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Review

Recent progress on the organoids: Techniques, advantages and applications

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ABSTRACT

Organoids are a cutting-edge technology in the life sciences field, with applications in precision medicine, bionic organs, and toxicological evaluations of chemicals. Their 3D structure closely resembles that of real organs, allowing more accurate functional mimicry. The 3D organoid culture system can simulate the growth state of cells *in vivo* and establish a suspension culture system for organoid 3D culture by using scaffold-less or scaffold technology to avoid direct contact between cells and plastic culture vessels. Furthermore, organoids can simulate the pathophysiological state of tissues and organs *in vitro*. This paper primarily discusses the construction methodologies, as well as the advantages and disadvantages of 3D culture systems for both scaffold-free organoids and scaffolded organoids. This review also summarizes the application of organoid models in chemical toxicology evaluation, drug screening and functional evaluation, establishment of *in vitro* disease models, and research on disease occurrence and potential mechanisms. The aim is to provide a reference for the research and practical applications of organoid-related scientific fields.

1. Introduction

In life sciences research, animal models and traditional two-dimensional cell models are commonly used to simulate human processes and structural functions at the morphogenetic and molecular levels. They are utilized to explore intercellular signal communication and conduction, study the targets of compounds in the body or tissues, and elaborate on their mechanisms of action [1]. However, as human research in the field of life sciences deepens, these models face challenges in clinical applications and have limitations in accurately reflecting physiological phenomena in the body's tissues and organs. Therefore, many efforts have been made to establish new research models that can bridge the gap between the cellular and organ levels present in most current model systems. The aim is to better simulate the function of real organs *in vitro* and obtain clinical models that reflect the real tissue physiology of the body.

Organoids, also known as mini-organs, are self-assembled three-dimensional cell clusters cultured *in vitro* that are differentiated from stem cells or tumour cells, with the cell type and similar spatial structure of the corresponding organ, and can simulate part of the function of the organ [2]. Compared with traditional 2D cell culture models, the organoids formed by *in vitro* culture technology can maintain individualized heterogeneity, better simulate the interaction between cells under physiological conditions, and have stronger proliferation ability. Compared with animal models, it has strong visualization, high operability, timeliness and economy, and can achieve high-throughput screening with low ethical risk [1,3,4]. It can provide a new research and application platform for the screening of human efficacy factors, toxicological evaluation of drugs, disease pathogenesis and drug mechanism of action.

With the development of science and technology, great progress has been made in the fields of materials science, cell biology, clinical medicine, and preventive medicine, which has greatly promoted the development of organoid technology. The organoid technology has also promoted the process of basic scientific research, which has shown important value in disease research, drug screening and regenerative medicine, and accelerated the research process of life sciences. Here, we briefly summarize the development process and limitations of organoid technology, and the techniques for constructing organoid models, mainly including the establishment of three-dimensional (3D) organoid models without scaffolds and with scaffolds. This paper focuses on the diverse applications of human organoid models, including their role in

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establishing *in vitro* disease models, investigating disease onset and underlying mechanisms, facilitating drug screening and functional assessment, enabling gene editing and precision medicine, as well as contributing to regenerative medicine. This review is expected to provide a theoretical basis for the development and utilization of new human organoid models.

2. Development of organoids

The traditional 3D cell culture model is a 3D spherical structure formed by mixing cells, cell growth factors, and suitable backbones in a single system. As a three-dimensional cell complex formed by the self-assembly of stem cells and tumour cells *in vitro*, organoids are different from traditional spheroid cultures. Rather than being simple, homogeneous 3D structures, organoid models exist in a variety of tissue cell types. Although they are not actual human organs, organoids can simulate real organs in terms of tissue structure, physiological function, and anatomical features [5,6]. According to their different sources, organoids can be divided into embryonic stem cell (ESC) organoids derived from pluripotent ESCs, induced pluripotent stem cell (iPSC) organoids derived from iPSCs, adult stem cell (ASC) organoids derived from adult stem cell (ASC) and tumour organoids derived from patient tumour tissues (Fig. 1) [3,7,8]. Among them, organoids derived from ESCs and iPSCs are formed by *in vitro* induction of stem cells. ESC-derived organoids have advantages in mimicking in vivo organ and tissue development [9]. In contrast, iPSC-derived organoids can maintain a high degree of cell diversity and tissue similarity [10]. ASC-derived organoids are isolated from mature tissue cells or organs and then are expanded and induced to undergo differentiation *in vitro* to form adult stem cells, which have high clinical relevance and stable genetic phenotypes [11,12]. Tumour organoids, which have the advantages of clinical prediction and are suitable for personalized treatment, target verification and drug sensitivity prediction in precision medicine research in the study of human disease, are usually formed by the proliferation and differentiation of stem cells in tumour tissue or tumour cells with stem cell characteristics [13].

The 2D monolayer cell model is a traditional approach widely used in life science research due to its simple modeling and mature cell culture technology. However, the human body is an organic structure composed of different specific cellular structures and their surrounding microenvironment, resulting in complex pathological and physiological responses. Organoid models have emerged as a pivotal solution to the pressing need for innovative cellular models capable of accurately simulating the microenvironment surrounding actual human tissues and organs, as well as replicating their pathophysiological characteristics [14].

Wilson's reported organoid technology in 1907 for the first time, when he made attempt to regenerate organisms *in vitro*. He discovered that sponge cells, when separated mechanically, could be regrouped and become new sponge organisms with normal function through self-organization [15]. However, scientists were only able to isolate and culture embryonic stem cells derived from human blastocysts for the first time in 1998 and establish lines of human embryonic stem cells, which opened up new avenues for disease modeling [16].The progress of organoid research has been relatively slow due to limitations in scientific and technological development. However, the robust advancements in life sciences and material sciences during the 21st century have laid a solid foundation for the progress of organoid technology. In 2009, Sato et al. [17] discovered that adult intestinal stem cells expressing G protein-coupled receptor 5 (Lgr5 +) and a single leucine-rich repeat sequence could self-organize and differentiate into crypt villus structures even without mesenchymal ecological niches. This discovery marked a new era in organoid culture. Organoid technology is currently the focus of international basic research and high-tech applications. In recent years, research related to organoids has been increasing year by year, frequently achieving remarkable results in key areas of life science research. Researchers have successfully constructed various types of organoids, such as retinal organoids [18], liver organoids [19], pancrea organoids [20], skin organoids [21], and gastrointestinal organoids [22], along with their corresponding tumor organoids. These models offer invaluable insights into the tissue physiology of organisms.

3. Construction of organoid models

The 3D organoid culture system is created through a suspension culture method that includes extracellular matrix and necessary factors to maintain organoid ecology and differentiation. This system is established using scaffold-less or scaffolding technology to prevent direct

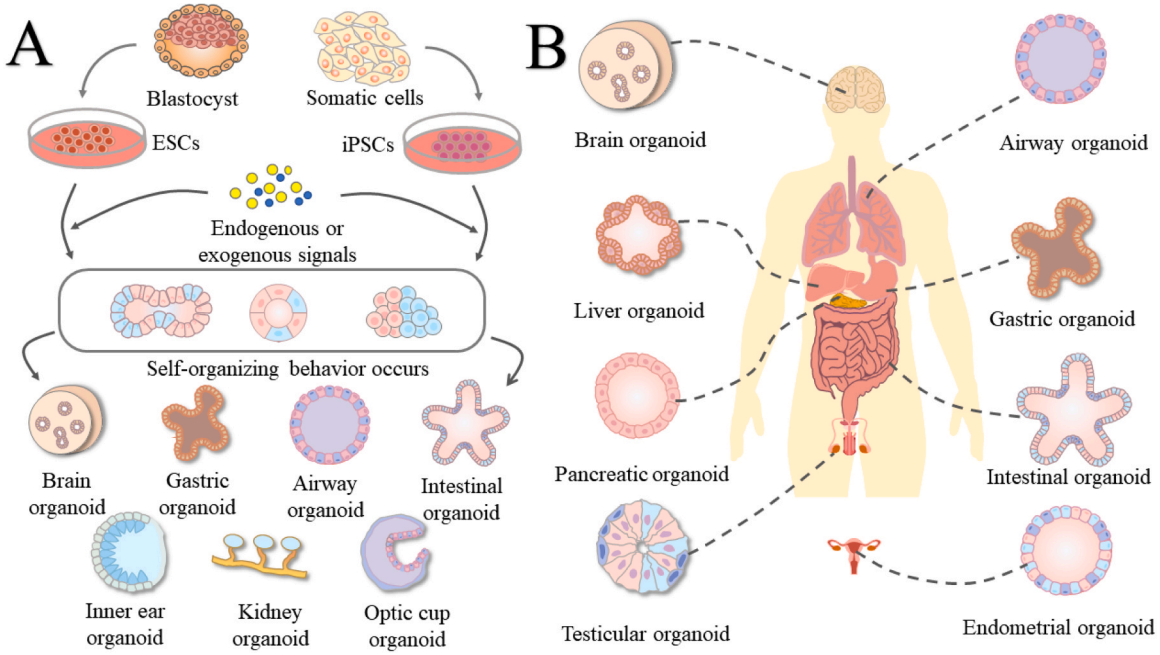


Fig. 1. Schematic diagram of ESCs, iPSCs and ASCs induced in vitro to generate corresponding tissue organoids.

contact between cells and the plastic culture vessel [23].

3.1. Scaffold-free 3D organoid models

A variety of methodologies can be employed to develop scaffold-free 3D cell culture models, also known as 3D multicellular spheroid models. These methods include magnetic levitation, gas-liquid interface, suspended droplet culture, and bioreactor methods [24,25].These strategies rely on the gentle reattachment of cell surfaces through the cells self-organizing ability to form stable and complex structures without the use of any external. polymeric material scaffolds or encapsulated gel materials, as well as external forces [24,26,27]. Under scaffold-free conditions, the cells form their own extracellular matrix, which better mimics the *in vivo* microenvironment. This facilitates the formation of multicellular spheres that more accurately mimic the tissue morphology and cellular state found within the organ.

The main principle of magnetic levitation technology is to achieve equilibrium of cells at a specific levitation height and force them to assemble during migration from high to low magnetic field areas. Under these equilibrium conditions, tissue cell morphology and physiology can be adjusted through non-contact operations during the organoid formation process (Fig. 2A). This provides an optimal micro-environment for 3D cell growth while maintaining organoid cell viability [28].

In contrast, the air-liquid interface, a classic method for 3D cell culture, does not fully immerse the cells in the medium. Instead, it exposes the top layer of cells to the air while the bottom layer remains in contact with the liquid medium (Fig. 2B) [29]. The air-liquid interface culture model of epithelial cells is a crucial tool in biomedical research. It simulates the behavior of external pathogens in the human respiratory tract and helps to expand our understanding of respiratory diseases by assessing the toxicity of exposure to these pathogens and deepening our understanding of their pathogenesis [30]. Additionally, the cell culture model at the gas-liquid interface enables a differentiated transformation of the structure and function of the barrier between normal and diseased tissues. This could have significant implications in clinical applications, such as toxicity testing and cancer research [31].

Suspension droplet culture is a process that involves creating a loose single-cell suspension of adherent cells using enzymes. The resulting single cells are then placed in suspension culture under the physiological conditions of suspension droplet culture, as depicted in the Fig. 2C [32]. In addition to being subject to gravity, the corresponding 3D cell sphere model can form spontaneously without any synthetic material or force. It has the characteristics of fast cultivation speed, requires minimal tissue debris, and generates 3D cell spheres with uniform morphology. Djomehri et al. [33] successfully constructed human mammary organoids *in vitro* using the suspension drop technique. Specifically, they formed one organoid per suspension drop. The study found that breast organoids share similar histophysiological characteristics with normal breasts and can provide reproducible quantitative parameters for assessing the relationship between microenvironmental factors and tumorigenesis.

Dynamic bioreactor models for organoid culture can support the proliferation and differentiation of organoids from a wider range of tissue types, as well as provide fine control and manipulation of the *in vitro* environment, unlike static culture methods [25]. Rotating bioreactors are commonly used for cell culture as they can simulate the microgravity environment required and improve oxygen delivery and nutrient utilization (Fig. 2D). This provides an optimal environment for cell culture [34]. In disease modeling, drug discovery, and regenerative medicine, it can be used to efficiently generate sensitive and reliable *in vitro* models of human brain organoids, inner ear organoids, and human intestinal organoids. These models summarize the key structures and components of tissue growth and development [35–37]. In simulating neurodevelopment and neurological diseases, organoid models formed through electrical stimulation are capable of adequately responding to the functional connectivity within neuronal networks and can monitor transmembrane conductance activity rhythms in real time [38]. Researchers have used a non-invasive and biosafe approach to establish uniform electric fields that can efficiently amplify organoids *in vitro* [39]. The study suggests that the microenvironment has the ability to imitate the human brain's function, which can facilitate the application of bioelectrical interface technology and artificial intelligence [40,41]. Microfluidic bioreactors are a platform for dynamic organoid culture that allows for precise manipulation of fluids through microchannels,

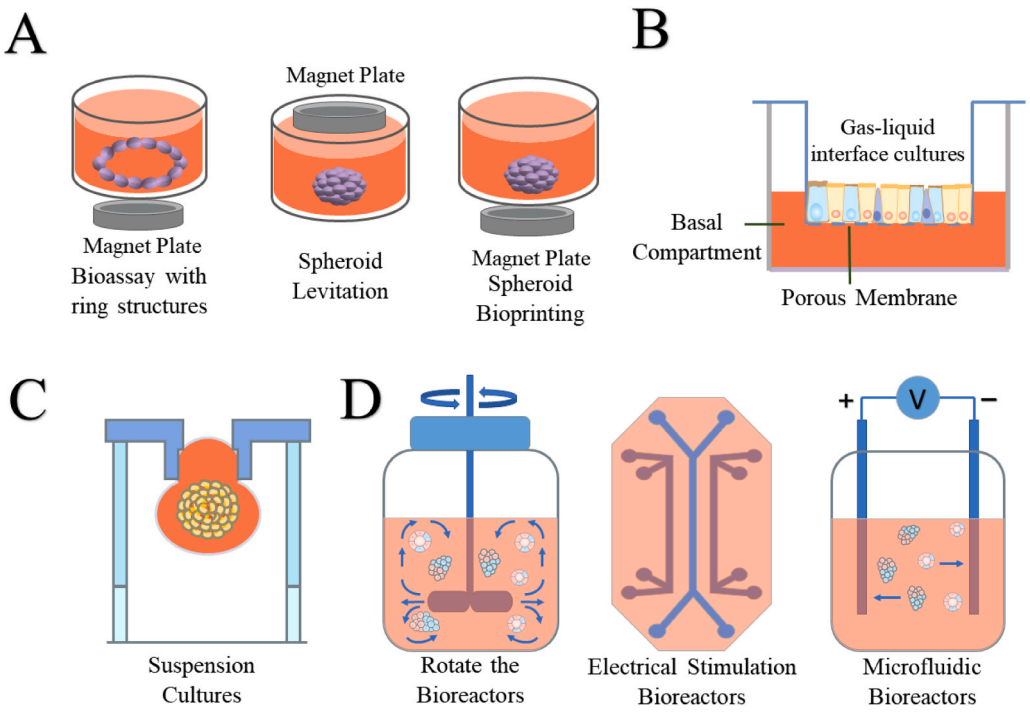


Fig. 2. Scaffold-free 3D organoid models. A, 3D cell culture magnetic levitation model; B, 3D cell culture "gas-liquid interface" model; C, 3D cell suspension model; D, 3D cell culture bioreactor model: rotating bioreactor, microfluidic bioreactor, electrically stimulated bioreactor.

resulting in accurate control of the microenvironment. The bioreactors' flexible mechanical properties enable timely nutrient provision and metabolic waste removal, while their precise spatial distribution minimizes the low homogeneity and unpredictability of the organoid culture process [42,43]. Microfluidic devices are widely used in disease modeling, drug discovery, and screening. They can simulate physiological parameters of body tissues, reproduce pathogen-body interactions and drug-drug interactions [44,45].

3.2. 3D organoid model with scaffold

3D cell culture using scaffolds is a hybrid culture of target cells with cell growth factors, reconstructed matrix proteins, and hydrogel scaffolds to mimic the environmental characteristics of cell growth *in vivo* [46]. Commonly used 3D cell scaffolds are shown in Table 1. Compared to scaffold-free 3D cell culture models, scaffolded 3D cell culture models can enhance cell differentiation and guide cell behavior by utilizing their mechanical properties and spatial geometric characteristics [47,48].

3.2.1. Matrigel

As the mainstream culture material currently used in organoid research, Matrigel has a wide range of biomaterial properties, making it an effective structural support for nearly all types of epithelial and endothelial cells.

In the study of the transition of stem cell biology to an *in vitro* model of organogenesis, Matrigel matrix gel promotes the efficient generation of low heterogeneity, highly sensitive sac-shaped organoids for disease and regeneration studies by modulating the organoid culture microenvironment to promote the efficient generation of animal-derived ear vesicles [49]. Its organoid formation efficiency reaches 90 %. In the study of retinal organoids, embedding retinal organoids in matrix gel can protect retinal organoid photoreceptors from external mechanical shear [50]. Similarly, 3D matrix gels can provide critical signals and the necessary ecological niche environment required for cell differentiation, influencing the behavior of cell signaling dynamics based on providing structural support for epithelial cells [51]. Although matrix gel has a wide range of applications in organoid culture, according to the recent analysis of proteomics and bioinformatics studies on pancreatic organoids, matrix gel has significant differences in physical properties such as mechanical properties and molecular physiology from real organoid substrates *in vivo* during *in vitro* culture, as well as disadvantages such as poor reproducibility from batch to batch, which makes its application challenging [20,52].

3.2.2. Decellularized matrix

In contrast to Matrigel, decellularized matrix (dECM) hydrogels are created through the chemical, physical, or enzymatic decellularization of tissue cells. This process yields hydrogels with excellent biocompatibility and non-immunogenic properties [53,54].

Kim et al. [22] used dECM hydrogel as an alternative to Matrigel for *in vitro* modeling of gastrointestinal organoids and analyzed the morphology, formation efficiency, functional properties, and specific gene expression levels of the constructed gastrointestinal organoids by scanning electron microscopy, proteomic analysis, immunocytochemical staining, and qPCR analysis, which were significantly better than the organoid model established by Matrigel. In studies of stem cell differentiation into islet organoids, a microenvironment consisting of rat pancreatic cells depleted of extracellular matrix can significantly increase the expression of key pancreatic transcription factors and maintain glucose homeostasis in iPSC self-organized islet organoids [55]. The use of dECM in the study of peritoneal metastatic disease of colorectal cancer can provide the physiological and biochemical conditions necessary for the growth and development of tumor organoids organs. This approach faithfully reflects the clinical phenotypes and typical pathobiological features of peritoneal metastatic disease [56].

Table 1
Commonly used organoid scaffolds.

Scaffold type	Advantages	Disadvantages	Refs.
Matrigel	It may act as a three-dimensional substructure for cell adhesion and motility, generating and transmitting mechanical signals to activate intracellular signaling pathways. Additionally, it may act as a growth factor reservoir to promote cell adhesion.	The material is ineffective in providing a natural microenvironment for organoids. It is costly, has poor mechanical properties, exhibits high batch-to-batch variability, and displays spatial heterogeneity and immunogenicity.	[46, 52, 177]
Decellularized matrix	The biomolecular profile and composition must match the characteristics of the natural microenvironment, with kinetic and physiological properties that match the organoid spatio-temporally.	The chemical composition of the material in question is uncertain, which makes it difficult to precisely adjust its physical and biochemical properties. Additionally, there is considerable inter-tissue variability.	[53, 178]
Natural hydrogel scaffolds	It contains cell adhesion structural domains, exhibits the structure of the natural tissue microenvironment, and is capable of promoting cell growth and differentiation. It is biocompatible with abundant bioactive sites and exhibits low immunogenicity.	The material in question exhibits poor mechanical properties, low stability, susceptibility to physical and chemical factors, and a proclivity for degradation.	[48, 177, 179]
Synthetic hydrogel scaffolds	The material is less susceptible to batch-to-batch variation, demonstrating good biocompatibility and physiological inertia. It exhibits good mechanical properties and strain stiffness, and has been chemically functionalized to incorporate biological ligands, signaling molecules, and cross-linking sites, allowing for precise tuning of physical and biochemical ecological niches.	The material lacks cell adhesion sites and bioactivity, and it exhibits poor biodegradability.	[177, 179]
Hybrid hydrogel scaffolds	The material exhibits structural and stability properties that are not easily degraded. It can accurately mimic the ecological niche of stem cells in vitro. Furthermore, it can be customized to meet the performance requirements of different organoids through the control of biodegradability, reactivity, and stiffness.	The lack of recyclability for secondary applications represents a significant challenge.	[48, 178, 180]

Additionally, it reveals the potential application of tumor ECMs in drug research and gene editing. In the context of renal organoids in disease modeling and regenerative medicine, dECM hydrogels provide mechanical support and an immune microenvironment for cell growth and reproduction. They also facilitate the formation of vascular networks within human renal organoids and support renal organoid morphogenesis, thereby enhancing researchers' understanding of renal

development and the underlying mechanisms of renal diseases [57].

3.2.3. Natural hydrogel scaffolds

Hydrogels are 3D structures made of cross-linked polymers such as proteins and polysaccharides, and are biocompatible, non-immunogenic, and mechanically stable [48,58]. Natural hydrogel chemical composite materials have properties and fiber structures similar to natural ECM. They can exhibit unique biological activity during cell growth and differentiation. Commonly used natural polymers in the formation of hydrogels in cell culture include alginate, chitosan, hyaluronic acid, and collagen.

Alginate hydrogels are hydrophilic polysaccharides that are low-cost and safe for use in biomedical materials. Araszkiewicz et al. [59] evaluated the physicochemical properties of alginate-based hydrogels through various assays, including rheological, osmolality, morphology, cell viability, and magnetic resonance imaging. They confirmed that alginate hydrogels are essential for cellular delivery and transplantation. As a natural hydrogel, chitosan has good biodegradability, high mechanical strength and excellent biomimetic function [60]. In applied research related to bone tissue regeneration, chitosan can provide a microenvironment that is highly similar to the bone structure. It can induce the differentiation of pericyte MSCs from adipose tissue into skeletal tissue without the use of any external bone differentiating agents or growth factors [61]. Furthermore, the modified technology used to prepare functional porous chitosan gel scaffolds can address the limitations of the original method in cell transplantation. This can enhance the generation of neuronal cells and the formation of the vascular system in peripheral nerve regeneration [62]. The findings of this study provide valuable insights for cellular replacement therapy research. Hyaluronic acid is glycosaminoglycans formed by the covalent linkage of β-1,3 bonds, which are highly chemically modified and cross-linked [63]. Therefore, it can not only act as a scaffold carrier but also regulate cell behavior in the process of *in vitro* model of organoids [63,64]. In the study of oncological diseases, hyaluronic acid can affect the behavior of tumor cells and the generation of vasculature through specific receptors on the surface of target cells [65]. This correlation links the hyaluronic acid-rich extracellular microenvironment with various inflammatory behaviors. As a hydrophilic hydrogel with low antigenicity and biodegradability, collagen is considered one of the most promising biomaterials for life science research [66]. Due to its strong mechanical plasticity, collagen can be used to induce branch elongation in human mammary gland organoids during organoid culture [67] as well as to direct neurite outgrowth in 3D neuronal cultures [68].

3.2.4. Synthetic hydrogel scaffolds

In contrast to natural hydrogels of biological origin, synthetic hydrogels can be used to tune the biological and mechanical properties of the gel solids by varying the type of crosslinking agent, the size of the molecular weight, and the type of crosslinking [69].

Majumder et al.[70] discovered that Polyethylene glycol (PEG) hydrogel scaffold cultures exhibited more advanced neurological function, a less enriched neurological vasculature structure, and a better response to the pathophysiological behaviors of neuroinflammation compared to matrix gel in the study of neural organoids. As a novel biomaterial, polyisocyanate peptide (PIC) hydrogels not only have good mechanical properties, but also have unique thermal response properties that can directly influence the viability and differentiation of 3D organoid cells [71]. PIC hydrogels exhibit strong strain properties, which can be used as cellular scaffolds to guide intercellular behavior during 3D organoid culture. In addition, they promote the spontaneous formation of complex vascular systems in organoids *in vitro* and regulate cellular activity according to temperature changes [72]. PIC hydrogels are a highly promising biomaterial for cellular immunomodulation. They can be delivered minimally invasively by exploiting their reversible thermosensitizing behavior. Additionally, they are capable of stimulating the *in vitro* expansion of T cells under ex vivo conditions and their *in vivo* overtransfer [73]. Polyacrylamide hydrogels are commonly used in biomedical research because of their exceptional mechanical properties and near-perfect elasticity [74].

3.2.5. Hybrid hydrogel scaffolds

Due to the limited biological characteristics retained in a single biomaterial, it is difficult to achieve this combination. Developing hybridized hydrogels is necessary to combine the advantages of natural and synthetic materials, providing a more suitable microenvironment for the growth and development of organoids in the *in vitro* culture process.

Research has demonstrated that the hybridized hydrogel created by combining PIC and hydroxyapatite not only retains the biological properties of the original hydrogel, but also exhibits superior material stiffness and stress sensitivity compared to a single hydrogel [75]. The hybridized hydrogel material, fabricated by cross-linking polyethylene glycol (PEG) with hyaluronic acid through a glutamine transferase cross-linking system, possesses both hydrogel properties and bioactivity. This material can be used to mimic the microenvironment that sustains cell growth *in vitro* and reduce macrophage infiltration *in vivo*, while also better preserving the stem cell phenotype [76]. Hybridized hydrogel scaffolds formed by crosslinking alginate with collagen primary scaffolds can be constructed with tunable mechanical plasticity and fibrous microstructure to regulate tumor cell migration [77]. In cell culture and tissue engineering applications, alginate is usually hybridized with polyacrylamide to obtain good mechanical properties, and the polymer ratio is changed to obtain the optimal hydrogel for biomedical applications [78]. In addition to crosslinking pairs of natural active materials to form hybridized hydrogels, it is also feasible to crosslink more than two active materials to develop multi-network hydrogels. To achieve this, researchers employed green solvents and photocrosslinking techniques to interconnect gelatin, polyacrylamide, and carboxymethyl chitosan, thereby creating a tri-network hydrogel [79]. The mechanical properties, differential scanning calorimetry, swelling, and MTT measurements also demonstrated the potential for tissue engineering applications.

4. The biomedical applications of organoid

Organoids have been fully validated as the "smallest system" *in vitro* that can encompass the biological functions of organs in real organisms [4,80]. Consequently, these straightforward and accessible *in vitro* organoid evaluation models can be established to fulfill the requirements of contemporary biomedical research, tailored to the specific needs of the research objectives (Fig. 3).

4.1. Establishment and application of disease models

The primary purpose of establishing disease models is to enhance the understanding of diseases and identify the key mechanisms of disease development. Disease treatment drugs often have toxic side effects, so appropriate disease models can be established to study these effects and achieve precise drug administration. This can help reduce the toxic side effects of drugs on patients and improve therapeutic outcomes. Animal models are crucial in aiding our comprehension of the function of identified mutant genes in disease phenotypes. However, studying human-specific cognitive and behavioral disorders with existing animal models is challenging due to significant species differences. These differences include variances in developmental programs, cellular structure, cellular composition, and genetic backgrounds, which limit their similarity to human diseases [81,82]. On the other hand, the traditional 2D cell culture model culture conditions are too simple and lack cell-cell and cell-substrate interactions to generalize the physiological behavior of the organism, and lead to genomic instability as the cells pass through generations [83,84]. These differential behaviors make 2D cell models insufficient for effectively understanding and treating diseases. In

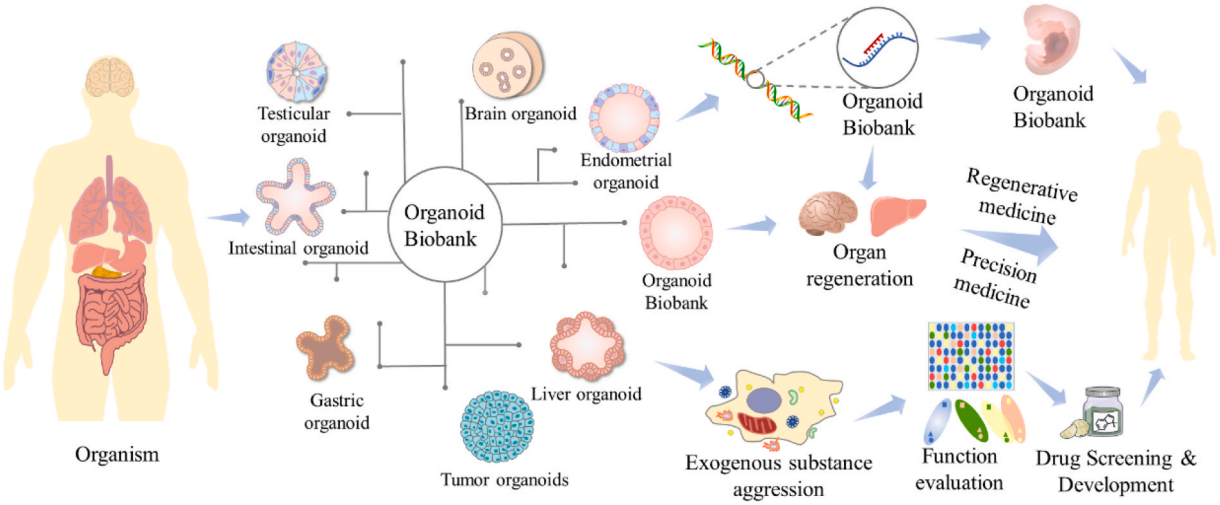


Fig. 3. Tissue organoids are associated with disease modeling, drug evaluation, gene editing, and regenerative medicine by establishing organoid biobanks *in vitro*.

contrast, organoids have the potential to become a powerful tool for the study and precise treatment of human diseases. They offer significant advantages over traditional animal models and 2D cellular models, including rapid growth, stable differentiation, and near-physiological characteristics [85,86]. By utilizing organoids, researchers can overcome the shortcomings of other models and gain a better understanding of human diseases.

In the study of congenital diseases that require a specific tissue microenvironment in which development occurs, the establishment of appropriate disease modeling systems has been central to the approach to such problems. Organoid models can be used not only for generalization of organ structure and function, but also for preservation of the original genetic characteristics and epigenetic traits of tissues, which can be used for gene editing of monogenic or polygenic genetic diseases [11]. Cystic fibrosis (CF) is a genetic disorder characterized by a diverse array of clinical phenotypes, resulting from mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) [87]. CF is highly genetically heterogeneous and drug efficacy depends on CFTR mutation and function as well as the genetic background of the patient, which is difficult to assess clinically in animal models [88]. The combination of organoid models with primary organ-specific cell types and structural features, along with gene editing technology, provides new opportunities to uncover the intricate molecular mechanisms of CF for treatment. To facilitate CF research, Maule et al. [89] demonstrated the potential of using AsCas12a to facilitate allele-specific repair of CF splice mutations. This approach offers a promising theoretical foundation for developing corrective measures for genetic diseases. Geurts et al. [90] established a biobank of CF intestinal organoids and demonstrated that an adenine base editor could theoretically achieve efficient correction of nonsense mutations in the CFTR gene in 20 % of these organoids.The establishment of the CF intestinal organoid biobank provides a theoretical and practical basis for drug discovery and personalized treatment of CF. It also paves the way for the treatment of genetic diseases. In addition, the researchers simulated the embryonic heart development under gestational diabetes conditions by constructing a cardiac organoid system. They uncovered the modifications in endoplasmic reticulum function and ultra-long-chain fatty acid metabolism that occur under conditions of impaired cardiac cell type specificity with the support of imaging and lipidomics [91]. In the study of autosomal recessive renal tubular dysplasia, researchers have explored the mechanisms underlying the complete absence or severe reduction of proximal tubules in renal tubular dysplasia by using induced pluripotent stem cells to differentiate into kidney organoids that are transplanted and vascularized [92].

In the realm of infectious diseases, the mechanisms of host-pathogen interactions have not been fully explored due to the absence of physiologically relevant organ models. Additionally, the understanding of host-pathogen interactions induced by microenvironmental stimuli remains limited [93,94]. To further investigate host-pathogen organism interactions in an organ-specific manner, researchers have established infectious models by intracellularly injecting various microorganisms via microinjection techniques [95], and by creating organoid models with antipodal polarization and co-culturing them with pathogenic organisms *in vitro* [96,97]. To model the infectiousness of a pathogenic organism infecting a host, the organoid model structures can be disrupted mechanically or enzymatically and then incubated with pathogens [98]. Alternatively, organoid model-derived cell monolayers can be co-cultured with pathogenic organisms *in vitro* [99]. And these infection models are used to explore the pathogenesis and potential molecular mechanisms triggered by invasive infections due to pathogens such as viruses [100–103], bacteria [102–104] and protozoa [103,105,106] (Fig. 4). Based on these cellular and molecular pathways, this text identifies key molecules for cellular recognition, pathogen invasion, and host response. This provides more physiologically relevant and predictive theoretical guidance for drug screening and antimicrobial therapy of pathogens of interest. Guided by these theories, researchers have modeled the interactions between a variety of pathogens and their hosts, including Cryptosporidium and small intestinal and lung organoids organs [105], SARS-CoV-2 virus and lung organoids and brain organoids [107], Helicobacter pylori and gastric organoids [108], and Chlamydia and endometrial organoids [109]. These disease models can enhance our understanding of trends in the occurrence, development, and spread of epidemics, as well as aid in the containment of their spread.

Cancer-derived organoids are preferred over traditional 2D cancer cell lines because they better retain tumor heterogeneity, enabling more accurate *in vitro* modeling of human cancers. In this study, researchers constructed preclinical cancer models with high fidelity and utilized the differences between lesion types to explore the evolutionary trajectory and tumor heterogeneity of individual colon tumors [110]. These tumor organoids, which are heterogeneous in nature, can be analyzed using transcriptomics and proteomics to uncover the signaling mechanisms involved. This analysis can aid in the development of more effective tumor drugs by exploring drug sensitivity responses. In addition, it can play a significant role in predicting patient responses and improving tumor cure rates [110–112]. CRISPR-Cas9 genome editing tools can also be used in conjunction with organoid technology to develop molecular tumor organoids with specific pathologies that recapitulate the pathogenesis and process variations of neoplastic diseases [113]. Sun et al. [19] established an organoid with liver structure and function by directly reprogramming human hepatocytes, and systematically

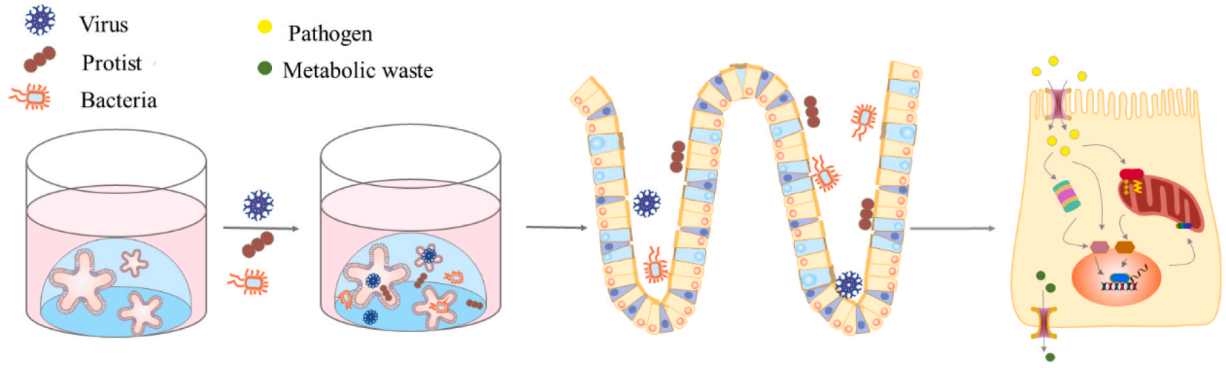


Fig. 4. By incubating the causative factors such as viruses, bacteria, and protists with organoids *in vitro*.

analyzed the genetic lesions involved in cancer development and progression by genetic engineering techniques, revealing the role of c-Myc in inducing hepatocellular carcinoma initiation associated with mitochondria-associated endoplasmic reticulum membrane alterations, as well as RAS-induced intrahepatic cholangiocarcinoma through the combined Notch and JAK-STAT inhibition pathway. In the lung cancer organoid study, researchers used malignant plasma exudates to construct lung organoid models that were validated based on their pathological and molecular characteristics to accurately predict clinical response to treatment in patients with advanced lung cancer and to develop personalized therapeutic strategies [114]. Similarly, in the study of bladder cancer organoids, researchers have introduced urologic oncology research by constructing urogenital bladder cancer organoids that retain the histological and molecular characteristics of the parent tumor [115]. This approach mimics the pathologic behavior and pharmacologic response of primary bladder cancer, which provide a non-invasive approach for modeling tumor pathogenesis, longitudinal drug response monitoring, and treatment [115,116]. It is used to predict and diagnose the development of bladder cancer, and to help guide the treatment of the disease and the development of related drugs.

4.2. Drug screening and toxicology evaluation

Currently, the physiological functions of cell lines commonly used in 2D laboratory cell culture differ significantly from those of multicellular tissues in vivo. As a result, they cannot effectively simulate the microenvironment of cells in the human body [117]. Animal experiments are time-consuming, expensive, and subject to medical ethics, and there are problems such as species differences [118]. These issues make it difficult to predict adverse drug reactions for most drug candidates for clinical application. They often remain in phase I experiments without approval for clinical trials. Most drug candidates have unpredictable adverse reactions when used in clinical applications. Toxicological testing is an important basis for conducting health risk assessment of chemicals and plays a critical role in hazard identification and characterization. Toxicological testing is an important factor in risk management and risk decision-making [119]. To evaluate exogenous general toxicity in clinical settings, the whole animal model is commonly used to conduct *in vivo* acute, subacute, chronic, and subchronic toxicity experiments under strictly controlled exposure conditions. However, to study the mechanism of toxicity and the metabolism of the chemical transformation process in the body, it is necessary to analyze its toxicological behavior using an isolated organ or cellular model (Table 2).

The development of organoid technology can greatly assist in toxicological research on the mechanisms of chemical toxicity and metabolic transformation processes in organisms. Quantitative analysis of test chemicals in organoid models can aid in determining the exposure dose in target cells. This can be combined with toxicokinetic modeling to establish a more accurate dose-effect relationship. The result is an enhanced basic research capacity of toxicology and its support for chemical risk evaluation and management. Additionally, it enhances the evaluation of chemical health risks through the application of *in vitro* toxicology modeling. Furthermore, it establishes environmental health effect-related and sensitive toxic effect endpoints, alongside high-throughput methods for both quantitative and qualitative detection [118,120].

Table 2
Commonly used organoid models of toxicology evaluation.

Organoids	Cell source	Project	Drugs	Cellular targets	Refs.
Testicular organoids	ASCs	Environmental reproductive toxicants and contaminants testing	Abiraterone	CYP17A1	[181]
Ovarian organoids	ASCs	Drug screening and toxicology testing	Salinomycin	Mitochondria	[122]
Lung cancer organoids	Surgical sample or transbronchial biopsy of the patient	Provide a new perspective on the discovery of clinical drugs	Halofuginone	Prolyl - tRNA synthetase	[128]
Skin organoids	ASCs &iPSCs	Insights into skin-to-skin transmission of Ebola virus and drug development	Ebola virus	AXL&NPC1	[182]
Intestinal organoids	hiPSCs	Explore the molecular mechanisms of human adenovirus and its effective therapeutics	Adenovirus	Saatchivirus-adenovirus receptor	[183]
Brain organoids	ASCs	Assess the neurotoxicity of severe psychotropic and other environmental pollutants	$CdCl_2$	Central nervous system	[184]
Kidney organoids	hPSC	Feasibility is judged by nephrotoxicity assessment during drug development	Tenofovir & aristolochic acid	Mitochondria & OAT	[185]
Liver organoids	HepaRG	Toxicity assessment of chemical components in Chinese herbal medicine complexes	Stilbene isomers	Mitochondria	[186]
Airway organoids	Bronchial epithelial cells	Assess respiratory toxicity induced by dust pollutants in the atmosphere	Black carbon or elemental carbon	-/-	[187]
Colon tumor organoids	Surgical samples from patients	Demonstrate dual-targeted nanomedicine design	Ferritin nanocages	Integrins and TfR1	[188]

Since the turn of the 21st century, scientific and technological advancements have resulted in the creation of numerous chemical substances that pose reproductive hazards to humans. To evaluate the reproductive toxicity of these substances, risk assessment can be conducted by constructing corresponding organoid models, which can help reduce the occurrence of risky behaviors. In terms of male reproductive toxicology, chemical damage to male reproductive function can be explored by establishing an epididymal organoid model *in vitro*, and the toxic mechanism of toxic substances in the epididymis can be clarified, thereby reducing the incidence of male infertility [118,121], and similarly, in female reproductive toxicology, Li et al. [122] constructed a 3D ovarian organoid model by culturing stem cells derived from the female reproductive system *in vitro*, and successfully applied it to reproductive toxicology to study the occurrence of infertility. Organoid models can be a useful tool for combining neuroscience and toxicology research. They simulate the signaling of functional neurons in organisms and can overcome differences in gene expression between different species in neurotoxicity research [123,124]. Traditional 2D cell cultures and cell co-cultures have been used in neurotoxicology studies to avoid species differences in animal models. However, these models are simpler and lack cellular diversity, making it difficult to reflect neurotoxic behavior in the context of cellular diversity. As human-derived cells, brain organoid models can mimic the development process of the human brain and the fine structure of the human brain. It not only overcome the limitations of animal models and traditional 2D cell models in neurotoxicology research, but also explain and evaluate neurotoxicants from the mechanism of toxicity through morphological analysis, multi-omics analysis and electrophysiological analysis, explain the functional changes of neurons, and organically combine neuroscience and toxicology [125].

During the development of new drugs, patient-derived organoids can maintain the genetic characteristics of parental tumor tissues while undergoing stable proliferation and differentiation [126]. This allows for the establishment of accurate, valid, and highly reproducible clinical data models during drug development. In addition, it enables the ability to perform all-around, high-throughput activity evaluation of new drugs. Patient-derived organoids are a valuable tool in drug development. They can serve as a link between *in vitro* and *in vivo* clinical trials for drug screening and functional evaluation, allowing for pharmacokinetic assessment of drug uptake and metabolism. This can lead to more precise predictions of clinical outcomes. Recent studies on lung cancer organoids have found that Solamargine and flufuranone have synergistic effects with cisplatin. This can change the sensitivity of cancer cells to cisplatin by inhibiting the expression of the hedgehog signaling pathway and blocking the occurrence of the PI3K/AKT and MAPK signaling pathways [127,128]. These findings provide a new perspective for clinical drug discovery. Furthermore, organoids can be employed for cancer genetic biomarker identification, enabling the assessment of drug sensitivity and therapeutic efficacy of anticancer agents based on the genetic expression of these biomarkers. This facilitates targeted drug screening and optimization of treatment regimens [129]. To develop liver drugs, researchers have blocked the uptake and excretion of bile acids in human liver organoids by knocking out the bile acid transporter gene [130]. This allows for a more accurate representation of drug toxicity and physiology in the human body. A large-scale drug screening is then performed using a dynamic assay with real-time imaging to determine drug susceptibility. Epigenetic research plays an important role in early disease recurrence and clinical testing of cancer drug resistance, and thus epigenetic drugs have significant potential for cancer therapy [131]. Based on this, Zhou et al. [132] immunocultured the constructed pancreatic ductal tumor organoids with T cells to establish a corresponding epigenetic drug screening platform based on immune checkpoint inhibitory therapies, which can be used to screen out the best and most efficient anti-tumor drugs among the library of tumor therapy candidates. Wetering et al. [133] utilized a comprehensive data library of organoid culture models to screen a range of drugs, including those currently undergoing clinical trials as well as those that have been or are presently being evaluated in clinical trials. They also correlated drug susceptibility with genomic signatures to identify molecular signatures associated with altered drug responses.

4.3. Gene editing and precision medicine

In recent years, organoid models have rapidly developed. They not only use new modeling technologies but also apply them to personalized therapy and immunotherapy based on the particularity of diseases. This provides new ideas for the development of novel and personalized antitumor treatments. Patient-derived tumor organoids accurately replicate the genomic, proteomic, and histopathological characteristics of parental tumors, overcoming inter- and intratumoral heterogeneity more effectively than monoclonal-derived cancer cell lines [134–136]. Recent studies on liver cancer have led to the establishment of a biobank of human liver cancer organoids. The purpose of this biobank is to summarize the histological and molecular characteristics of primary cancer tissues and identify relevant proliferative and metabolic organoid subtypes [137,138]. In addition to harvesting tumor organoids directly from patients, gene editing can be used as a feasible method for rapid large-scale production of organoids (Table 3). Researchers employed base editors to create sgRNA libraries targeting a spectrum of oncogene activation and tumor suppressor gene inactivation mutations. They then induced the engineering of human adult stem cell-derived organoids to develop tissue-specific, complex tumor models [139]. Drug-protein genomic analysis of these different subtypes of tumor organoids can be used to identify different patient-specific drug sensitivities, explore potential therapeutic targets and biomarkers of different subtypes of primary tumor diseases, and achieve precision treatment of human primary tumor diseases [138,140,141].

Gene editing technology can provide therapeutic targets for clinical disease research by precisely changing genetic information and phenotype types. Additionally, it can be used to engineer the generation of tumor organoids and explore changes in molecular pathways and drug targets during tumorigenesis and development, deepening our understanding of diseases. In a study of severe combined immunodeficiency, researchers discovered that the development of T lymphocytes reverted to normal levels following the knock-in of promoter-free RAG2 cDNA at the endogenous locus within the human thymic organoid system in patients deficient in RAG2 [142]. In the study of congenital amaurosis caused by CRXT155INS4/+ or CRXK88Q/+ mutations, gene editing technology is combined with retinal organoids to visualize the changes in the genetic phenotype of congenital amaurosis during retinal development [143]. Photoreceptor-specific defects were analyzed by histology and transcriptomics methods, specific cell types and gene targets for gene therapy that cause photoreceptor maturation defects were deciphered and verified, and photoreceptor dysfunction caused by photoreceptor-specific defects was improved and restored by using CRISPR/Cas9 tools to specifically knock out mutation-causing alleles [143,144]. Cherubini et al. [145] were the first to establish an organoid model for screening optimal housekeeping genes. They analyzed the gene expression levels and pancreatic marker expression levels of pancreatic organoid housekeeping genes.

Recently, a growing number of studies have shown that the tumor microenvironment plays a key role in tumor treatment by controlling cancer progression and drug response in anti-tumor immunity [146, 147]. The extracellular microenvironment comprises cells, signaling factors, extracellular matrix, and vasculature [148,149]. It guides intercellular behavior, regulates the quality of organoid formation, and provides small molecular determinants of organoid growth and development. Therefore, it is important to enhance our understanding of the extracellular microenvironment and its impact on the physiological function and anatomical properties of organoids. Due to the differences in the microenvironment and physiological local conditions *in vivo*, there is a certain heterogeneity between different tumors and within a single tumor tissue. Due to differences in microenvironment and

Table 3
The applications of organoid gene editing.

Organoid	Disease	Cell source	Gene editing tools	Target genes	Refs.
Intestinal organoids	Cystic fibrosis	iPSCs	CRISPR/Cas12a	CFTR	[89]
Thymic organoids	Severe combined immunodeficiency	iPSCs	CRISPR/Cas9	RAG1&RAG2	[142]
Retinal organoids	Congenital amaurosis fugax	iPSCs	CRISPR/Cas9	CRX	[144]
Kidney organoids	Congenital nephrotic syndrome	ESCs &iPSCs	CRISPR/Cas9	NPHS1	[189]
Dorsal root ganglion organoids	Friedrich's ataxia	iPSCs	CRISPR/Cas9	FXN	[190]
Vascular organoids	Cerebral autosomal dominant arteriopathy	iPSCs	Adenine base editors	NOTCH3	[191]
Lung organoids	-/-	ESCs	CRISPR/Cas9	SOX9	[192]
Kidney organoids	Hereditary polycystic kidney disease	iPSCs	CRISPR/Cas9	PKD1&PKD2	[193]
Brain organoids	AUTS2 syndrome	iPSCs	CRISPR/Cas9	AUTS2	[194]
Mammary organoids	Breast tumors	ASCs	CRISPR/Cas9	TRP53	[195]
Brain organoids	Microcephalia	hESCs	CRISPR/Cas9	MCPH17	[196]
Kidney organoids	Organ transplant immune rejection	iPSCs	CRISPR/Cas9	B2M	[197]
Kidney organoids	Glomerular disease	iPSCs	CRISPR/Cas9	APOL1 G0&G1	[198]
Pancreatic cancer organoids	Pancreatic cancer	Surgical samples from patients	CRISPR/Cas9	SHCBP1	[199]

physiological conditions in vivo, there is heterogeneity between tumors and within a single tumor tissue. To determine the most effective treatment plan for cancer, researchers conducted high-throughput screening on four different tumor organoids isolated from the same tissue [150]. This allowed them to observe the real-time response of the organoids to anticancer drugs and determine their sensitivity. Based on these findings, an optimal treatment regimen was determined. Zapatero et al. [151] discovered that cancer-associated fibroblasts alter patient-derived tumor organoid-specific drug responses in the corresponding tumor microenvironment during personalized cancer therapies using the Trellis dendroanalysis technique. A study on immunotherapy for pancreatic cancer found that incorporating T cells into pancreatic tumor organoids creates a microenvironment that is physiologically relevant and immunosuppressive to the original tumor tissue [132]. This microenvironment mimics the physical barrier of tumors, facilitates T cell infiltration, and enables high-throughput screening of anti-tumor drugs based on immune-cancer interactions while preserving the characteristics of the original tumor tissue [132, 152]. In addition, in the research process of precision medicine, the microenvironment can also be designed as an organoid model for the reproduction of the microenvironment and disease treatment [153].

4.4. Regenerative medicine

Regenerative medicine aims to develop functional biological alternatives to promote tissue healing or produce replacement tissues for the restoration or establishment of organ-specific functions through cytological and materials science methods [154,155]. Compared to traditional regenerative medicine, this model is highly heritable and contains a variety of cell types, allowing for cell-to-cell interactions [156]. It can be used for organ transplantation and cell replacement (Table 4).

Accurate and reliable retinal disease models can be developed using hiPSC-derived retinal organoids, particularly for permanent visual impairment resulting from retinal ganglion cell death. Screening cells of retinal organoids using different genetic markers and integrating the selected cell subtypes into the mouse retina can significantly improve retinal diseases caused by photoreceptor damage [157,158]. The brain is the highest nerve center and serves as the material basis for all mental activity. The 3D brain organoids induced by ESCs or iPSCs are highly similar to the brain in terms of cell histology and anatomical properties. They can mimic the complex structure and function of the human brain, making them useful for studying neurological and stroke diseases [159, 160]. In the study of traumatic brain injury, researchers transplanted

Table 4
Clinical application of organoids as a substitute for organ transplantation.

Organoid	Cell source	Receiver	Orthotopic or Ectopic	Site of transplantation	Application	Refs.
Retinal organoids	hiPSCs	Healthy and diseased adult C57BL/6 mice	Orthotopic	Retina	Retinal ganglion degeneration	[158]
Brain organoids	hPSCs	Female SCID mice	Orthotopic	Medial prefrontal cortex	Traumatic brain injury	[161]
Brain organoids	hPSCs	Stroke SCID mice	Orthotopic	Middle cerebral artery	Reconstruct the infarct tissue	[162]
Mammary organoids	ASCs	Female NSG mice	Orthotopic	Mammary fat pads	Simulate breast development and cancer	[200]
Neural organoids	hiPSCs	Thymic nude mouse pups	Orthotopic	Somatosensory cortex	Deepen understanding of brain development and function	[201]
Skin organoids	hiPSCs	Immunodeficient BALB/c nude mice	Orthotopic	Frostbite skin tissue	Frostbite	[202]
Endometrial organoids	ASCs	Female C57 mice	Orthotopic	The basal layer of the endometrium	Intrauterine adhesions	[203]
Lung organoids	hPSCs	Immunocompromised NSG mice	Ectopic	Epididymal blood vessels and fat pad	-/-	[163]
Liver organoids	hiPSCs endoderm, endothelial cells, and mesenchymal cells	Acute liver failure Albu-Terek/ West German mice	Ectopic	Renal subcapsular space	Acute liver failure	[164]
Salivary gland organoids	human submandibular gland stem/progenitor cells	Nude mice	Ectopic	Kidney capsule	Salivary gland disease	[165]
Kidney organoids	hPSCs	Chicks	Ectopic	Chorioallantoic membrane	Kidney development and disease	[166]
Colon cancer organoids	Colon tumor tissue	Female BALB/c nude mice	Ectopic	Shoulder	Develope of personalized nanomedicines	[188]
Intestinal organoids	ESCs	NSG mice	Ectopic & Orthotopic	Renal subcapsular space and mesentery	Characterizate the implant site and effect	[204]
Hepatobiliary organoids	hiPSCs	Cynomolgus monkeys	Ectopic & Orthotopic	Submesentery, subcapsular of the liver	Treat liver fibrosis and liver regeneration	[205]

organoids to the lesion area and observed normal proliferation and differentiation, as well as expression of STEM121 and human-specific cell markers [161]. This has the potential to greatly improve local nerve injury and promote nerve repair. Similarly, for motor and cognitive dysfunction caused by neuronal loss resulting from stroke, organoids can be transplanted to reconstruct infarct tissue in the stroke infarct core [162]. This can repair the infarct core and restore axonal communication in the cerebral cortex by integrating the transplanted cerebral organoids into the host neural circuit, thereby improving sensorimotor function. From a clinical application perspective, human induced pluripotent stem cell-derived organoids can be employed as a substitute for organ transplantation through ectopic transplantation. Briana et al. [163] transplanted lung organoids into epididymal fat pads in immunocompromised NSG mice by utilizing microporous scaffolds to model adult airway structural homeostasis and establish a platform for disease research. Furthermore, liver organoids, salivary gland organoids, and kidney organoids were transplanted into Alb-TRECK/SCID mice, nude mice, and chicks through ectopic transplantation, which greatly facilitated the research process of regenerative medicine [164–166].

Cell therapy is a promising therapeutic modality that can offer unique advantages in treating diseases that are considered incurable by traditional therapies. It can also be used to elucidate disease mechanisms of action, infer disease treatments, and identify targets for more effective and precise treatments [167]. To investigate the safety and reliability of organoid cell therapy, researchers have conducted biological identification and safety evaluations of various organoid models [168,169]. This has established the role of organoid cell therapy in regenerative medicine research. Valente et al. [170] addressed autoimmunodeficiency resulting from thymic dysfunction by developing a thymic organoid system and utilizing organoid cell therapy. Jeong et al. [171] isolated stem cells from normal lacrimal gland tissue to construct a 3D lacrimal gland organoid model. This model was stimulated using pilocarpine, and proteomic analysis confirmed that its functional and structural properties align with those observed in vivo. Implantation into the lacrimal gland tissue of mice afflicted with dry eye disease has demonstrated that the 3D lacrimal gland organoid model can effectively repair and reconstruct damaged glands, thereby alleviating the symptoms of dry eye disease [172]. Organoid models can be used in the study of type I diabetes to overcome the difficulty of generating mature functional β cells *in vitro* and the autoimmune attack of individuals in ordinary stem cell therapy to reverse immunomodulatory pancreatitis [173].

5. Conclusion and outlook

Organoid models are more physiological, intuitive, and controllable. They can highly simulate the structure, spatial morphology, and function of real organs and tissues *in vivo*, thereby addressing the limitations of traditional models and effectively bridging the gap between *in vitro* and *in vivo* environments. However, it is important to note that each biological model has its inherent advantages and limitations. While organoids are a promising avenue of research, they are not exact replicas of human organs and are still in the exploratory stage. Therefore, there are several challenges and problems that need to be addressed urgently.

Organoids, as microtissues cultivated *in vitro*, can reflect the physiological characteristics of tissues. However, they lack the interaction between organoids and other tissues and organs *in vivo*, and cannot reproduce the pathophysiological characteristics of the whole organism in the process of disease modeling. Additionally, they cannot reflect the physiological and behavioral changes of the whole organism in the process of drug treatment [174,175]. Secondly, many organoids do not have a corresponding vascular system or immune cells in the culture system. This results in an incomplete extracellular microenvironment, which in turn affects the growth and development of organoids. Without corresponding immune regulatory mechanisms, organoids cannot exchange substances with the external environment of cells in a timely manner, which endangers the growth and reproduction of cells [42,176,177]. Furthermore, the limited reproducibility of organoid models is a crucial factor that hinders their clinical application. Organoids are formed through selective differential expression and differentiation of tissue cells in a specific microenvironment. This process is dynamic and influenced by factors such as cell-to-cell interactions, extracellular microenvironment, and cell physiological conditions. As a result, organoids lack stability and reproducibility, making commercial applications difficult.

Despite the challenges, the rapid advancement of technology and the ongoing refinement of modeling methodologies make it feasible to establish standardized organoid culture models. This can be achieved by combining multiple key fields such as clinical medicine, public health and preventive medicine, cell biology, and materials science in a multidisciplinary manner. The objective is to address the current limitations of organoid culture models, thereby fostering their gradual maturation and refinement.

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Declaration of Competing Interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Data availability

No data was used for the research described in the article.

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