Burns & Trauma, 2026, **14**, tkag015
https://doi.org/10.1093/burnst/tkag015
Published: 16 February 2026
Review

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Organoid research: new concepts and new technologies

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Abstract

As three-dimensional biomimetic systems developed from stem cells or somatic cells, organoids have gradually evolved into important links between basic research and clinical application. Compared with traditional two-dimensional culture and animal models, organoids can better reflect the structure and function of tissues and organs in terms of physiological relevance and prediction efficiency. This review explores the integration of organoid technology with microfluidic technology, gene editing, and artificial intelligence, focusing on the prospects of these technologies for application in precision medicine, disease modelling, drug screening, and toxicology. In addition, the latest progress in organoid research, including organ-on-a-chip, organoid biobank construction, and related applications of regenerative medicine, is summarized. The development of key technologies is reviewed in detail. These technologies include improvements in culture systems, innovations in microfluidic and matrix materials, gene editing and modification, and imaging and multiomic analyses. These achievements improve the application value of organoids in disease modelling, drug screening, and toxicology. Despite challenges such as standardization, batch-to-batch consistency, and clinical translation, the integration of interdisciplinary technologies has provided a new impetus for the development of organoids. In the future, the combination of organoids with artificial intelligence, personalized medicine, and regenerative medicine is expected to drive research progress on disease mechanisms and precision treatment.

Keywords Organoid, Organ-on-a-Chip, Organoid imaging, Precision medicine, Regenerative medicine

Highlights

- This article focuses on the latest progress in organoid research, with a particular emphasis on its applications in disease modelling, drug screening, and regenerative medicine, as well as the integration of precision medicine and organoid technology.
- Combining organoids with microfluidics, gene editing, and imaging technologies enhances the role of organoids in drug development and precision therapy.
- Organoid biobanks provide patient samples over the long term, facilitating the translation of basic research into clinical applications.

Received: 4 November 2025. **Revised:** 13 February 2026. **Accepted:** 13 February 2026
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Background

Reliable model systems are platforms for studying disease mechanisms, evaluating intervention outcomes, and promoting clinical translation [1]. The development of preclinical models that simulate the human *in vivo* microenvironment is a research focus with the goal of minimizing the risk of clinical trial failure [2]. Traditional two-dimensional (2D) cell culture and animal models have limitations in preclinical evaluation [3, 4]. Although 2D culture systems offer the advantage of being high throughput, their overly simplified artificial microenvironments cannot replicate the three-dimensional (3D) architecture and physiological functions of human tissues [5]. They often fail to predict drug efficacy and toxicity [6]. Animal models offer a more intricate *in vivo* environment. Interspecies biological differences pose challenges in accurately representing the specific pathological and physiological characteristics of humans. Most drugs validated in animal studies fail in clinical trials because of insufficient efficacy or unexpected toxicity [7] and high maintenance costs, and the long duration of animal experiments also limits the efficiency of drug development [8, 9]. In recent years, stem cell technology has advanced the construction of human-derived models. Stem cells have the ability to self-renew and undergo multilineage differentiation. They are an ideal source material for building organ and tissue models [10, 11]. In a novel 3D model, organoids are generated through the self-organization of stem cells, progenitor cells, or differentiated cells, and they resemble the structure, function, and genetic characteristics of native tissues *in vitro* [12].

As early as 1946, Smith and Cochrane first introduced the term ‘organoid’ when describing cases of cystic teratoma [13]. In 2009, Sato and colleagues constructed the first functional 3D epithelial organoid. They generated this by isolating G-protein-coupled receptor 5–positive (Lgr5+) intestinal stem cells rich in leucine repeat sequences and cultivating them in Matrigel with specific factors such as epidermal growth factor (EGF), Wnt3A, and R-spondin1 [14]. Their research demonstrated that even in the absence of mesenchymal cell support, a single Lgr5+ adult stem cell could self-organize into a mini-gut structure with distinct crypt–villus polarity. The generated tissues mimic the cellular composition and spatial arrangement of the natural intestine [14]. Moreover, biochemical signals—including lineage-specific genetic programs—have been integrated into 3D organoid cultures. By introducing morphogens, growth factors, or morphogen inhibitors, researchers use embryonic stem cells (ESCs) or adult stem cells (ASCs) to construct various organoid models [15]. Zhang *et al*. encapsulated tumour cells and matrix materials in hydrogel microspheres. They used microfluidic droplet encapsulation technology to direct the growth of patient-derived tumour organoids (PDTOs) [16]. By physically constraining cellular interactions, they simulate the mechanical tumour microenvironment *in vivo*, reducing the phenotype shifts caused by *in vitro* amplification. Han and colleagues introduced an acoustic virtual 3D scaffold (AV-Scaf) system that allows tumour organoids to grow without the need for Matrigel. They established a dynamic coculture platform for tumour organoids and T cells using this system [17]. In contrast to traditional static coculture methods, this approach uses acoustic force to guide immune cell positioning and migration, enhancing infiltration into tumour organoids. This system also recapitulates immune suppression in the tumour microenvironment [12]. This method avoids interference from physical scaffolds and reconstructs intercellular interactions in a highly biomimetic manner, relying on acoustic field mechanics to create a dynamic and realistic culture system.

As emerging 3D models, organoids simulate *in vivo* tissues in terms of structure and function and provide a platform for disease modelling, drug screening, and regenerative medicine. With the integration of advances across multiple disciplines, the application of organoids in precision medicine and personalized treatment is becoming increasingly prominent. This article reviews the latest concepts and key technologies in organoid research. The current applications and future development trends of organoids are also discussed.

Review

Basic concepts and characteristics of organoids

Definition and classification of organoids

Organoids are 3D miniature tissue structures produced under *in vitro* culture conditions that can simulate the morphological characteristics, spatial structure, and physiological functions of original tissues [18, 19]. By recreating cell–cell interactions and dynamic cell–matrix relationships, these models reproduce the microenvironment of tissues *in vivo* and have the advantage of being capable of long-term stable passage [20]. On the basis of their cellular origin, organoids can be broadly classified into pluripotent stem cell (PSC)–derived organoids and ASC-derived organoids (Figure 1). PSC-derived organoids are developed following the principles of embryonic development. When the factors maintaining PSC pluripotency are removed from the culture, the cells can spontaneously form 3D embryoid bodies (EBs) containing cells of the three germ layers. [21]. By precisely introducing specific growth factors and cytokines, these EBs can be directed to differentiate towards a particular germ layer or tissue type, ultimately forming organoids with specialized functions [22]. For example, Lancaster and colleagues induced human pluripotent stem cells (iPSCs) to self-organize into brain organoids with layered cortical structures, neural progenitors, and neurons by sequentially adding neural induction factors and self-organization signals in a scaffold-free suspension culture system. This successfully recapitulated the early stages of human brain development [23]. Similarly, Zhong and colleagues guided iPSCs to differentiate into retinal progenitor cells by carefully regulating retinoic acid and other differentiation signals. Using 3D suspension culture, they constructed retinal organoids with distinct outer nuclear and photoreceptor layers. The photoreceptor cells demonstrated light response capabilities, providing a highly representative model for studying degenerative retinal diseases [24]. Rossant and colleagues were the first to report the generation of lung organoids from iPSCs and further developed an air–liquid interface culture for their maturation. Through stepwise modulation of signalling pathways, they successfully directed PSCs to differentiate into functional airway epithelium [25]. In contrast, ASCs are key regulators of tissue homeostasis and have shown great potential in regenerative medicine, such as liver injury repair. Unlike PSC-derived organoids, ASC-derived organoids are constructed by mimicking the native tissue microenvironment. By recreating the conditions of the stem cell niche during

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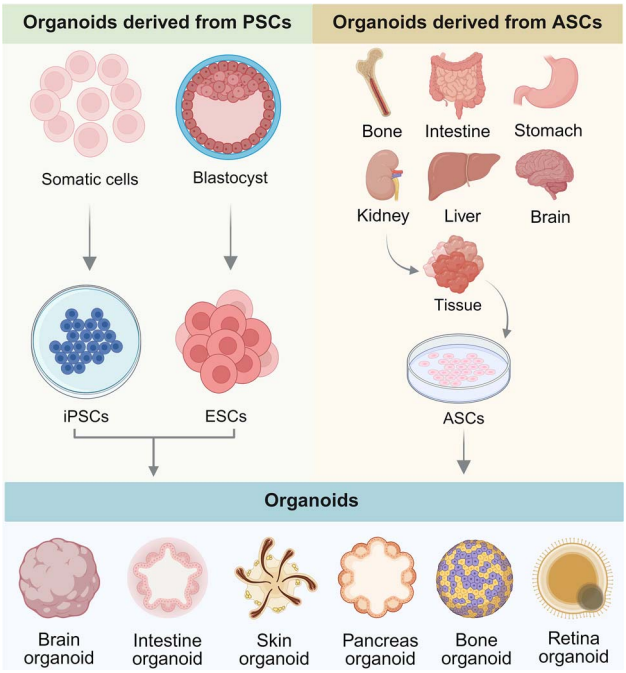


Figure 1 Organoids can be derived from the pluripotent stem cells of humans and animals, such as iPSCs and ESCs, or from ASCs. Created by BioRender. *iPSCs* induced human pluripotent stem cells, *ESCs* embryonic stem cells, *ASCs* adult stem cells

normal tissue renewal or injury repair, ASCs are driven to self-organize into 3D structures [26]. Although these organoids are composed primarily of epithelial cells and lack vascular or neural components, they maintain high levels of cellular heterogeneity and tissue-specific spatial architecture, making them ideal for studying normal physiology and disease mechanisms [27]. For instance, Manzano and colleagues described a method for generating intestinal organoids using adult stem cells. Epithelial cells were isolated from healthy or diseased small intestine and colon biopsy samples and cultured with basement membrane extract (BME) supplemented with specific growth factors such as Wnt3A and R-spondin1, creating a 3D intestinal organoid culture system [28]. To date, a wide variety of organoid models have been successfully established, including those representing the lung, liver, kidney, brain, pancreas, prostate, breast, bladder, ovary, and stomach. These 3D culture systems have demonstrated tremendous potential in disease modelling, biobanking, tumour initiation and progression research, drug testing, immunotherapy development, and regenerative medicine.

Structural and functional characteristics of organoids

Owing to their microscopic structural similarity to that of native tissues and organs, the cellular composition, spatial organization, and extracellular matrix (ECM) environment of organoids are highly similar to those of native tissues and organs [29]. In terms of cellular composition, different types of cells can be included in organoids, which are consistent with the cell types present in target organs. For example, Li *et al*. refined an efficient organoid culture protocol to obtain a controllable human small intestinal organoid model with dynamic control of stem cell self-renewal and differentiation [30]. When cultured in this protocol, organoids can consistently generate multiple types of intestinal cells with their *in vivo* counterparts, including stem cells, absorptive enterocytes, goblet cells, Paneth cells, and enteroendocrine cells. Not only were the proportions of different cells similar to those of the intestine *in vivo*, but the spatial distribution pattern and marker expression of different cells also reflected the ability of the organoids to correctly reproduce the diversity and lineage hierarchy of the target organ, the intestine. In terms of cellular organization, compared with real tissues, organoids can also self-organize into 3D structures with similar organization. For example, Mun *et al*. generated mature liver organoids from PSCs [31]. When cultured, organoids can consistently generate multiple types of liver cells, including hepatocytes, bile duct cells, and Kupffer cells, *in vivo*. These liver organoids exhibited a morphology that was almost indistinguishable from that of epithelial organoids generated from adult liver tissue, with tightly packed cell clusters and obvious polarity. Upon further culture, the molecular profile of liver organoids progressively converged towards that of native liver tissue. These organoids retained highly conserved functions characteristic of the mature liver, including serum protein production, drug metabolism and detoxification, active mitochondrial bioenergetics, and regenerative and inflammatory responses. This highly ordered arrangement is reflective not only of the functional compartmentalization of tissue but also of the intricate cell–cell interactions and polarity that emerge during development. With respect to ECM reconstruction, organoid culture methods have adopted 3D scaffold materials that can closely recapitulate the biochemical and physical properties of the native basement membrane. Various scaffolds, such as Matrigel and composite hydrogels formed from sodium alginate and gelatine, have been reported. Matrigel is a protein matrix consisting of laminin, type IV collagen, and heparan sulphate proteoglycans; these mimic the natural ECM components *in vivo* and provide important adhesion, migration, and polarization signals [32]. Shao *et al*. formed composite microspheres by fabricating Matrigel beads and then subjecting them to stepwise induction with factors such as SB431542 and CHIR99021 in SRM, leading to the formation of spinal cord organoids [33]. In this case, Matrigel promoted the attachment of stem cells, whereas the composite scaffold recreated the spinal cord microenvironment to enable the derivation of spinal cord organoids. Hendriks *et al*. reported that human foetal brain tissue can self-organize into foetal brain organoids (FeBOs) with complex 3D structures *in vitro* [34]. The FeBOs generated their own ECM-like matrix, maintaining tissue integrity while enabling long-term expansion and maintaining structure. Thus, these results demonstrate that organoids have a high degree of fidelity to primitive organs in terms of cell type, spatial organization, and ECM *in vitro* and *in vivo* physiology.

Organoids display the architecture of *in vivo* organs and have the functional properties of the original organ, including physiological and pathological functions [35]. Liver organoids (LOs) can recapitulate the liver microenvironment, such as drug metabolism and bile secretion. Zhu *et al*. isolated ASCs from healthy human livers, formed expandable bile duct organoids, and subsequently induced further differentiation into LOs [36]. The LOs formed lobule-like structures and exhibited the functions of mature hepatocytes, including albumin secretion, glycogen storage, urea cycle activity, and cytochrome P450-mediated drug metabolism. Intestinal organoids replicate the absorptive and secretory functions of the small intestine. In these organoids, absorptive enterocytes take up nutrients, while goblet

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cells secrete mucus to maintain the intestinal barrier. Xu *et al*. integrated human primary macrophages into intestinal organoids, creating macrophage-augmented organoids (MaugOs) [37]. These MaugOs contained typical intestinal epithelial cell types, such as Lgr5+ stem cells, goblet cells, and enteroendocrine cells. They also recreated the dynamic interactions between immune cells and epithelial cells found in the intestinal mucosa. This includes macrophage chemotaxis and migration, pathogen recognition, and the secretion of antimicrobial peptides, providing a more complete model of intestinal immune responses. Pancreatic organoids are also capable of simulating the endocrine functions of the pancreas, such as insulin secretion. Andersson-Rolf *et al*. developed a novel 3D suspension culture system to isolate and expand pancreatic stem cells with multilineage potential from human foetal pancreatic tissue, generating functional pancreatic organoids [38]. Through stepwise regulation of signalling pathways, these organoids further differentiated into β cells, α cells, acinar cells, and ductal cells. The mature insulin expression in β cells, glucagon secretion from α cells, which plays a role in counterregulating glucose, secretion of digestive proteinases such as trypsin from acinar cells, and branched network-like structures formed by ductal cells secreting pancreatic fluid were detected. From a pathological viewpoint, tumour organoids possess the genetic, phenotypic, and behavioural features of primary tumours, including mutations, proliferation, drug responses, and invasiveness. For example, Kim *et al*. constructed lung cancer organoids by extracting epithelial cells from lung cancer patient samples and culturing them in Matrigel-based 3D culture systems with growth factors such as EGF, FGF, and R-spondin [39]. These organoids maintained the architecture and cellular heterogeneity of the original tumours, expressed high levels of epithelial–mesenchymal transition (EMT) markers, and generated invasive pseudopodia, modelling local tumour invasion. Similarly, Broutier *et al*. generated liver cancer organoids from primary hepatocellular carcinoma (HCC) patient tissue [40]. These organoids expressed high levels of HCC-associated genes, such as AFP and CK19, and components of the upstream signalling pathways, including Wnt/β-catenin and PI3K/Akt, which are critically involved in HCC initiation and progression. In summary, these examples illustrate that organoids can recapitulate not only the morphology of native tissues but also their tissue-specific physiological and pathological functions, making them flexible and versatile tools for both basic and clinical studies.

Comparison of organoids with other research models

Tissues and organs are extremely complex 3D structures, but current investigations into their development, functions, and pathological processes are largely conducted in 2D cell culture dishes or animal models [41]. This may be referred to as a '2D research approach' in contrast to the actual 3D spatial organization of living bodies. Conventional 2D cell culture dishes allow the culture of cells in a single monolayer on a flat surface. Because of the lack of 3D organization, 2D culture models cannot recapitulate the spatial organization of cells in living tissues or their interactions with the microenvironment [42, 43], which may be major limitations for cell behaviours. Furthermore, the culture conditions in 2D models are relatively static and lack signalling and metabolic interactions among different cell types, limiting the application of these models in multicellular interaction and tissue-level function studies [44, 45]. In comparison with 2D culture models, animal models may better mimic the dynamic physiological and pathological processes of human biological systems. The advantages of animal models do not lie in recapitulating pathological phenotypes in limited local regions but in simulating complicated biological processes, including systemic pathological responses and interactions among multiple organs. Experimental animal models are usually expensive and time-consuming, and the results may be limited by species differences [46–48].

As a newly developed 3D culture system, organoids can recapitulate the structure and function of various human organs *in vitro*, especially the physiological and pathological environment [25, 49, 50]. Organoids can maintain the 3D structure of tissues, mimic the microenvironment with multiple cell types, and present physiological and pathological phenotypes similar to those of the human body [51]. For instance, Akbari *et al*. obtained liver organoids by using endoderm-derived progenitor cells, ECM scaffolds, and stage-specific growth factors [52]. The organoids self-organized into a microphysiological system with bile duct networks and a lobule-like structure and correctly exhibited the liver functions of albumin secretion, bile production, and drug metabolism [53]. Organoids can also act as realistic disease models [54–56]. Mao *et al*. developed colorectal cancer organoids by isolating epithelial cells from patient tumour tissues using mechanical and enzymatic digestion. The cells were embedded in a 3D Matrigel scaffold and cultured with essential factors such as EGF and Wnt pathway activators [57]. These cancer organoids self-organized into glandular-like structures and retained the histological characteristics and driver gene mutations of the original tumours. This provides a high-fidelity pathological model for drug screening. The efficiency and translational potential of organoids promote their application in drug screening [58]. Organoids provide a 3D microenvironment closer to the human body than animal models do. They also enable high-throughput testing in drug screening, which shortens the experimental cycle [59, 60]. Therefore, the use of organoids is seen as a promising tool for future disease research and personalized medicine.

New concepts in organoid research

Organoid research under the concept of precision medicine

Precision medicine (PM) is a new medical concept and health care model based on an individual's genome, environment, and lifestyle [61]. Advancements in genome sequencing, combined with the integration of bioinformatics and big data, have accelerated the development of this field. In PM, a patient's genetic information, medical history, and other physical characteristics are evaluated to create a treatment plan tailored to that patient (whereas traditional medicine involves a 'one-size-fits-all' approach) [62]. This facilitates the development of individualized treatment plans to maximize the efficacy of a drug and reduce potential side effects.

Drug sensitivity testing (DST) is an essential part of PM. PM enables personalized drug selection on the basis of the genetic information of the patient and tumour [63]. Organoids have become a dynamic research tool for personalized medicine, as they can replicate the pathological features and molecular heterogeneity of primary tissues. By performing DST with patient-derived organoids, treatment success rates can be improved, and the risk of side effects can be reduced. Organoid models for DST

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have also been established *in vitro* and used to study different solid cancers, such as breast cancer [64], gastric cancer [65], and colorectal cancer [66]. For instance, Broutier *et al*. established liver cancer organoids derived from patient tumour tissues and accurately recapitulated the tumour microenvironment, which is composed of cancer cells and stromal components [40]. An automated, high-throughput drug screening platform was constructed to evaluate the activity of chemotherapy agents and molecularly targeted drugs *in vitro. In vitro*, this study revealed differences in drug sensitivity among tumour subgroups with different genetic backgrounds or abnormal signalling pathways. By comparing these results with clinical treatment responses in patients, experimental guidance for individualized therapeutic strategies was provided on the basis of the experimental results. Similarly, Zhao *et al*. established 57 patient-derived gastric cancer organoids from tumour samples of 73 gastric cancer patients [67]. These organoids displayed the histological phenotype of the original tumour and different responses to various chemotherapeutic drugs. RNA sequencing analysis demonstrated that tumour-suppressive genes and pathways were upregulated in organoids sensitive to 5-fluorouracil (5-FU) or oxaliplatin. Drug-resistant organoids showed enrichment of genes and pathways related to proliferation and invasion. On the basis of these findings, they were able to predict which patients were likely to respond to 5-FU and oxaliplatin and which were resistant [67]. These studies demonstrate the value of patient-derived organoid models as practical tools for investigating drug sensitivity and resistance mechanisms.

Integrating the concepts of organ-on-a-chip and organoids

Organs-on-a-chip (OoCs), based on microfluidic technology, are advancing the field of biomedical and chemical research [68]. By combining microfluidic engineering and cell biology, OoC systems can reproduce the physiological and pathological microenvironments of target organs *ex vivo* [69]. The models display typical features of local diseases at a specific site but also permit close regulation of the environmental parameters that affect cell behaviour, thus offering a cost-effective and high-throughput biological platform [70]. In 2010, Huh *et al*. produced the first 'breathing lung' model by using microfluidic technology and successfully recapitulated the structure and function of the human lung [71]. This work has paved the way for the rapid development of OoC technology. Advances in OoC technology have enabled us to reconstruct the basic physiological functions of human organs *in vitro*. Ho *et al*. applied enhanced-field dielectrophoresis (DEP) to collect and assemble parenchymal and nonparenchymal liver cells in a circular microfluidic chip. Rapid spatially controlled coaggregation of heterogeneous cell populations was achieved to recapitulate the microtissue heterogeneity of liver lobule structures [72]. Jang *et al*. developed a dual-layer OoC device with fluid shear stress incorporated into the design to mimic the architecture of the proximal tubule of the kidney. The chip was formed by stacking upper and lower microchannels with a porous membrane in between; primary human renal tubular epithelial cells were cultured in the two microchannels to form a polarized and functional monolayer barrier [73]. Ren *et al*. developed a capillary endothelial barrier formed by two parallel microcolumn arrays and investigated the dynamics of hypoxia-induced cardiac injury under well-controlled microenvironmental conditions [74]. This chip effectively mimics the architecture and function of myocardial tissue. OoC technology mimics the physiology and pathology of physiological functions in multiple organs using a microfluidic-based biomimetic design.

Blending organoids and OoC technology has resulted in the emergence of 'organoid-on-a-chip' systems that maintain the biological advantages of organoids while leveraging the controlled environment offered by the microfluidic system [75]. Achberger *et al*. combined stem cell-derived retinal organoids (ROs) and a multilayer microfluidic chip to construct a biomimetic human retinal model [76]. This system simulated the complex neural signal transmission pathways of the retina. It also replicated functional features such as the blood–retinal barrier and pathological conditions, including hypoxia-induced vascular leakage and photoreceptor apoptosis. This study has advanced the understanding of the pathogenesis of degenerative eye diseases. Zhang *et al*. cultured human iPSC-derived cardiac organoids within a microfluidic chip to investigate the cardiotoxic effects of polystyrene nanoplastics (PS-NPs) [77]. OoC technology uses microfluidics to replicate the *in vivo* microenvironment. When integrated with organoids, it creates a biomimetic and dynamically controllable 'microphysiological system' [78]. This combined platform offers advantages for accurately modelling complex physiological and pathological processes, analysing cell–cell interactions, and studying substance transport.

Construction and application concepts of organoid biobanks

For many years, tumour biobanks have been platforms for storing large numbers of high-quality, well-annotated human cancer samples. These biobanks provide researchers with valuable resources for studying cancer biology [79]. With the advancement of 3D culture technologies, living organoid biobanks have emerged, now encompassing a wide range of solid tumour types and serving as important complements to traditional tumour biobanks [80]. The investigators employ a standardized organoid culture system that allows each sample to maintain its unique histological and biological properties, enabling high-throughput screening (HTS) for drug sensitivity and resistance in different sarcoma types (Figure 2a) [81]. These organoid biobanks systematically preserve and manage precious biological samples from diverse sources and tissue types, especially patient-derived tumour tissues, rare disease samples, and normal tissues, offering long-term and stable material support for future research and clinical applications [82]. More interestingly, as 3D culture systems that highly resemble the structure and function of organs *in vivo*, organoids can faithfully reproduce tissue heterogeneity, physiological function, and pathological phenotype [83]. Therefore, they are ideal models for disease mechanism research, drug screening, and toxicity analysis and can help promote the development of precision medicine and personalized therapy. For example, Krijn *et al*. constructed and applied a tumour organoid biobank derived from patients with nonsmall cell lung cancer (NSCLC) to elucidate immune escape mechanisms at the tumour subclonal level. On the basis of this organoid biobank, this study revealed the differential immune escape strategies of intratumor subclones in antigen presentation and the regulation of immune-related pathways (Figure 2b) [84]. Tiriac *et al*. constructed a patient-derived pancreatic cancer organoid model. First, tumour tissues were obtained from patients

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through surgical resection or biopsy. Then, the tumour cells were digested by enzymes and dissociated into single cells. After that, the organoids were constructed and cultured. The organoids could preserve the original tumour heterogeneity and biological function. In addition, the organoids could be cryopreserved for later use. Therefore, a heterogeneous and durable pancreatic cancer organoid biobank was constructed [85]. Using this biobank, one can test the response of tumours from different patients to chemotherapy drugs and determine the difference in drug sensitivity for pancreatic cancer, further providing a basis for personalized treatment.

Setting up an organoid biobank involves the following critical steps: collection and sample processing, such that the tissues are fresh, noncontaminated, and rapidly subjected to the first steps of dissociation to preserve viability [86]. The cells are isolated and cultured under specific 3D culture conditions to obtain organoids from different tissue sources [87, 88]. Once established, the organoids are subjected to a standardized characterization step, consisting of phenotypic analysis, molecular profiling, and functional assays to validate their stability [89]. Finally, the organoids are frozen using a cryopreservation protocol and a cryoprotective agent to guarantee the viability and recovery of the organoids upon thawing [90]. Helen *et al*. constructed an organoid biobank derived from human gastric cancer patient samples. These organoids maintain morphological, genomic, and expression characteristics that are highly consistent with those of the original tumour over long-term culture, enabling them to serve as high-fidelity *in vitro* models. This organoid biobank provides resources for the study of molecular mechanisms and tumour heterogeneity analysis in gastric cancer (Figure 2c) [65]. Abdullah *et al*. developed organoid models from PDTO samples of low-grade gliomas [91]. These organoids not only provided a more accurate representation of each tumour's biological characteristics but also became part of a larger organoid biobank. Vlachogiannis *et al*. collected 110 fresh tissue samples from 71 patients and established an organoid biobank derived from metastatic, posttreatment colorectal cancer, and gastroesophageal cancer samples using a 3D culture system [92]. Organoid models generated from PDTO tissues reflect the biological features of tumours, facilitating mechanistic cancer research and the evaluation of therapeutic strategies [93, 94]. With the continuous advancement of technology, the application of organoid biobanks will continue to expand [95].

As *in vitro* high-fidelity biobanks, organoids are widely used to explore the mechanisms of many diseases, especially cancers, genetic diseases, and infectious diseases [96]. These organoids provide a platform for investigating biological processes, such as pathological progression and signalling pathway abnormalities [97]. Moreover, because of the ability to perform high-throughput culture and analysis, organoids are indispensable for drug screening, toxicity and mechanism of toxicity analysis, and resistance mechanism analysis, which promote the efficiency and accuracy of early drug development [98]. Some researchers have established a colorectal cancer tumour organoid biobank, which highly reproduces the characteristics of the original tumour and normal tissue on the basis of the morphological, histological, and genetic variation profiles and covers the main colorectal cancer (CRC) molecular subtypes, resulting in good *in situ* representation. The establishment of this biobank has promoted the study of tumour molecular mechanisms and large-scale drug screening (Figure 2d) [66]. Ebisudani *et al*. established a patient-derived lung cancer organoid (PDLO) biobank and analysed the dual value of these models in drug screening and pathological mechanism research [99]. By using PDLOs, the study team further performed genotype–phenotype correlation analysis. They reported that the transcription factor NKX2-1 promotes tumour malignancy by activating the Wnt/β-catenin signalling pathway. Additionally, they further screened compounds targeting the NKX2-1–Wnt axis from a biobank, revealing a new mechanism of drug resistance in lung adenocarcinoma. These findings provide evidence for the optimization of precision treatment strategies. Yu *et al*. used an organoid biobank containing ARID1A-deficient samples to analyse changes in the expression of human endogenous retrovirus H (HERVH) [100]. They reported that HERVH, which is associated with pluripotency, was abnormally activated in CRC samples. Inhibiting the expression of HERVH in CRC cells and patient-derived organoids inhibited tumour growth. These results further demonstrated the application value of PDTO biobanks in the exploration of tumorigenesis and development mechanisms. Through the construction of PDTO biobanks, researchers can discover new anticancer drugs and minimize the risk of clinical trial failure. This method can save time and cost in drug development and holds broad potential and profound biomedical significance for advancing personalized medicine and therapeutic innovation [101, 102].

Regenerative medicine and organoid-based repair concepts

Organoids derived from stem cells or organ-specific progenitor cells are self-organizing, self-renewing, multicellular 3D structures capable of mimicking the structure and function of the tissues from which they originate [103]. Owing to these characteristics, the use of organoids has great potential in regenerative medicine [104]. Organoids can not only reproduce the hierarchical structure and functions of human tissues but also exhibit strong proliferation, differentiation, and self-repair abilities. They are suitable as seed cells for repairing and regenerating damaged tissues and organs [105, 106]. At present, research on the repair of tissues is concentrated on how to transplant organoids and how they can be integrated with host tissues [107]. Compared with 2D cultures, organoids have a certain 3D structure, which is more similar to natural human tissue, and it is possible to create a physiological environment to culture organoids [108]. Researchers have made efforts to assess the feasibility of organoid transplantation therapy [109]. Watson *et al*. used human pluripotent stem cells (hPSCs) to produce human intestinal organoids (HIOs) *in vivo* [110]. HIOs can recapitulate the functions of absorption and mucus secretion in the human intestine. When HIOs were transplanted into host mice, the transplanted organoids could expand and mature. Their study provided new ways to test therapies for intestinal diseases and organ transplantation. McLelland *et al*. used retinal organoid sheets derived from human embryonic stem cells (hESCs) to treat retinal degenerative diseases [111]. They transplanted retinal organoid sheets into a rat model of retinal degeneration. The results showed that the transplanted organoid sheets could differentiate into retinal cells and integrate with the host retina. Transplantation improved visual function in rats, which demonstrated the feasibility of using organoids to regenerate damaged retinas. Wang *et al*. transplanted brain organoids derived from hPSCs into the infarction core and

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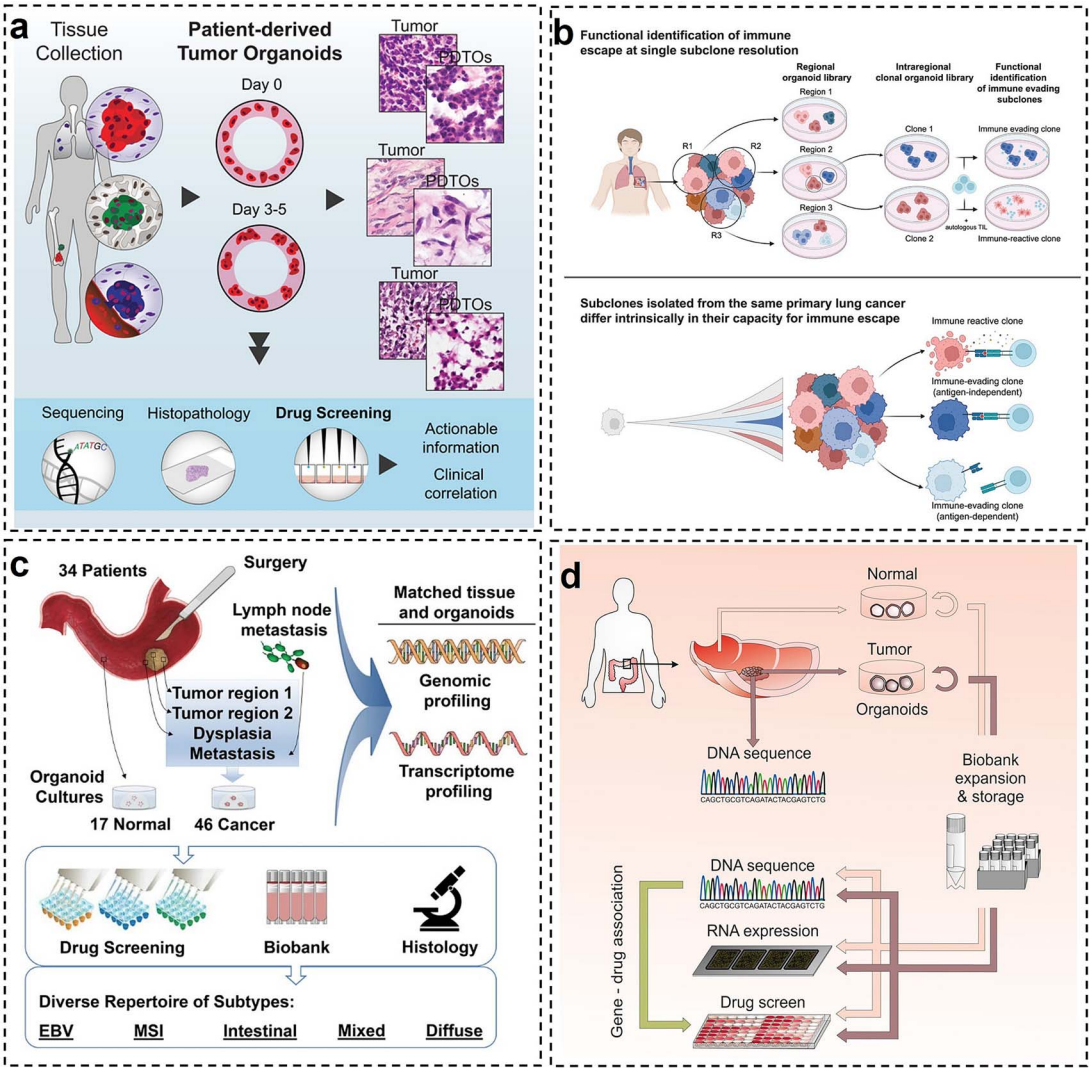


Figure 2 Construction and application of different organoid biobanks. (**a**) A PDTO biobank was constructed to characterize the drug resistance and sensitivity of sarcoma using PDTOs, which can be used for drug screening and disease mechanism research. Adapted from [81] with permission. Copyright © 2024, Cell Stem Cell. (**b**) Regional and organoid biobank analyses identified differences in immune escape mechanisms among tumour subclones, revealing both antigen-dependent and antigen-independent immune evasion pathways. Adapted from [84] with permission. Copyright © 2025, Cancer Cell. (**c**) A live organoid biobank derived from primary colorectal cancer and matched liver metastases captured molecular fingerprint heterogeneity both within and between patients, supporting personalized treatment and biobank expansion. Adapted from [65] with permission. Copyright © 2018, Cell Stem Cell. (**d**) Colorectal cancer tumour organoids were established to replicate the subtypes and genomic changes of primary tumours and are suitable for high-throughput drug screening and gene–drug association testing. Adapted from [66] with permission. Copyright © 2015, Cell. *PDTOs* patient-derived tumour organoids

peri-infarction area of mice after stroke [112]. The transplanted organoids survived well inside the damaged area and then differentiated into mature neurons and extended long axons to form synaptic connections with the host neural circuitry. Finally, the damaged tissue was repaired, and the sensory and motor functions of the mice improved. These studies have demonstrated that through transplantation and integration, organoids provide new ways to repair the intestine, retina, and brain, opening new directions to develop therapies for diseases affecting these organs and to advance organ transplantation strategies.

Given the extreme scarcity of donor organs available for transplantation, organoid technology offers an important complementary research strategy for regenerative medicine by generating functional tissues or organs *in vitro* [113]. Wang *et al*. combined digital light processing (DLP) bioprinting technology with a new hybrid bioink made from GelMA/AlgMA/HAP. They developed a large-scale self-mineralizing bone organoid scaffold. This scaffold supports the long-term culture and progressive maturation of bone organoids, promotes multicellular differentiation, and mimics the properties of natural bone through its inherent self-mineralization, significantly enhancing bone repair both *in vitro* and *in vivo* (Figure 3a) [114]. Shen *et al*. performed cartilage repair based on silk fibroin–DNA hydrogel-based cartilage organoid precursors (COPs). COPs mimic the structure and function of natural cartilage tissue, and after implantation into a cartilage defect model, the COPs could significantly promote cartilage regeneration and effectively repair the defect area, showing a strong repair ability (Figure 3b) [115]. On the basis of COPs,

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Shen *et al*. created a DNA–silk fibroin hydrogel sustained release system in which mature cartilage organoids were cultivated for 4 weeks. By activating MAPK signalling, they successfully regenerated rat joint cartilage defects for 8 weeks (Figure 3c) [116]. Zhu *et al*. constructed woven bone organoids (WBOs) that are highly mineralized and osteogenic. After WBOs were implanted into bone defect models, they significantly promoted the rapid regeneration and repair of bone tissue, especially in the defect area, achieving efficient bone regeneration, demonstrating the strong potential of braided bone organoids in bone repair and providing an effective tissue engineering strategy for bone defect repair (Figure 3d) [117]. Organoids can be expanded outside the body. Once transplanted, they play a role in the tissue repair process, and in some cases, this method can even be substituted for organ transplantation [118]. There are still many issues that need to be resolved before organoids can be widely used in clinical practice. First, it is still technically challenging to produce organoids on a large scale to meet clinical demand [119]. The cultivation of these organoids requires highly specific conditions and controlled cell sources, and maintaining their structural and functional integrity over long periods is especially challenging [120]. Second, it needs to be verified that the long-term survival of transplanted organoids and their integration with host tissues are possible and that they can perform true biological functions after transplantation [121]. Finally, it is still quite challenging to recreate a microenvironment during organoid development that is similar to that of human tissues [122]. In summary, organoids are effective tools in regenerative medicine with immense potential in tissue repair and organ regeneration.

New technologies in organoid research

Innovations in organoid culture techniques

Culture media and signalling molecules In comparison with traditional 3D culture systems, organoids generated from PSCs or ASCs can yield highly organized 3D structures with high cellular heterogeneity [123]. To improve the efficiency of organoid formation and enhance the function of organoids, researchers have made comprehensive improvements to culture medium components and screening of target growth factors and small molecules [124, 125]. The right medium can provide the organoids with the necessary nutrients and signalling support to promote their growth and development. Growth factors promote the formation and maturation of organoids by stimulating cell proliferation and differentiation, target growth factors can induce the generation of specific types of cells, and small molecules guide cells into specific differentiation processes by regulating cell fate determinants (Figure 4). For example, Lee *et al*. fabricated a 3D culture method to generate human spinal cord organoids (hSCOs) that could reproduce the morphogenetic process of the human embryonic neural tube [126]. By finely optimizing the culture protocol and sequentially adding the growth factors CHIR99021 and SB431542, hSCOs were able to recapitulate the typical morphological processes of human spinal cord development. These processes include neural plate formation, neural tube closure, neuron and glial differentiation, and the formation of functional neural circuits. Volmert *et al*. combined a 3D suspension culture system and temporal regulation of the morphogenetic pathway to induce cell self-organization and differentiation using bone morphogenetic protein (BMP), Wnt, and retinoic acid signals without any external scaffolds [127]. Through this technique, cells can self-organize to construct patterned human primitive heart organoids with ventricular structures, directed conductance, and spontaneous contractile activity. These organoids can reconstruct the complex structural morphogenesis and basic functions of the early human heart in an *in vitro* modelling system, providing a powerful tool for cardiac developmental research, disease modelling, and drug screening. By finely regulating culture conditions and tightly controlling signalling pathways, organoid technology has made significant progress in reproducing human developmental processes and tissue structures [128].

Advanced scaffold materials With the development of research on organoids, the 3D culture systems used as the basic support framework of organoids have gradually tended to become more efficient and more biomimetic [129, 130]. As the mainstream culture material used in current organoid research, Matrigel has a wide range of properties [83]. In the study of the translation of stem cell biology into *in vitro* models of organogenesis, Matrigel matrix gels promoted the efficient generation of animal-derived ear sacs by promoting efficient generation with low heterogeneity and modulating the microenvironment of organoid culture [131]. These cystic organoids exhibit high sensitivity in disease and regenerative medicine research, with organoid formation efficiency reaching 90%. According to the latest proteomic and bioinformatic studies of pancreatic organoids, matrix gels differ significantly from real organoid matrices *in vivo* in terms of physical properties, such as mechanical properties and molecular physiology during *in vitro* culture, and exhibit disadvantages such as poor batch-to-batch reproducibility, which make their application challenging [132]. Compared with that of Matrigel derived from Engelbreth–Holm–Swarm mouse sarcoma, the widespread application of other matrix gels is severely limited because of their heterogeneity, high batch variability, and high production costs. Owing to their unique 3D network structure and easily modulated properties, hydrogels have been recognized as ideal candidates for replacing Matrigel in organoid culture. New types of 3D culture materials, including natural/synthetic hydrogels and functionalized bioscaffolds, can offer mechanical support and biochemical information to cells similar to the *in vivo* environment, which can further guide the spatial assembly, self-arrangement, and functional maturation of cells [133]. For example, Khan *et al*. designed a tuneable hybrid hydrogel system to improve 3D culture materials for bone marrow organoids [134]. Based on traditional hydrogels containing high amounts of human type I and IV collagen, they introduced both natural polymers, such as polyethylene glycol (PEG), and synthetic polymers, such as hyaluronic acid (HA), and used transglutaminase (TG) to realize the crosslinking process. The physical strength and biocompatibility of the hydrogels greatly improved as did the *in vivo* microenvironment. The improved hydrogel enhanced the formation of vascular-like structures and multilineage cells in the bone marrow organoids. The improved bone marrow organoids more closely simulated the human bone marrow microenvironment and provided a more stable and reliable *in vitro* platform for disease modelling and target discovery. The improved hydrogel also greatly enhanced the spatial assembly of bone marrow cells and promoted the formation of vascular-like structures in the bone marrow organoids.

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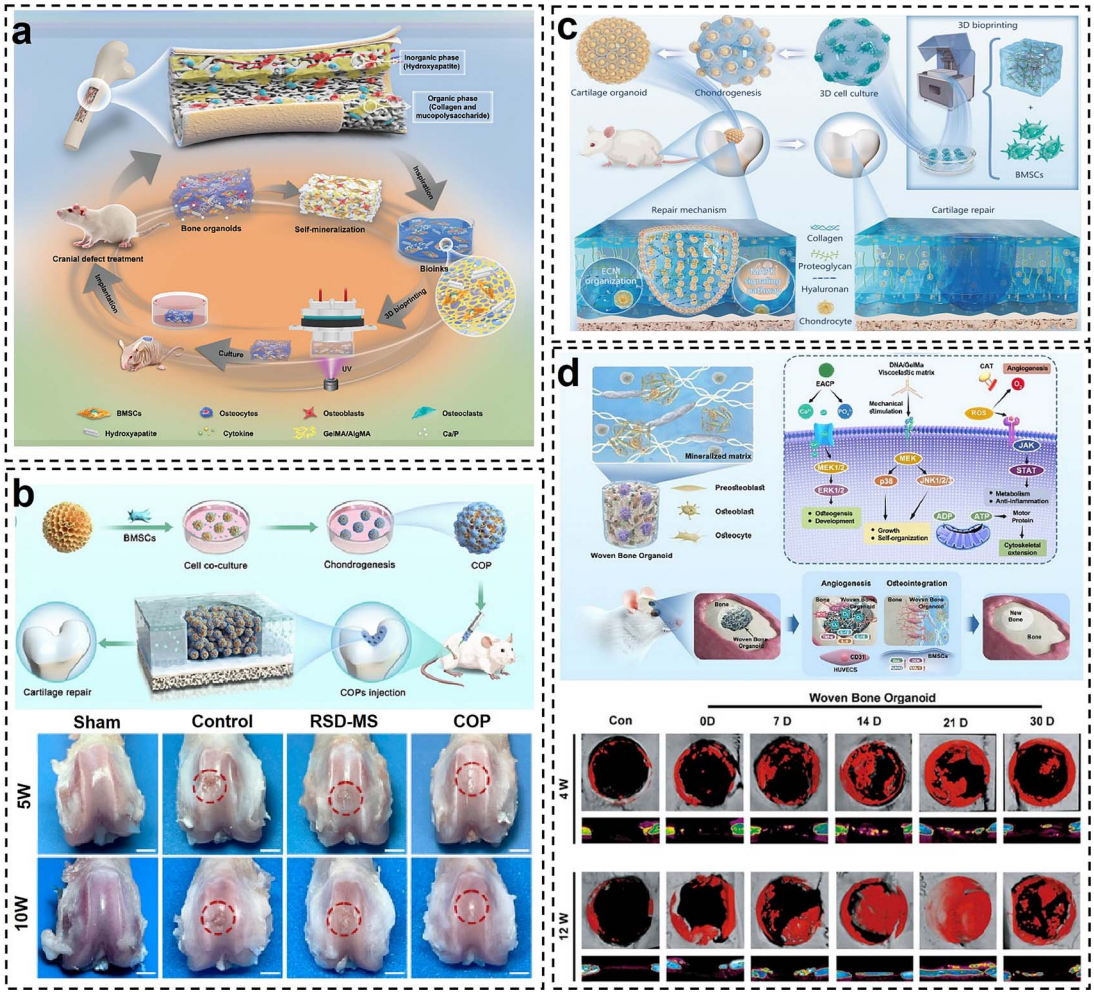


Figure 3 Applications of organoids in regenerative medicine. (**a**) Bone organoids with self-mineralizing ability and cellular differentiation ability can repair bone tissue both *in vitro* and *in vivo*. Adapted from [114] with permission. Copyright © 2024, Advanced Materials. (**b**) RGD–silk fibroin–DNA hydrogel microspheres (RSD-MSs) were prepared on the basis of a microfluidic system integrated with photopolymerization and self-assembly processes to repair and regenerate cartilage. Adapted from [115] with permission. Copyright © 2024, Bioactive Materials. (**c**) A DNA-silk fibroin hydrogel sustained release system was used to culture mature cartilage organoids. The rat joint cartilage defect was efficiently repaired. Adapted from [116] with permission. Copyright © 2025, Military Medical Research. (**d**) A versatile bone-mimicking matrix was prepared to construct woven bone organoids with structural features and functional activity, providing a new idea for bone tissue engineering and regenerative medicine. Adapted from [117] with permission. Copyright © 2025, Advanced Materials

Zhu *et al*. designed a catalase-conjugated GelMA/DNA/EACP (CGDE) hydrogel to improve the 3D culture materials for bone organoids [117]. CGDE was constructed by hydrogen bonding interactions between DNA and gelatine methacryloyl (GelMA), and then, GelMA network crosslinking resulted in the formation of a dual-network structure with appropriate mechanical strength and enhanced viscoelasticity. During the 21-day 3D culture process, CGDE further promoted the migration and self-assembly of cells, ultimately leading to the formation of WBOs through intramembranous ossification. The WBOs exhibited spatiotemporal organization that supported dynamic mineralization and tissue remodelling. This innovative design not only optimizes the biophysical properties of bone organoids but also provides a new platform for more accurate simulation of the dynamics of bone tissue. These findings provide a solid basis for further research on the mechanisms of bone diseases and the development of new targeted therapeutic strategies.

Microfluidic and bioreactor systems In addition to advanced culture materials, microfluidic chip systems are also used to strictly control culture medium flow, establish a stable gradient, and even maintain a stable microenvironment. A microfluidic chip system is a 3D-engineered microsystem designed to reproduce the functional units of an organ *in vitro* for cell culture. The system typically consists of individually contactable, perfused, (sub)millimetre-scale chambers that can integrate and culture different types of cells and dynamically regulate the culture environment [135]. Unlike traditional 2D monolayer culture-based chip platforms, microfluidic chip platforms are used to culture 3D tissue structures such as cell aggregates, spheroids, and organoids [136]. These 3D tissues can be integrated into microfluidic chips in a variety of ways to facilitate the culture and growth of organoids. In addition, microfluidic platforms allow for the coculture of different types of organoids, capturing complex interactions and communications between organs in the body. By applying

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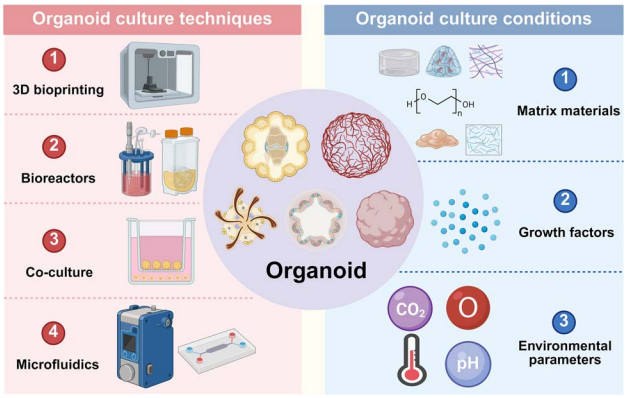


Figure 4 Organoid culture methods and culture conditions organoid culture methods include microfluidic technology, cell coculture, bioreactor technology, and three-dimensional printing technology. Organoid culture conditions include optimizing matrix materials, adding appropriate growth factors, and controlling culture conditions. Created using BioRender

geometric constraints and spatial cues [137], the microfluidic platform can not only control the morphology of organoids but also simulate the dynamic transport of nutrients and signalling molecules *in vivo*, thereby further improving the controllability of the culture process. Similarly, rotating bioreactors could also improve nutrient and oxygen exchange through continuous stirring and a low shear environment and further improve the uniform growth and volumetric expansion of organoids [138]. Gabbin *et al*. also dynamically cultured cardiac microtissues and kidney organoids on a microfluidic chip and reported that these two types of organoids maintained their structure and function and that a microfluidic chip could be used to establish a combined heart–kidney model [139]. This platform allows investigation of the heart–kidney axis, promoting research on heart- and kidney-related diseases, and offers a controlled environment to assess drug-induced cardiorenal toxicity and promote the discovery of safer and more efficacious drugs. Qian *et al*. obtained forebrain-specific organoids from hPSCs using a miniaturized spinning bioreactor (SpinΩ) and applied them to study Zika virus (ZIKV) infection [140]. In combination with the SpinΩ system, region-specific brain organoids were used to establish a platform to model human brain development and disease and to screen drugs. This platform has facilitated the search for antiviral compounds against the ZIKV. Sustained advances in culture materials and devices have provided solid support for further enhancing the *in vitro* structural complexity, differentiation efficiency, and physiological functions of organoids.

Coculture strategies Coculture strategies for organoids are emerging quickly. By coculturing organoids with other cell types, researchers can better mimic cell–cell interactions and the microenvironment *in vivo* [141]. Schuth *et al*. successfully cocultured patient-derived PDAC organoids with cancer-associated fibroblasts (CAFs) in a 3D culture system [142]. This system recapitulated tumour cell–stromal cell interactions and the tumour microenvironment. The coculture system involving tumour and stromal cells, i.e. organoids and fibroblasts, enhanced tumour cell proliferation and chemotherapeutic drug resistance, mimicking chemoresistance in the tumour microenvironment. Jiang *et al*. constructed a coculture model with macrophages and pancreatic cancer organoids to investigate treatment for macrophage-associated gemcitabine resistance (GR) in pancreatic cancer [143]. Human pancreatic cancer organoids were cocultured with macrophages. This system mimicked cell–cell interactions in the tumour microenvironment. Pancreatic cancer organoids cocultured with macrophages formed a more dynamic 3D system, which enabled a closer examination of the interactions between tumour cells and immune cells. This more physiologically relevant *in vitro* system could be used to investigate drug resistance and potential treatment strategies for resistant tumour microenvironments. In summary, coculture technology can be used not only to investigate cellular signalling and interactions but also to construct more complex organoid models. With the continuous development of coculture technology, we believe that organoid research will be more widely used in future medical research and precision medicine.

Organoid gene editing and modification techniques Gene editing technology uses CRISPR/Cas9 technology to genetically modify organoids, which significantly improves the application value of these organoids in regeneration and disease modelling [144]. Organoids are 3D culture materials obtained *in vitro* and can simulate human organs to a high degree. They provide highly biomimetic experimental models for exploring the functions and mechanisms of certain genes in the morphogenesis of tissues, diseases, and drug effects [145]. As early as 2006, Avansino *et al*. transplanted intestinal mucosal stem cells isolated from neonatal transgenic mice expressing green fluorescent protein (GFP) into the intestine of adult GFP-expressing mice to obtain transgenic intestinal mucosal organoids [146]. This study laid the foundation for the construction and functional study of organoids, demonstrating the traceability of organoids through genetic markers. On this basis, the emergence of CRISPR/Cas9 technology has further promoted the development of organoid research. CRISPR/Cas9 technology uses RNA guidance to induce accurate double-strand breaks in the DNA sequence, thereby achieving the knockout of target genes [147]. If a homologous template is added, the system can also introduce foreign genes or point mutations at a specific site through homologous recombination to achieve gene knock-in or precise gene modification at a specific site. By using this technology, researchers can choose target genes in organoids for editing and explore their functions in processes such as cell differentiation, proliferation, tissue morphogenesis, and disease evolution. Hendriks *et al*. established culture conditions for the long-term expansion of human foetal liver cells and used CRISPR/Cas9 to knock out target genes, including TP53, PTEN, and NF1 genes, in liver organoids [148]. The findings demonstrated the feasibility and potential applications of using organoids as models to investigate human liver diseases. In colorectal cancer research, Roper *et al*. introduced CRISPR/Cas9-mediated mutational events targeting driver genes such as APC, TP53, and KRAS directly into mouse colon epithelial cells, resulting in tumours *in situ* [149]. They then grafted genetically edited intestinal organoids into immunodeficient mice and observed tumour emergence and progression over time. This research recapitulated the multistage process of CRC development, ranging from initial mutational events to invasion and metastasis. CRISPR/Cas9 clearly plays a key role in modelling tumour initiation and progression. The construction of specific mutation models, exploration of disease phenotypes, and dynamic mapping of drug action pathways

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using organoids in combination with CRISPR/Cas9 technology have become powerful approaches for studying human diseases, precision medicine, and tissue regeneration [150].

In addition to CRISPR/Cas9, other genetic modification approaches, including gene transduction and transposon systems, can also be used to generate organoid models with specific phenotypes or functions [105]. These precise genetic approaches enable organoids to mimic real pathological phenotypes. In addition, organoids could also serve as reliable *in vitro* models for drug development.

Imaging and analysis techniques for organoids Imaging approaches are used in the analysis of both the complex structure and the dynamic processes of organoids [151]. To observe and image the self-organization, morphogenesis, and differentiation of organoids, different imaging methods have been widely used in organoid research. These methods range from basic bright-field (BF) imaging and routine confocal microscopy to advanced light-sheet fluorescence microscopy (LSFM) and high-content imaging systems. These imaging methods can provide more information for understanding cellular behaviours and signalling networks in organoids. BF microscopy supports real-time imaging but lacks 3D spatial resolution and sensitivity to tissue/cell function. Electron microscopy has the ability to analyse chemical and elemental data and supports real-time dynamic imaging, but its imaging requires complex samples, does not allow visual visibility inside the sample, and does not allow for functional imaging (Figure 5a) [152]. In contrast, confocal microscopy uses point-by-point scanning and optical sectioning to obtain high-contrast 3D images of organoid tissue structure, and observing cell arrangement, hierarchical structure, and spatial distribution is feasible (Figure 5b) [153]. Compared with confocal microscopy, conventional optical microscopy has many disadvantages, such as shallow penetration, photobleaching, and phototoxicity [152]. However, super-resolution microscopy can overcome the diffraction limit of conventional optical microscopy and directly observe subtle changes in subcellular structures, such as the cytoskeleton, microtubules, and organelles, in organoids.

A live-cell imaging system uses fluorescence labelling and time series acquisition and can be used to observe cell proliferation, migration, differentiation, and apoptosis in organoids in real time, as well as further explore the development process of organoids and cell interactions. Ramli *et al*. detected the bile canaliculus function and structure of liver organoids derived from hPSCs with high spatiotemporal resolution using a live-cell imaging system [154]. By combining a cell-permeable fluorescent probe, which can be hydrolysed to CDF inside the cells and then transported into the bile canalicular network through MRP2, they could observe the accumulation of fluorescence in the bile canaliculi and the gradual outflow of fluorescence to bile duct–like structures over time [154]. When drug or free fatty acid treatment conditions were used, the dynamic change of the bile canalicular network breaking, fluorescence reflux, and shrinkage could also be captured by the system, and the precise process of damage to organoid function and the disease model could be observed. In addition to live-cell imaging, other imaging techniques, such as optical coherence tomography (OCT), fluorescence lifetime imaging microscopy (FLIM), and Raman spectroscopic imaging (RSI), also have important applications in organoid research (Figure 5c–e). OCT is a time series analysis–based imaging technique that can be used to study tissue and intracellular activity [155]. OCT uses near-infrared light as a probe to achieve depth profile imaging through coherent gating and has the advantages of being nondestructive, label-free, and noninvasive. It can image depths of more than 1 mm and has high spatial resolution at the micron level, enabling the observation of thick tissue structures at the cellular scale. Therefore, OCT has important potential for application in organoid evaluation [156]. However, OCT also has certain limitations: its dynamic range is small, and it is difficult to distinguish small structures in deep tissues; thus, this method is insufficient for analysing cell structures, with effects on contrast and resolution. FLIM supports multiparameter and multidimensional five-dimensional imaging, enabling the study of intracellular and intercellular dynamic processes using biomarkers and a growing number of physicochemical parameters related to cellular function, such as inflammation and cellular metabolism [157]. Since FLIM is independent of fluorescence intensity, it is able to effectively distinguish between different fluorescent molecules and probes, thereby reducing background interference. This enables dynamic observation of complex biological systems, particularly organoids, by FLIM [158, 159]. RSI is a nonlinear optical microscopy technique that enables label-free chemical imaging by targeting molecular vibrational signatures. It provides nondestructive molecular-level imaging that directly reveals the chemical composition and structural information of organoids [160]. In addition, RSI has high spatial resolution, enabling precise characterization of microstructures inside and outside cells, making it particularly suitable for dynamic monitoring in complex biological systems [161]. Browne *et al*. also used a multimodal live-cell imaging system to dynamically observe the structural and functional characteristics of retinal organoids [162]. Through the integration of optical coherence tomography (OCT), fluorescence lifetime imaging microscopy (FLIM), and hyperspectral imaging, they observed, without damaging the sample, the layered structure, metabolic status, and the distribution of key molecules in organoid development *in situ* with high spatiotemporal resolution. By continuously monitoring the growth and differentiation of organoids at different time points, the system could provide noninvasive, real-time, and high-precision technical support for the maturity and function of organoids.

In addition, single-cell sequencing technology can be used to amplify and sequence RNA or DNA from single cells with high throughput and obtain the gene expression profile and epigenetic modification or mutation status of each cell [163]. Different cell subpopulations and their development processes can be distinguished. Single-cell sequencing can be used to identify the types and fractions of cells in organoids, track the differentiation process of cell lineages, study the effects of reprogramming or disease mutations on cell fates, and explore the reconstruction of gene regulatory networks. Uchimura applied single-cell RNA sequencing (scRNA-seq) technology to analyse kidney organoids derived from hPSCs. Researchers can map the types and differentiation states of cells in organoids at the single-cell level [164]. The results revealed a large number of different cells at different maturity states in the organoids, including 'off-target' cell types, such as neurons, muscle cells, and melanocytes, which did not exist in the corresponding mature kidney tissue. These results indicate that current differentiation methods cannot completely reflect the characteristics of mature adult kidneys. Although

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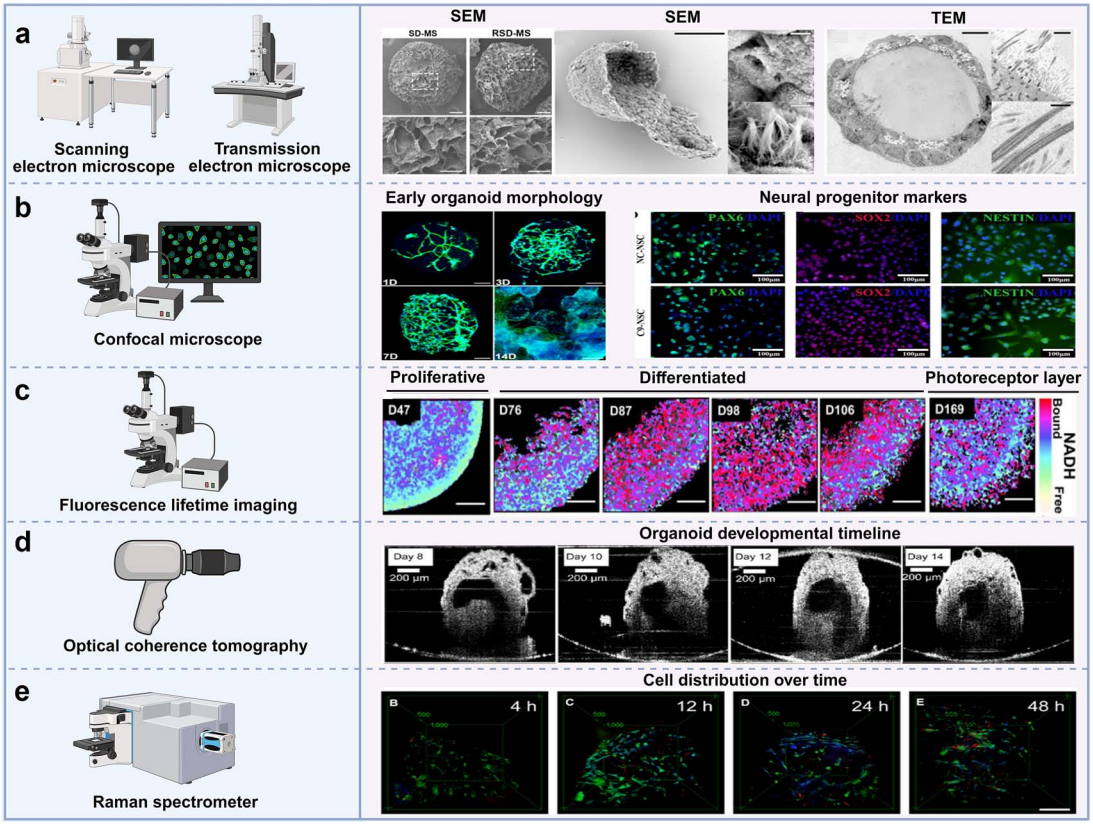


Figure 5 Schematic of imaging and analysis techniques for organoids. (**a**) Surface characterization: Electron microscopy can be used in surface analysis and is applicable for obtaining high-resolution morphological information of organoid structures. Adapted from [152] with permission. Copyright © 2024, Advanced Drug Delivery Reviews. (**b**) Protein expression: Confocal microscopy can be used to visualize the spatial distribution and expression pattern of proteins in organoids. It is ideal for high-resolution imaging of fluorescent protein expression. Adapted from [153] with permission. Copyright © 2024, Molecular Neurobiology. (**c**) Long-term real-time imaging: Fluorescence lifetime imaging microscopy can be used to visualize the spatial distribution of metabolic activity in RtOgs regions and to validate overall cell viability, as well as to study long-term dynamic changes. Adapted from [159] with permission. Copyright © 2021, Frontiers in Cellular Neuroscience. (**d**) Cross-sectional imaging: Optical coherence tomography enables strong tissue penetration and noninvasive deep imaging of thick samples and is an ideal tool for characterizing the cross-sectional structure of organoids. Adapted from [156] with permission. Copyright © 2022, Biosensors & Bioelectronics. (**e**) Raman imaging: Raman spectroscopy was used to study the molecular composition of acute gout synovial organoids and their intercellular interactions. Raman images of cells were acquired at different time points, and the dynamic changes in cell distribution were studied to provide information on organoid function and development. Adapted from [161] with permission. Copyright © 2024, Research (Washington, D.C.)

single-cell sequencing has high sensitivity and resolution and can provide a comprehensive transcriptional profile of cells, many challenges still exist in its application in practice. These challenges include a large amount of data noise, sample handling bias, and complicated analysis algorithms. These issues should be considered when interpreting organoid models. On a larger scale, multiomic analysis involves analysing genomic, transcriptomic, proteomic, and metabolomic information to obtain a global understanding of organoids. Combined analysis could identify core genes or signalling pathways driving the development of organoids or pathological changes. It could also be used to explore the relationships between protein modifications and metabolic fluxes and phenotypic outcomes [165]. For example, Ji *et al*. conducted a multiomic integration study on 65 liver cancer organoids. They integrated genomic, epigenomic, transcriptomic, and proteomic information to construct a molecular characteristic map of these organoids. They reported four subtypes with different metabolic and signalling pathway features. Furthermore, they integrated high-throughput drug screening and found that different subtypes presented different drug response patterns [166].

Using an elastic network regression model, researchers successfully predicted drug sensitivity and resistance. In summary, multiomic analysis enables precise subtyping, functional analysis, and drug response prediction of organoids. By integrating multiple levels of omic information, we can not only gain more insights into the biological behaviour of tumours but also pave the way for personalized treatment [167].

Applications of organoid research

With the advent of organoids, this technology has been widely used in other areas of biology and human diseases; for instance, using organoids derived from patient tissues, the development and progression of tumours and the functions of key genes involved in tumour heterogeneity and metastasis can be modelled [168]. Furthermore, organoids also serve as excellent *in vitro* models for investigating viruses and pathogens that cause diseases in humans [169]. In this section, we cover the use of organoids to elucidate disease phenotypes, screen drugs, and performed developmental biology and toxicology studies (Figure 6).

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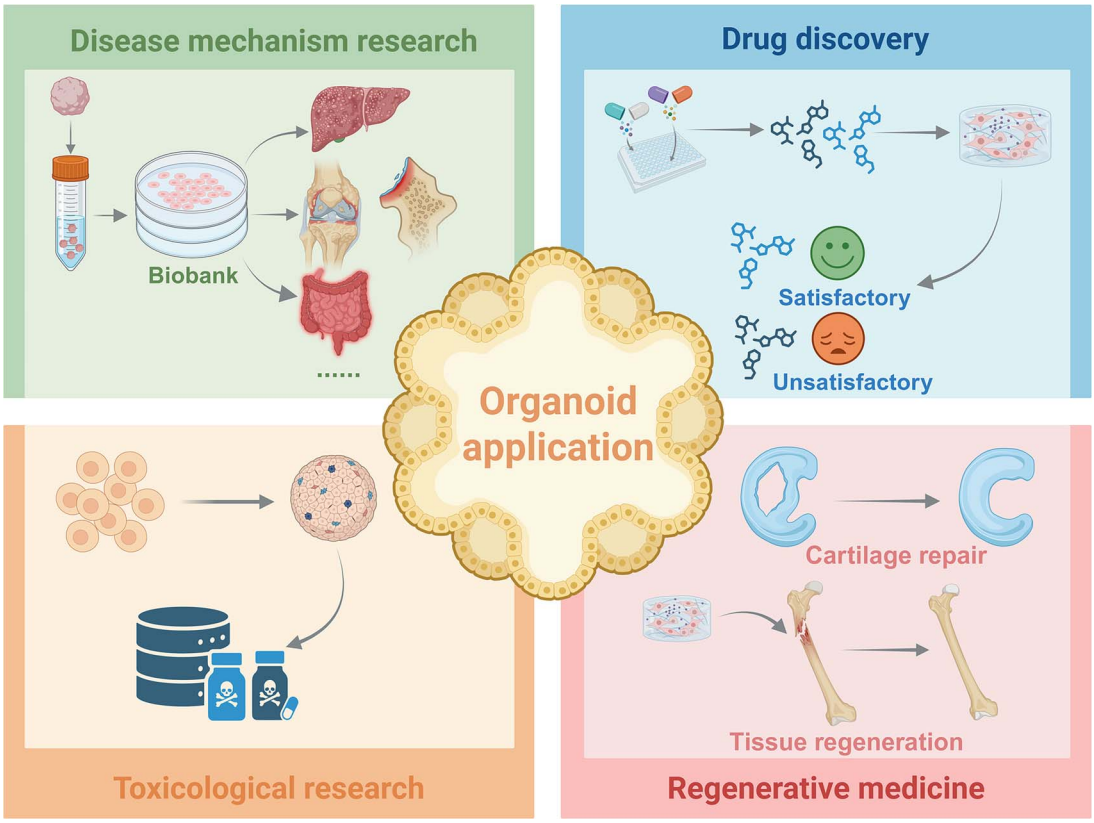


Figure 6 Schematic of organoid applications. Organoids effectively replicate the physiological characteristics and functions of tissues, providing a more realistic and efficient model platform for research. They show significant potential for applications in areas such as disease mechanism analysis, drug development, toxicology assessment, and regenerative medicine. Created using BioRender

Disease mechanism research

Organoid structures are relatively simplified, and complete tissue hierarchy and system complexity are lacking; thus, accurate simulations of multiorgan interactions or systemic characteristics of certain diseases are limited. However, as miniature biomimetic tissues cultured *in vitro* in 3D, organoids have shown great potential for modelling various diseases, especially in the field of cancer, because of their ability to closely mimic the structure and function of organs *in vivo* [170]. In cancer research, tumour-derived organoids (TDOs) can maintain the gene mutation profiles, phenotypic characteristics, and heterogeneity of the patient's tumour tissue and reproduce both the tumour's evolution and dependence on the microenvironment [171]. For example, Duan *et al*. constructed pancreatic cancer organoids to elucidate the pathogenesis of pancreatic cancer driven by KRAS mutations. Based on this platform, researchers have conducted comparative analyses and drug interventions on organoids with different KRAS genetic backgrounds. These experiments were used to evaluate signalling pathway activities and cell proliferation responses, thereby revealing key pathogenic mechanisms associated with KRAS mutations (Figure 7a) [172]. Kyungtae *et al*. used human foetal alveolar organoids as an *in vitro* development model to systematically analyse the mechanism of respiratory diseases in newborns. By simulating the abnormal state of lung development, this organoid model can be used to study lung developmental disorders and related diseases by elucidating the pathogenic role of cellular fate imbalance in neonatal respiratory diseases (Figure 7b) [173]. Additionally, colorectal cancer organoids (Figure 7c) [174] and liver cancer organoids (Figure 7d) have been established [175]. Lee and his group applied bladder cancer PDTOs to systematically study tumour evolution and drug responses. They reported that compared with those of primary tumours, the histology, gene mutation profiles, transcriptional features, and phenotypic characteristics of these organoids were retained and could reflect the tumour heterogeneity of bladder cancer [176]. Moreover, the drug sensitivity characteristics of these organoids were essentially consistent with clinical patient responses, indicating that these organoids could be applied in research on disease mechanisms. Furthermore, Yan and his group constructed a human organoid biobank involving multiple molecular subtypes of gastric cancer [65]. They analysed the signalling pathways, mutation profiles, and phenotypic features of the organoids from different gastric cancer subtypes by genomics and transcriptomics and revealed the diversity of these aspects in gastric cancer. The results demonstrated that organoids could not only stably preserve the key molecular characteristics of tumours but also act as a dynamic model to track tumour evolution and treatment responses, providing a powerful means to explore the pathogenesis of gastric cancer [65]. Therefore, as a highly biomimetic *in vitro* model, organoids can not only maintain the molecular characteristics and heterogeneity of tumour tissues well but also serve as an ideal platform for exploring the mechanism of cancer development, investigating mechanisms of resistance, and predicting individualized treatment responses [177]. As organoid biobanks continue to grow and multiomic technologies become more integrated, their value in basic cancer

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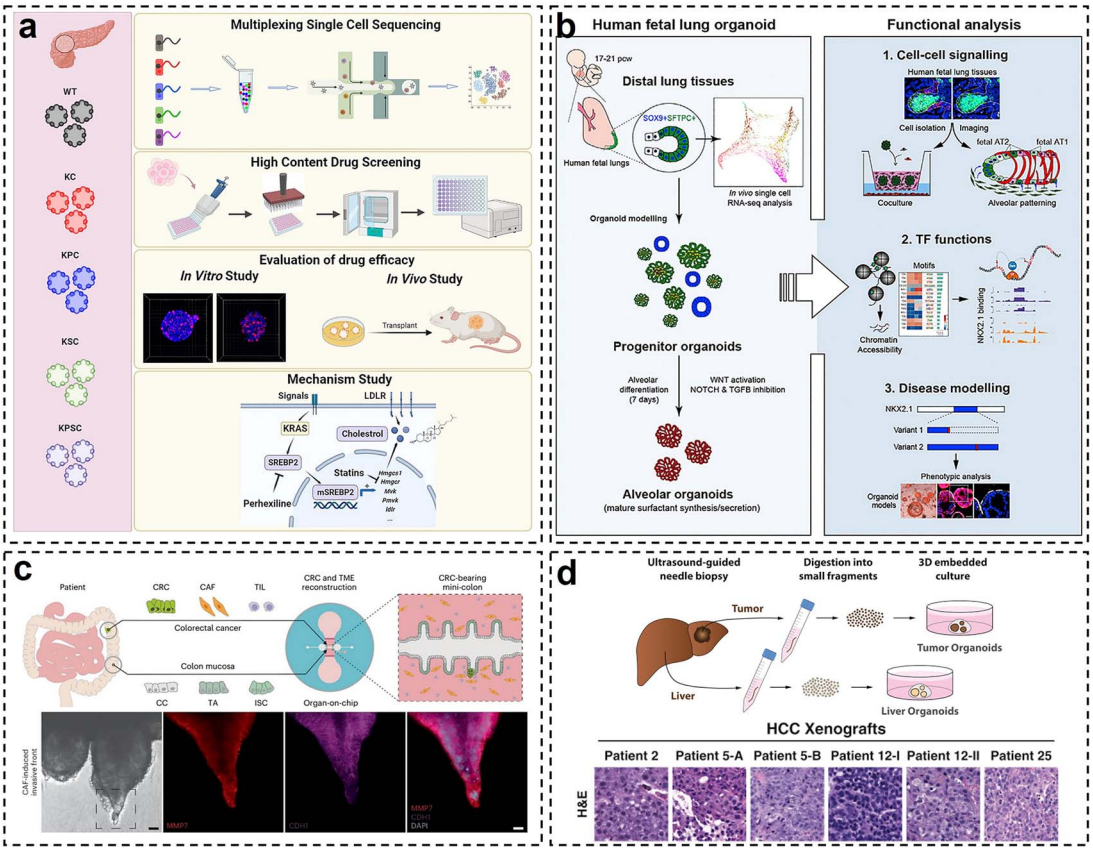


Figure 7 Applications of organoids in disease mechanism research. (**a**) Using multiplex single-cell sequencing and high-throughput drug screening platforms, the study of KRAS mutation-specific inhibitors in pancreatic cancer organoids provides deeper insights into the mechanisms of pancreatic cancer. Adapted from [172] with permission. Copyright © 2024, Cell Stem Cell. (**b**) A bone marrow organoid model of TGF-β-induced myelofibrosis allows for inhibitor screening, aiding in the exploration of disease mechanisms. Adapted from [173] with permission. Copyright © 2023, Cell Stem Cell. (**c**) The establishment of colorectal cancer organoids and reconstruction of the tumour microenvironment help in the study of the interactions between tumour cells and their microenvironment, revealing the role of proinflammatory signalling in driving epithelial–mesenchymal transition and invasion in CRC. Adapted from [174] with permission. Copyright © 2025, Nature Biotechnology. (**d**) Hepatocellular carcinoma organoids retain the morphology and expression patterns of liver cancer biomarkers, as well as the genetic heterogeneity of the primary tumour, making them useful for personalized treatment and liver cancer research. Adapted from [175] with permission. Copyright © 2018, Cell Reports

research and clinical translation will become increasingly evident, advancing the progress of precision medicine and targeted therapies [178].

In recent years, organoid technology has shown broad potential in various fields, including neurological diseases, metabolic disorders, and infectious diseases [83]. Compared with traditional 2D cell lines and animal models, organoids better preserve tissue specificity, cellular differentiation states, and microenvironment interactions, allowing for more accurate simulations of human pathological processes [179]. In addition, they are applicable for revealing the dynamic processes of complex diseases. For instance, Mariani *et al*. constructed brain organoids from iPSCs from autism spectrum disorder (ASD) patients that can recapitulate the *in vitro* development of the early embryonic forebrain cortex in humans [180]. They further applied the model to investigate the neurodevelopment process in patients with severe idiopathic autism [180]. The ASD organoids displayed an accelerated cell cycle and overproliferation in inhibitory neurons, supporting the important role of organoid systems in revealing the mechanisms of neurodevelopmental processes in disease. In addition to ASD, brain organoids are widely used to develop models for diseases such as Alzheimer's disease and microcephaly. These organoids can be used to construct models for these diseases and further identify signalling pathways or cell populations that are involved in abnormal neural development [181, 182]. Olijnik and colleagues constructed a human bone marrow organoid model that can biomimetically simulate the pathological microenvironment of blood system diseases, especially bone marrow fibrosis [183]. By activating the TGF-β signalling pathway or implanting fibrosis-derived cells from patients, they induced matrix remodelling and fibrosis deposition within the organoids. This recapitulated the pathological process and provided an important platform for studying haematopoietic-related diseases [183]. Wang and colleagues created expandable liver organoids derived from hESCs and integrated human foetal liver mesenchymal cells to develop a system suitable for modelling alcoholic liver disease (ALD) [184]. Under ethanol treatment, this model accurately recapitulated several key pathological features of ALD, including enhanced oxidative stress, lipid degeneration, inflammatory factor release, and liver fibrosis, validating its usefulness and effectiveness in liver injury mechanism studies. Additionally, pancreatic and liver organoids

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are extensively used in diabetes and nonalcoholic fatty liver disease research to simulate metabolic disorders [185]. Moreover, intestinal organoids have become a useful model for studying the mechanisms of intestinal pathogen infections and host responses [186]. Thus, organoids provide a controlled, reproducible, and high-fidelity model platform for disease mechanism research [187]. Organoid technology is gradually facilitating the translation of basic research into clinical applications, accelerating the discovery of new mechanisms and the development of targeted intervention strategies.

Drug screening

Organoids are cultured from stem cells or tissue-specific cells in 3D and exhibit cellular heterogeneity, spatial organization, and intercellular communication, as well as functional similarities to how human tissues absorb, metabolize, and display toxicity to drugs [188]. Compared to conventional 2D culture or animal models, organoids have greater accuracy and clinical relevance for predicting drug effects and toxicity and can be particularly valuable in personalized medicine, modelling rare diseases, and high-throughput drug screening [189]. A personalized organoid model can be established using tissue samples from patients or iPSCs derived from patients to model the disease condition of patients *in vitro*, which will help predict the response of patients to different drugs and treatment regimens [188]. For instance, Kim *et al*. generated organoids from tumour tissue from a paediatric ovarian cancer patient [190]. They used an organoid model to perform high-throughput drug screening with multiple drugs and discovered that methotrexate had strong antitumour activity. The authors further explored the effect of methotrexate on tumour cells and reported that methotrexate could inhibit tumour cell proliferation and induce apoptosis and thus be useful in treating paediatric ovarian cancer. Similarly, Wei and colleagues used a human organoid–based drug screening platform to identify α2-adrenergic receptor signalling as a potential therapeutic target for cartilage regeneration (Figure 8a) [191]. The research team screened various drugs using patient-derived cartilage organoids and discovered that activating the α2-adrenergic receptor promoted cartilage cell proliferation and tissue repair [191]. These findings provide a new drug target for the treatment of cartilage damage and degenerative diseases and may offer a theoretical foundation for clinical cartilage repair therapies.

In personalized drug development, organoids play a crucial role as tools for predicting therapeutic drug responses. By building PDTOs, sensitivity to chemotherapy or targeted drugs can be evaluated, providing a scientific basis for personalized medication and individualized treatment [192]. Nuciforo *et al*. constructed a human liver cancer organoid model based on tumour biopsy samples to simulate the histological and molecular characteristics of liver cancer and conduct drug screening studies. In this study, a drug screening experiment was conducted on the multikinase inhibitor sorafenib to evaluate the differences in the response of liver cancer organoids from different sources to sorafenib (Figure 8b) [175]. Seidlitz *et al*. generated organoid models from human gastric cancer patients and subsequently tested the drug sensitivity of these models to commonly used chemotherapy drugs, including 5-fluorouracil, irinotecan, oxaliplatin, docetaxel, and epirubicin [193]. The results demonstrated that gastric cancer organoids from different sources displayed different sensitivities to these drugs, and the results recapitulated the individualized treatment response profiles among patients [193]. Broutier *et al*. constructed liver cancer organoids to mimic the biological characteristics of liver cancer and conduct drug screening studies. On the basis of this model, the researchers performed *in vitro* treatment with a variety of targeted drugs and chemotherapy drugs to analyse the differences in drug sensitivity of different patient-derived organoids by assessing organoid growth inhibition and survival response (Figure 8c) [40]. Li *et al*. established a patient-derived organoid model of upper tract urothelial carcinoma (UTUC). On the basis of this model, the researchers conducted *in vitro* drug screening of a variety of commonly used and potential therapeutic drugs, such as cisplatin, carboplatin, and gemcitabine, verifying the practical value of the organoid platform in individualized medication decision-making and precision treatment research for UTUC (Figure 8d) [194]. These results further support the ability of organoid platforms to predict chemotherapy response and guide personalized treatment. Additionally, Saito *et al*. screened 22 compounds among 339 clinically approved drugs for their ability to inhibit the growth of intrahepatic cholangiocarcinoma (IHCC) organoids. The results demonstrated that the MDM2 inhibitor nutlin-3a will be useful for treating refractory cancers with wild-type TP53 [195]. In addition, the antifungal drugs amorolfine and fenticonazole exhibited significant antitumour activity and could be used as novel therapeutic drugs for IHCC. Thus, organoid platforms have broad applicability and potential clinical value in drug screening and personalized treatment prediction [196].

Toxicological research

Adverse drug reactions are among the main reasons for the termination of drug development projects and the market withdrawal of already approved drugs. Therefore, minimizing the occurrence of these reactions is critical for improving patient drug safety [197]. Organoids can mimic the 3D structure and multicellular microenvironment of human organs *in vitro* and exhibit good tissue specificity and functional expression, thereby providing a more accurate representation of the true toxicity of compounds to human tissues [198]. For example, liver organoids can be used to evaluate the hepatotoxicity of drug metabolites. In one study, Shinozawa and colleagues used liver organoids derived from hPSCs and exposed them to several known hepatotoxic drugs. They observed indicators such as cell viability, the release of liver enzymes, and gene expression to evaluate the toxic effects of drugs on liver tissue [199]. The results demonstrated that this organoid model could accurately predict drug-induced liver damage, and the prediction results of this model were consistent with clinical observations and superior to those of 2D liver cell cultures. Liang *et al*. constructed liver organoids with structural and functional characteristics of the liver using hPSCs from healthy and diseased donor sources. They used this model system to evaluate the toxic effects of microplastics and tetrabromobisphenol A (TBBPA) alone and in combination on liver tissue. The results revealed that combined exposure to microplastics and TBBPA was significantly toxic to the liver, indicating that liver organoids are suitable for the study of environmental compound pollutant toxicity and individual susceptibility (Figure 9a) [200]. Mun *et al*. used hPSCs to construct long-term expandable hepatocyte-like liver organoid models, which maintained their liver phenotype and metabolic function after induced differentiation to stages under 3D culture conditions. The

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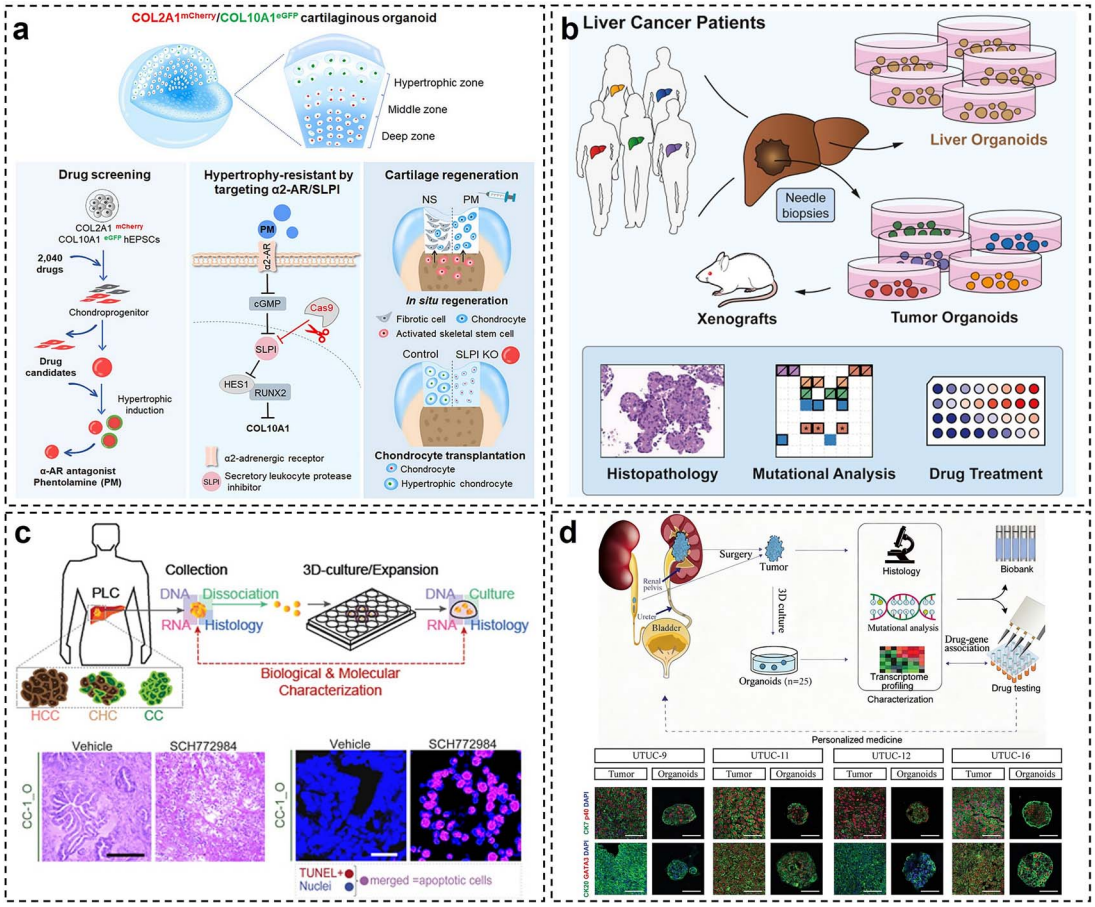


Figure 8 Applications of organoids in drug screening. (**a**) Human cartilage organoids were created to screen drugs that promote cartilage formation and inhibit hypertrophy. Adapted from [191] with permission. Copyright © 2024, Cell Stem Cell. (**b**) Hepatocellular carcinoma organoids were developed to test sensitivity to sorafenib. Adapted from [175] with permission. Copyright © 2018, Cell Reports. (**c**) Organoids derived from primary liver cancer were used for biomarker detection and drug screening. Adapted from [40] with permission. Copyright © 2017, Nature Medicine. (**d**) Upper tract urothelial carcinoma (UTUC) organoids were established for gene transcriptomic analysis, drug testing, and personalized treatment research. Adapted from [194] with permission. Copyright © 2022, Advanced Science

researchers selected a variety of representative drugs with clear clinical hepatotoxicity characteristics, such as acetaminophen, for testing, and the results showed that the liver organoids could better reflect dose-dependent toxic effects and human liver metabolism-related responses (Figure 9b) [31]. These studies demonstrate the accuracy and feasibility of using organoids as an *in vitro* platform for toxicological studies. In addition to hepatotoxicity, the application of organoids in neurotoxicity research is receiving increasing attention. Neurotoxicity manifests as neuronal damage or death, axonal degeneration, disruption of neurogenesis, and cognitive dysfunction. These effects are due to the harmful effects of different chemicals [201]. Brain organoids may have the potential to elucidate the mechanisms underlying these neurodevelopmental disorders [202]. Lu *et al*. constructed a 3D brain organoid model based on hPSC induction to systematically evaluate the potential neurotoxic effects of perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS) exposure on the human nervous system. Using this model, the researchers treated brain organoids with polyfluoroalkyl substances (PFASs) at different doses for different durations, and the results revealed that PFAS exposure can induce neuropathological changes similar to those observed in Alzheimer's disease, indicating that brain organoids are effective *in vitro* models for studying the neurotoxicity of environmental pollutants and disease-related mechanisms (Figure 9c) [203]. Nzou *et al*. employed human neural spheroids formed by functionalized blood–brain barriers to study neurotoxicity [204]. These spheroids can contact neurons, microglia, oligodendrocytes, and other types of cells. By examining cell viability and cell death and the response of organoids to functional changes in the blood–brain barrier, the effects of toxic exposure on the structure and function of organoids were investigated. Acute methylmercury and trimethyl tin exposure increased cell death, oxidative stress, and astrocyte reactivity in 3D neural tissue [204]. Furthermore, other types of organoids have been developed and used in toxicity testing. Crespo *et al*. used colon organoids derived from iPSCs for toxicity testing and assessed the effects of drugs on CRC models. Their findings revealed that rapamycin and XAV939 could damage healthy colon cells by impairing cell proliferation [205]. In another study, Zhou *et al*. constructed a human-derived airway organoid model and systematically evaluated the airway toxicity effects of the environmental pollutant 1-nitropyrene (1-NP) in combination with multimodal imaging technology (Figure 9d) [26]. Czerniecki

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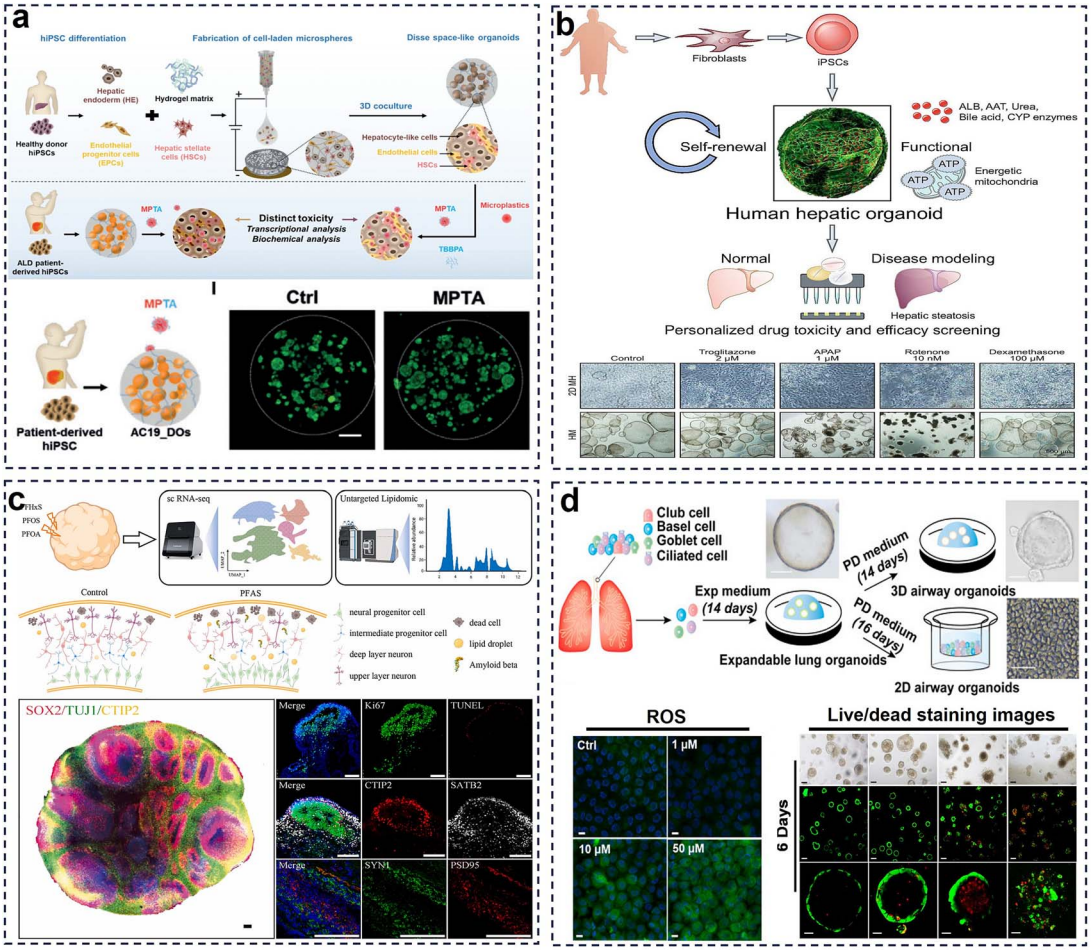


Figure 9 Applications of organoids in toxicological evaluation. (**a**) An individualized human liver organoid model was developed to demonstrate significant genetic variation in the toxic effects of microplastics and their adsorbed substances, revealing that liver fibrosis–related genes play a key role in mediating susceptibility to microplastic toxicity. Adapted from [200] with permission. Copyright © 2024, International Journal of Bioprinting. (**b**) iPSC-derived human liver organoids provide a liver model for toxicity prediction and drug screening. Adapted from [31] with permission. Copyright © 2019, Journal of Hepatology. (**c**) The potential neurotoxic effects of per- and polyfluoroalkyl substances (PFASs) on human brain organoids were studied. Adapted from [203] with permission. Copyright © 2024, Environmental Pollution. (**d**) Human airway organoids were used for toxicity assessment of 1-nitropyrene (1-NP) on the basis of multimodal imaging. Toxicity tests revealed morphological damage and oxidative stress in airway organoids after 1-NP exposure. Adapted from [26] with permission. Copyright © 2024, Environmental Science & Technology

and colleagues reported an automated HTS platform used to derive kidney organoids from hPSCs. This platform was applied to toxicity assessments and in-depth studies on polycystic kidney disease mechanisms [206]. Thus, the application of organoids in toxicology research provides a more reliable *in vitro* model for drug safety evaluation. Their unique 3D structure and multicellular environment allow for more accurate simulations of human responses, enhancing the precision of toxicity predictions.

Challenges and prospects

Despite the immense potential of organoid technology in the biomedical field, a range of challenges still exist in its development and application (Figure 10). First, while current culture systems can simulate certain structures and functions of organs, a significant gap remains compared with real organs. Cultivating organoids requires precise control over various growth factors, extracellular matrix components, and physicochemical environments, with even slight variations in these conditions potentially affecting the formation and functionality of the organoids. Additionally, the organoid culture process is time-consuming and challenging to standardize, making it difficult to ensure batch-to-batch stability. The root cause of the difference in organoid batches lies in the systematic shift in the starting cell state, culture microenvironment, and processing parameters across different batches. Heterogeneity in the donor source and cell composition, fluctuations in the ECM and growth factor batches, variations in oxygen and nutrient diffusion gradients, and subtle differences in the timing of operations can all impact organoid stability. The presence of these factors limits the widespread promotion and application of organoids in large-scale drug screening and clinical applications [207].

Another challenge is the standardization and quality control of organoid models [86, 208, 209]. Since the formation of organoids involves self-organization and differentiation of cells, their morphology and function may vary considerably under different experimental conditions. This leads to a lack of experimental reproducibility between laboratories and hinders the

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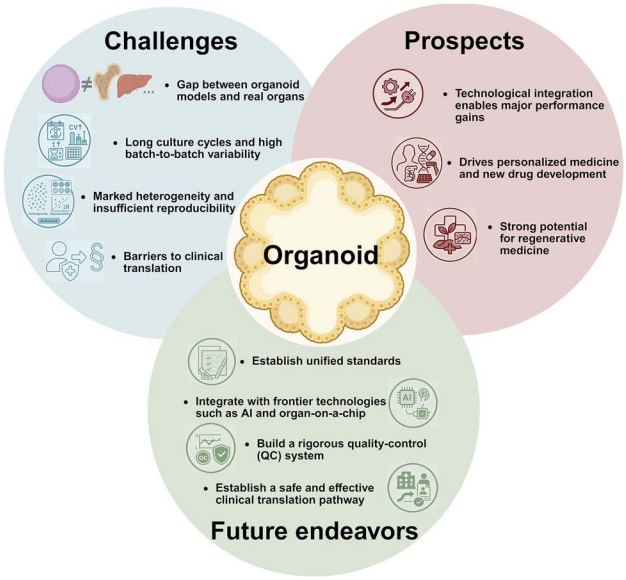


Figure 10 Challenges and prospects of organoid research. This figure summarizes the current challenges, including long cultivation periods, significant cellular heterogeneity, and difficulties in clinical translation. It also highlights the potential applications of organoids in personalized medicine, drug development, and regenerative medicine, as well as their future development directions. Created by BioRender

experimental standardization of organoid models. Therefore, it is necessary to establish standardized protocols for organoid culture and characterization, which should involve the identification of cell sources, standardization of culture medium components, and establishment of criteria to characterize organoid morphology and function. New, more efficient and precise tools for quality control must be developed to ensure the stability of organoids for both research and clinical use.

Furthermore, the clinical translation of organoid technology faces several obstacles [210–212]. While organoids can be used to simulate certain pathological processes *in vitro*, it remains uncertain whether they can perform similar functions *in vivo* and achieve effective tissue repair and regeneration. Issues such as immune rejection after organoid transplantation, long-term survival, and integration with host tissues are key factors influencing the clinical success of organoid-based therapies. Currently, clinical trials are still in the early stages, and more data and experience are needed to assess the safety and efficacy of organoid transplantation, as well as to optimize transplantation techniques and immunosuppressive protocols.

Looking ahead, with the integration of technologies such as gene editing, microfluidics, biomaterials, and multiomic analysis, organoid models are expected to make significant breakthroughs in terms of tissue complexity, functional maturity, and accuracy in disease modelling. The combination of organoids with cutting-edge technologies such as OoCs and artificial intelligence (AI) will further drive innovations in personalized medicine and drug development [213]. Organoid–AI fusion is showing several emerging directions, such as machine learning based on high-content phenotypes and multiomic data for drug response prediction, deep learning-driven organoid segmentation, and temporal kinetic tracking to improve screening throughput and reproducibility, and label-free phenotypic evaluation for culture quality control and batch correction, thereby enhancing model interpretability and translational value [214]. Currently, the concept of AI virtual organoids (AIVOs) has been proposed. AIVOs, also known as silicon organoids, refer to organoid-scale digital twins instantiated in the computational space [215]. AIVOs use virtual cells and, where appropriate, virtual organoids as the minimum executable unit to realize the dynamic simulation of the structure and function of biological organoids. This indicates that organoid technology has officially moved from the era of 'test tube experiments' to the era of 'silicon-based twins' [215]. In the field of regenerative medicine, optimizing culture and transplantation strategies may make organoids a crucial solution for addressing organ shortages.

Conclusions

As emerging 3D biomimetic models, organoids are increasingly bridging the gap between basic research and clinical applications. By replicating the structure and function of human organs, organoids have proven valuable in disease modelling, drug screening, toxicology assessments, and precision medicine, overcoming the limitations of traditional 2D cell cultures and animal models. With continuous advancements in organoid culture systems, microfluidic technologies, and gene editing, the potential for the use of organoids in basic research and regenerative medicine is steadily increasing. While challenges such as standardization and clinical translation remain, the integration of interdisciplinary technologies is expected to further enhance the role of organoids in personalized health care and disease treatment.

Abbreviations

2D, two-dimensional; 3D, three-dimensional; Lgr5+, G-protein-coupled receptor 5–positive; EGF, epidermal growth factor; ESCs, embryonic stem cells; ASCs, adult stem cells; AV-Scaf, acoustic virtual 3D scaffold; PDTOs, patient-derived tumor organoids; PSC, pluripotent stem cell; EBs, embryoid bodies; iPSCs, induced human pluripotent stem cells; BME, basement membrane extract; ECM, extracellular matrix; FeBOs, fetal brain organoids; LOs, liver organoids; MaugOs, macrophage-augmented organoids; EMT, epithelial–mesenchymal transition; HCC, hepatocellular carcinoma; PM, precision medicine; DST, drug sensitivity testing; 5-FU, 5-fluorouracil; OoC, organ-on-a-chip; DEP, dielectrophoresis; ROs, retinal organoids; PS-NPs, polystyrene nanoplastics; PDLO, patient-derived lung cancer organoid; HERVH, human endogenous retrovirus H; CRC, colorectal cancer; hPSCs, human pluripotent stem cells; HIOs, human intestinal organoids; hESCs, human embryonic stem cells; DLP, digital light processing; COPs, cartilage organoid precursors; hSCOs, human spinal cord organoids; PEG, polyethylene glycol; HA, hyaluronic acid; TG, transglutaminase; CGDE, catalase-conjugated GelMA/DNA/EACP; GelMA, gelatin methacrylamide; WBOs, woven bone organoids; BMP, bone morphogenetic protein; ZIKV, Zika virus; PDAC, pancreatic ductal adenocarcinoma; CAFs, cancer-associated fibroblasts; GR, gemcitabine resistance; GFP, green fluorescent protein; BF, bright-field imaging; LSFM, light-sheet fluorescence microscopy; OCT, optical coherence tomography; FLIM, fluorescence lifetime imaging; scRNA-seq, single-cell RNA sequencing; TDOs, tumor-derived organoids; ASD, autism spectrum disorder; ALD, alcoholic liver disease; IHCC, intrahepatic

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cholangiocarcinoma; UTUC, upper tract urothelial carcinoma; HTS, high-throughput screening; TBBPA, tetrabromobisphenol A; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFASs, polyfluoroalkyl substances; 1-NP, 1-nitropyrene; AIVOs, artificial intelligence virtual organoids; EBV, Epstein–Barr virus; MSI, microsatellite instability, BMSCs, bone marrow stromal cells, EACP, engineered artificial calcium phosphate; SEM, scanning electron microscope; TEM, transmission electron microscope; PLC, primary liver cancer; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; CHC, combined HCC/CC; ROS, reactive oxygen species.

Acknowledgements

We would like to thank the Institute of Translational Medicine, Shanghai University, for their support and encouragement in this article. We also appreciate Biorender for their support in the figures of this article.

Author contributions

All authors contributed to the writing of this manuscript. All authors have read and approved the final version of the manuscript. [JZ and YZW]: Conceptualization, Data curation, Writing—original draft, Writing—review & editing [equal]. [TZS, YRM, HJD]: Data curation, Writing—review & editingFigures. [YL, ZG, ZHL, JCS]: Conceptualization, Funding acquisition, Resources, Supervision, Writing—review & editingConception.

Ethics approval and consent to participate

The authors declare that their participation in writing this review and its publication is voluntary.

Consent for publication

All authors agree to publish this review.

Conflicts of interest

None declared.

Funding

This work was supported by the Key Project of the National Natural Science Foundation of China (82530072, 82230071), National Natural Science Foundation of China (32471395).

Data availability

Not applicable.

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