2022;17:1279–88.
96. Jin H, Zou Z, Chang H, Shen Q, Liu L, Xing D. Photobiomodulation therapy for hair regeneration: a synergetic activation of β-catenin in hair follicle stem cells by ROS and paracrine WNTs. Stem Cell Rep 2021; 16:1568–83.
97. Li J, Chu J, Lui VCH, Chen S, Chen Y, Tam PKH. Bioengineering liver organoids for diseases modelling and transplantation. Bioengineering (Basel, Switzerland) 2022;9:796.
98. Wang L, Owusu-Hammond C, Sievert D, Gleeson JG. Stem cell-based organoid models of neurodevelopmental disorders. Biol Psychiatry 2023;93:622–31.
99. Guy B, Zhang JS, Duncan LH, Johnston RJJ. Human neural organoids: models for developmental neurobiology and disease. Dev Biol 2021; 478:102–21.
100. Achberger K, Probst C, Haderspeck J, Bolz S, Rogal J, Chuchuy J, et al. Merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. Elife 2019;8:e46188.
101. Kim H, Kim GS, Hyun SH, Kim E. Advancements in 2D and 3D in vitro models for studying neuromuscular diseases. Int J Mol Sci 2023;24: 17006.
102. Garreta E, Kamm RD, Chuva de Sousa Lopes SM, Lancaster MA, Weiss R, Trepat X, et al. Rethinking organoid technology through bioengineering. Nat Mate 2021;20:145–55.
103. Licata JP, Schwab KH, Har-El Y-E, Gerstenhaber JA, Lelkes PI. Bioreactor technologies for enhanced organoid culture. Int J Mol Sci 2023;24: 11427.
104. Liu H, Gan Z, Qin X, Wang Y, Qin J. Advances in microfluidic technologies in organoid research. Adv Healthc Mater 2023;13:e2302686.
105. Tenreiro MF, Branco MA, Cotovio JP, Cabral JMS, Fernandes TG, Diogo MM. Advancing organoid design through co-emergence, assembly, and bioengineering. Trends Biotechnol 2023;41: 923–38.
106. Sidhaye J, Knoblich JA. Brain organoids: an ensemble of bioassays to investigate human neurodevelopment and disease. Cell Death Differ 2021;28:52–67.