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Review Article

Innovations in Organoid Engineering: Construction Methods, Model Development, and Clinical Translation

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Abstract

Organoids are derived from self-organizing stem cells and form three-dimensional structures that are structurally and functionally similar to *in vivo* tissues. With the ability to replicate the *in vivo* microenvironment and maintain genetic stability, organoids have become a powerful tool for elucidating developmental mechanisms, accurately modeling disease processes, and efficiently screening drug candidates, and have also demonstrated significant value in the field of traditional Chinese medicine (TCM)-including applications in screening active components of TCM, studying TCM pharmacodynamic mechanisms, evaluating TCM safety, and verifying the effects of traditional non-pharmacological therapies such as acupuncture and yoga. Organoids can be cultured using air-liquid interface systems, bioreactors, and vascularization techniques. They are widely used in drug screening, disease modeling, precision medicine, and toxicity assessment. However, current limitations include high costs, difficulty in accurately replicating the microenvironment, and ethical concerns. In this review, we systematically retrieve, synthesize, and analyze relevant literature to elucidate the culture methods of organoid technology, its diverse applications across various fields, and the challenges it faces. In the future, integration with artificial intelligence may provide new insights and strategies for drug development and disease research and the modernization of TCM.

Introduction

Organoids are collections of organ-specific cells cultured *in vitro*.[1] *In vitro* three-dimensional (3D) cultures of pluripotent stem cells (PSCs) and adult stem cells (ASCs) can form spatially structured cell aggregates that mimic the structure and physiological functions of organs *in vivo*.[1] According to their origin, organoids can be categorized into those derived from ASCs and those derived from PSCs.[2] PSCs include embryonic stem cells and induced pluripotent stem cells (iPSCs). In general, organoids derived from ASCs are commonly utilized to recapitulate the states of their tissue of origin under *in vivo* homeostatic or regenerative conditions and are primarily applied in disease modeling, including cancer and neurodegenerative diseases.[1,3,4] Additionally, PSC-derived organoids, which resemble fetal tissues, can self-organize into neural epithelial structures and complex neuronal networks.[1,5] These organoids are mainly used to study diseases related to developmental defects, such as microcephaly.[3,4]

In recent years, organoids have addressed many limitations of two-dimensional (2D) models by more accurately replicating the structure and function of *in vivo* organs while retaining the original tissue characteristics and genetic stability.[6] Organoid models derived from various organs have gradually emerged as a research focus.[7] However, systematic reviews of their construction methodologies and applications remain limited. This review discusses the construction methods and diverse applications of organoids, aiming to provide innovative insights for drug development and personalized therapeutic strategies.

Keywords: Organoid; Pluripotent stem cell; PSC; Tumor microenvironment; TME; High-throughput screening; HTS; Drug development.

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Organoid construction

Organoids form 3D structures that rely on matrix materials or supports to provide attachment and structural support for cells.[8] Three key factors must be considered in the construction of organoids. (1) The physical characteristics of the culture environment[3]: When developing 3D organoid models *in vitro*, it is necessary to simulate the characteristics of the extracellular matrix (ECM) in tissues.[9] The ECM regulates tissue development and homeostasis by releasing growth factors, cytokines, and chemokines.[9] Matrigel is the most widely used substrate in organoid cultures.[9] (2) Requirements of endogenous and exogenous signals[3]: For most organoids, the cell system needs to be stimulated with specific exogenous signals during derivation. For example, the Wnt/β-catenin signaling pathway is crucial for the development of specific anatomical regions in the mouse stomach during gastric growth.[10] This signaling pathway serves as the mechanistic basis for inducing the differentiation of human pluripotent stem cells (hPSCs) into 3D human gastric fundus organoids.[10] (3) Starting cell type[3]: Distinct initial cell types result in varied properties in the final organoids. For instance, neural ectoderm-associated optic cups,[11] brain organoids,[5] and mesodermal kidney organoids can currently only be derived from PSCs.[12] Additionally, organoids from somatic ectodermal lineages (especially glandular tissues) are mainly derived from ASCs or dissociated adult tissues.[13] Most endodermal organoids can be derived from both PSCs and ASCs, such as colonic organoids.[14,15] The selection of different components forms the foundation of organoid culture, with specific construction methods described in the following sections (Fig. 1a–c).[5,17–18]

Air-liquid interface (ALI) culture

The ALI is a 3D culture method that mimics the luminal environment of hollow organs.[19,20] In ALI culture, the permeable membrane of insert petri dish chambers is pre-coated with collagen before cell inoculation.[19] The apical cell surface is exposed to air, while the basal side interacts with the culture medium through the membrane, forming a 3D model that simulates the internal environment of cavity organs.[19] ALI culture can also preserve various infiltrating immune cell populations (e.g., T cells and B cells).[20]

ALI has been widely applied in tissue engineering and cell culture to study the effects of mechanical forces on cells.[21] When cultured using this system, airway epithelial cells form pseudostratified layers with tight junctions, cilia, and mucin synthesis, closely mimicking their *in vivo* counterparts (Fig. 1a).[21] This method is also valuable for studying organoid interactions with the tumor microenvironment (TME).[22] Scientists have used ALI culture to construct patient-derived organoids (PDOs) from immune-competent patients for TME studies in lung and colorectal cancer models.[22] Tumor-infiltrating lymphocytes in these organoids retained the T-cell receptor profiles of the original tumors,[22] offering insight into the immune characteristics of tumors and mechanisms underlying immune responses.[22] Despite its advantages, the ALI method has limitations. For instance, immune cells within ALI-cultured organoids exhibit a relatively short survival time, which limits long-term *in vitro* maintenance and successive expansions.[23,24] Moreover, as organoids grow beyond a certain size, nutrient availability becomes restricted.[23,24] To address these limitations and improve the realism of ALI organoid cultures, future research should focus on optimizing the culture environment to maintain immune cells and stromal components long-term, while more accurately replicating the cellular microenvironment.[23,24]

Bioreactor culture

Bioreactors enhance nutrient exchange by precisely controlling microenvironmental conditions and delivering oxygen, nutrients, and supplemental agents in a regulated manner (Fig. 1b).[16,25,26] Improved aeration and nutrient distribution support cell viability and promote the formation of complex organoid structures, particularly in brain organoid cultures, resulting in more complete morphological development.[16,25,26]

Bioreactor culture is a relatively straightforward method for supporting organoid structures, outperforming other techniques in simplicity and efficacy. Qian's team used 3D-printed components to develop *SpinΩ*, a miniaturized porous rotating bioreactor that enables the derivation of iPSCs into forebrain, midbrain, and hypothalamus organoids.[27] This system enhances nutrient uptake, offers a compact design, and reduces media costs.[27]

Bioreactor technology also helps maintain a highly undifferentiated state in organoids during long-term culture.[28] For example, suspension bioreactors enable high-throughput, scalable culture of mouse embryonic stem cells, supporting their large-scale expansion while preserving pluripotency.[28] However, most current organoid cultures use suspension formats, and bioreactors incorporating mechanical compression and tension are not yet available. Therefore, engineering bioreactors capable of applying programmable mechanical and reactive forces holds significant scientific and practical potential.[16]

Vascularization

Organoid vascularization is a process that emulates and recreates the vascular structure of organs through cellular self-reorganization, microvascular fragmentation, *in vivo* transplantation into the host, and microfluidics.[29,30] The central nervous system (CNS) of vertebrates develops in synchrony with the vascular system.[31] Since the CNS does not generate vascular progenitor cells, avascular brain organoids fail to survive when transplanted into the host.[31,32] Thus, vascularization is critical for neuronal nutrition, oxygen supply, and healthy development.[31,32] Vascularized organoids are increasingly used in disease models to study vascular-neural interactions. Human blood vessel organoids (hBVOs) produce vascular cells capable of penetrating brain organoids and forming vascular-like structures (Fig. 1c).[33,34] The integration of hBVOs with brain organoids has been demonstrated to generate vascularized brain organoids that model interactions between neuronal and non-neuronal components *in vitro*.[33,34] The presence of molecular markers associated with the blood-brain barrier (BBB) in vascularized brain organoids indicates that hBVOs can establish a neural-specific vascular network.[33,34]

Currently, the development of vascularized organoids faces challenges related to large discrepancies in the size and density of their vascular structures compared to those observed *in vivo*.[35] Additionally, issues of insufficient stability and lack of long-term reproducibility remain.[35] The integration of advanced 3D bioprinting techniques with *in vitro* methods for constructing external circulatory systems is expected to enable the creation of vascularized organoids possessing functional vascular networks and achieving complete maturation.[35] This advancement is poised to significantly impact pharmaceutical R&D processes.

In summary, current organoid construction methods have their advantages and limitations (Table 1).[16,19,21,22,27,28,33–35] ALI culture can simulate the environment of hollow organs but has limitations in cell survival and nutrient supply. Bioreactors optimize culture conditions but lack mechanical stimulation. Vascularization technology is crucial for organoid survival but faces challenges related to structure and stability. In the future, innovative technologies need to be inte-

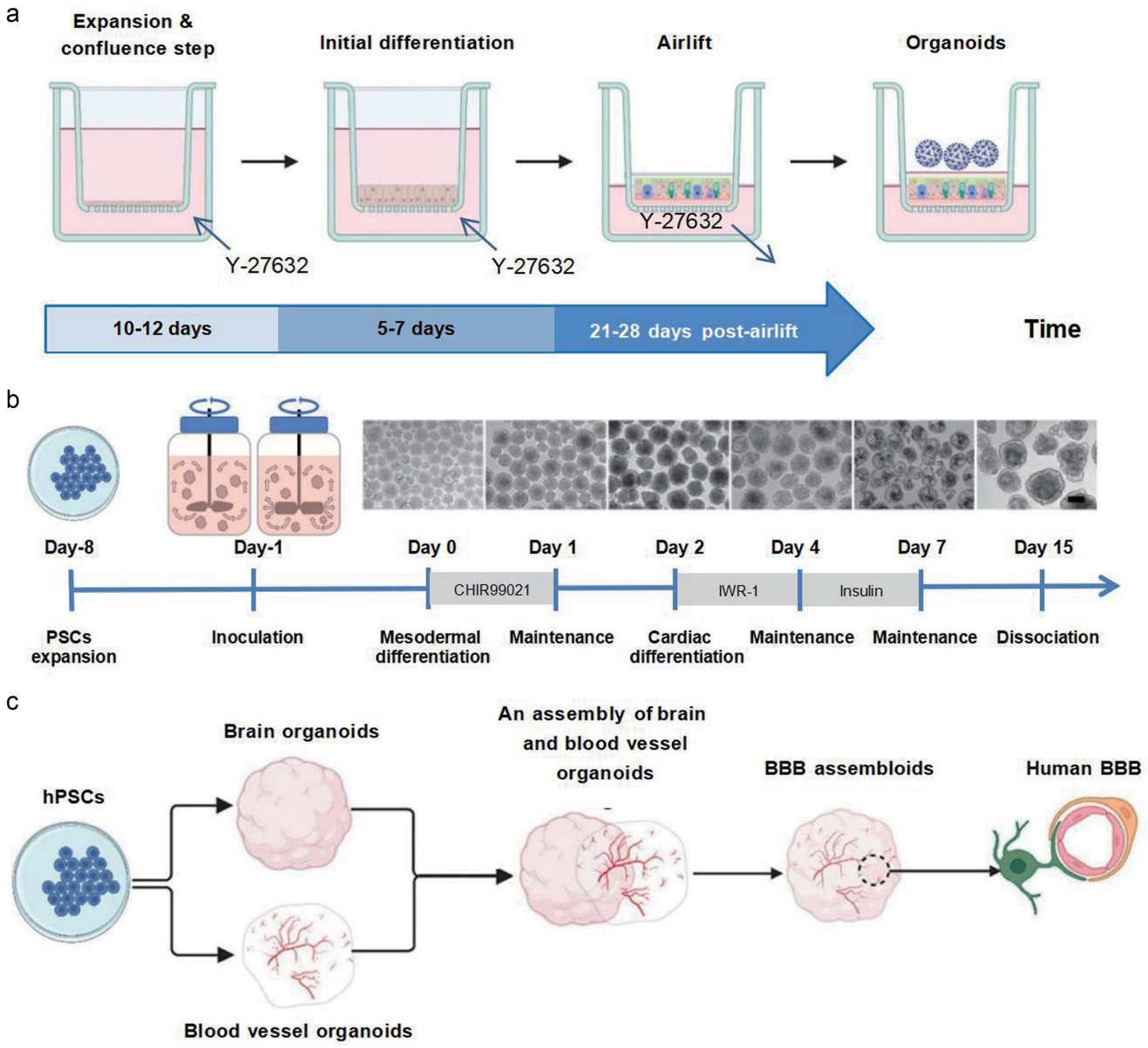

Fig. 1. Schematic diagram of organoid culture method.[5,16–18] (a) Experimental procedure for culturing human lung organoids by the ALI method. Cell expansion was carried out in submerged culture using medium supplemented with Y-27632. When confluence reached 100%, initial differentiation was induced by removing the apical medium and switching to air-lift culture to form pseudostratified epithelial cells. (b) Schematic of the optimized bioreactor cardiac differentiation protocol. PSCs were inoculated into Geltrex-pretreated T80 bottles and cultured in E8 medium until 90% confluence. After digestion, cells were resuspended and transferred to a stirred-tank bioreactor. Differentiation was initiated when the EB diameter reached 100–300 μm: CHIR99021 medium was applied on day 0, switched to inhibitor of Wnt response-1 (IWR-1) -endo medium on day 2, and 1:1000 insulin-containing medium was introduced from day 7. Cells were enzymatically digested and frozen on day 15. (c) Vascularized brain organoids after the construction of the human BBB process. Brain and vascular organoids were generated separately and subsequently fused to simulate neurovascular co-development, leading to the formation of BBB organoids by promoting telencephalic cell fusion with surrounding cells. ALI, air-liquid interface; BBB, blood-brain barrier; EB, embryoid body; hPSCs, human pluripotent stem cells; PDO, patient-derived organoid; PSCs, pluripotent stem cells. *Some images were created with BioRender.*

grated to overcome these bottlenecks and promote organoid technology for broader roles in scientific research and clinical applications.

Construction of representative models

Different diseases require specific organoid models to study their pathological mechanisms in depth. By combining various types of organ models, researchers can gain a comprehensive understanding of the pathological characteristics of different tissues and organs, thereby providing robust support for precision medicine (Table 2).[36–47]

Kidney organoid

The kidneys play a crucial role in maintaining fluid, electrolyte,

Table 1. Comparison of organoid construction methods

Methods	Applications	Advantages	Limitations
ALI	Cultivate epidermal and respiratory epithelial cells.[21] Studying organoid-tumor microenvironment interactions clarifies tumor immune traits and immune response mechanisms.[22]	Simulate the environments of hollow organs and tubes.[19]	Constructing PDO for tumor microenvironment research.[22]
Bioreactor	To build more complex brain organoids.[27]	Maintaining organoids in a highly undifferentiated state enables large-scale stem cell expansion.[28]	Enhance nutrient exchange to promote complex organoid formation.[16] Small footprint and low cost.[27]
Vascularization	A disease model for studying vascular-neural interactions.[33,34]	Simulate and reconstruct the organ vascular structure.	The size and density of vascular structures vary significantly *in vivo*.[35] Issues of stability, long-term maintenance, and lack of repeatability.[35]

ALI, air-liquid interface; PDO, patient-derived organoid.

Table 2. Methods for constructing representative organoid models

Organoids	Sources	Methods	Establishments	References
Kidney organoid	PSCs	CHIR99021-FGF pathway and BMP/FGF pathway	Renal unit-related cell types originate from the primitive streak, posterior mesoderm, and renal unit progenitor cells	45
Liver organoid	PSCs, ASCs	Monotypic cell culture	iPSCs were reprogrammed and differentiated into endoderm to form organoids, which matured in differentiation medium (DM)	36
		Polytypic cell co-culture	The cells were differentiated into hepatocytes and co-cultured with human umbilical vein endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) to self-organize into 3D aggregates	
Lung organoid	PSCs	Scaffold-free system culture method carrier-based scaffolding System method	PSCs form anterior foregut spheres. They differentiate into germ layers and mature into lung organoids via FGF and HH factors	37,38
	ASCs		Alveolar type II epithelial cells (AEC2s) were co-cultured with other cells to form alveolar-like organoids	46
	LCOs		Lung cancer cells were embedded in Matrigel with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to induce organoid formation	39
Brain organoid	PSCs	Unguided method	The gametophyte differentiates into neural ectoderm embedded in matrix gel for 3D culture, which is then transferred to a rotating bioreactor	40
		Guided methods	By regulating signaling pathways (e.g., BMP and Wnt), directed cell differentiation is induced. Adjusting factor intensity promotes tissue self-organization, generating functional brain organoids via long-term culture	41,42
Intestinal organoid	PSCs	–	Crypts from biopsy were embedded in matrix gel and cultured in normal colon medium with 50% Wnt3a for expansion, then generated organoids via normal colon medium culture and intestinal epithelial differentiation medium	
	ASCs			47
Bone organoid	PSCs	–	PSCs form mesoblastic aggregates, differentiate into vascular/hematopoietic cells, and generate mature bone organs under specific conditions	43
	BMSCs		A PCL-based 3D-printed scaffold embedded with GelMA/SFMA hydrogel and PTH@MSNs formed a porous scaffold for repairing large weight-bearing bone defects	44

3D, three-dimensional; ASCs, adult stem cells; BMP, bone morphogenetic protein; BMSCs, bone marrow mesenchymal stem cells; FGF, fibroblast growth factor; GelMA/SFMA, gelatin methacrylate/Methacrylated silk fibroin; HH, hedgehog; iPSCs, induced pluripotent stem cells; LCOs, lung cancer organoids; PCL, polycaprolactone; PSCs, pluripotent stem cells; PTH@MSNs, parathyroid hormone-loaded mesoporous silica nanoparticles.

and pH balance within the body by filtering plasma, facilitating the reabsorption of essential nutrients, and secreting metabolic waste.[48] Kidney organoids are 3D *in vitro* models derived from PSCs under 3D culture conditions.[49] These organoids contain epithelial renal unit-like structures expressing markers associated with podocytes, proximal tubules, medullary collaterals, distal tubules, and collecting ducts.[50] Organoids exhibit similarities to natural renal tissues in terms of cell types, morphology, and functional characteristics.[50,51] The construction of renal organoids is a multistage process involving differentiation of PSCs into primitive somite mesoderm.[36] The cells further differentiate into intermediate mesoderm, which then splits into two distinct embryonic tissues: the metanephric mesenchyme and the ureteric bud.[36] Currently, two primary methodologies are employed in the induction of renal organoids. CHIR99021, an activator of the Wnt signaling pathway, has been demonstrated to modulate both the fibroblast growth factor (FGF) pathway and the bone morphogenetic protein (BMP)/FGF pathway (Fig. 2a).[52–59] The Wnt signaling pathway plays a crucial role in maintaining the balance between nephron progenitor cell expansion and mesenchymal-epithelial transition.[60–62] The FGF signaling pathway is essential for maintaining nephron progenitor cells, and BMP4 promotes ureteric bud formation.[60–62]

Liver organoid

The liver performs essential functions such as metabolism, protein synthesis, and detoxification of xenobiotics.[63] It also exhibits remarkable regenerative abilities following severe injury. The primary functional cell types in the liver are hepatocytes of endodermal origin and cholangiocytes.[63] The formation of liver organoids can be conceptualized through two distinct culture systems: monotypic cell culture and polytypic cell co-culture.[64] In monotypic cell culture systems, liver organoids generated from iPSCs and ASCs typically further differentiate into mature hepatocytes or hepatobiliary organoids (Fig. 2b).[64] These cells differentiate into a high proportion of mature hepatocytes, which generally exhibit albumin production, urea synthesis, and cytochrome P450 activity.[64] The polytypic cell co-culture system is employed to create vascularized and transplantable organoids designed to preserve signaling interactions between mesenchymal and parenchymal cells, which are vital for normal tissue growth and functionality (Fig. 2c).[64] Compared to monotypic cultures, polytypic cell co-cultures more realistically mimic human liver metabolic processes. In long-term culture, primary liver organoids show less genomic structural variation than PSC-derived ones,[65,66] retaining and stably maintaining the genetic traits of their original donors.[65,66] Currently, liver organoids are primarily generated from the foregut endoderm through activation of the Wnt, FGF, BMP, and Activin/Nodal signaling pathways.[67] Hyperactivation of the Wnt signaling pathway leads to hepatic stagnation.[68]

Lung organoid

The lung is one of the most complex organs in the human body, consisting of more than 40 cell types that form a unique structure for its primary function of effective gas exchange.[69] Lung organoids are composed of a variety of cells, including lung epithelial cells, myofibroblasts, upper airway-like epithelial cells, and mesenchymal compartments.[53] The emergence of tracheal structures is a critical indicator of successful lung organoid construction.[53] Currently, there are two main lung organoid culture methods.[70] One is a scaffold-free system used in ALI cultures, designed to enhance cell-to-cell contact, leading to the formation of lung spheroids, which subsequently develop into lung organoids. The other approach involves a carrier-based scaffolding system, primarily utilizing Matrigel. The ECM within Matrigel provides essential nutrients and channel proteins, facilitating the growth and development of lung organoids (Fig. 2d). Lung organoids can be categorized into mature lung epithelial stem cell-derived organoids, PSC-derived lung organoids, and lung cancer organoids (LCOs). Mature lung epithelial stem cells are directly cultured *in vitro* and induced to generate lung organoids.[37,38] PSC-derived lung organoids induce PSCs to form anterior foregut spheroids, which then differentiate into specific germ layers via modulation of growth factors such as FGF and hedgehog signaling.[37,38] Further differentiation directs these germ layers towards mature lung organoids.[37,38] PSC-derived lung organoids provide a solution to the challenge of isolating lung stem/progenitor cells, especially in diseased lungs, and are currently the most commonly employed method for lung organoid cultivation. LCOs are created by transplanting lung cancer stem cells from patient tissues into Matrigel, with growth factors added to promote proliferation and differentiation.[39]

Brain organoid

Brain organoids are self-assembled 3D aggregates generated from PSCs, containing cell types and cellular structures similar to those of the embryonic human brain.[71] The construction of brain organoids can be categorized into two approaches: unguided and guided methods.[71] The unguided method, which aims to form the whole brain, does not utilize pattern-based growth factors (Fig. 2e). Instead, it focuses on enhancing growth conditions and creating an environment conducive to intrinsic signaling pathways that guide development.[72] Neural ectoderm is generated from embryoid bodies, then embedded in Matrigel for 3D culture, after which the Matrigel droplets are transferred to a rotating bioreactor.[40] This method allows rapid brain tissue development and can be used to study interactions between various brain regions. However, it can result in contamination by non-neural lineages such as mesoderm and endoderm and leads to high variability among organoids in terms of structure and cell composition.[73] In contrast, guided methods use exogenous patterning factors to direct neuroepithelial development, guiding hPSCs to recapitulate the structural organization of specific brain regions.[40] Examples include organoids modeling the cerebral cortex,[41] hippocampus,[42] and midbrain.[74] The organoid differentiation protocol, known as the bootstrap method, can be customized by adding external patterning factors exclusively at the early differentiation stage to allow hPSCs to become progenitors characteristic of specific brain regions (Fig. 2f).[71] Once successful patterning occurs, these factors are removed, and subsequent differentiation mirrors *in vivo* neural patterning.[71] Directed organoid cultures produce a relatively uniform mixture of cell types with minimal variation between batches and cell lines.[75]

Intestinal organoid

The intestines are a pivotal component of the digestive system, playing critical roles in food digestion, nutrient absorption, and metabolism.[76] Intestinal organoids are derived from crypt-like units containing $Lgr5^+$ stem cells,[76] which can originate from adult intestinal stem cells and PSCs. These organoids retain stem cell characteristics, reflecting the activity of intestinal epithelial stem cells with the capacity to proliferate and differentiate (Fig. 2g).[76–78] They can also be stably cryopreserved for long-term self-renewal.[76–78] The primary method for constructing intestinal organoids involves isolating crypts from human intestinal tissues, pre-

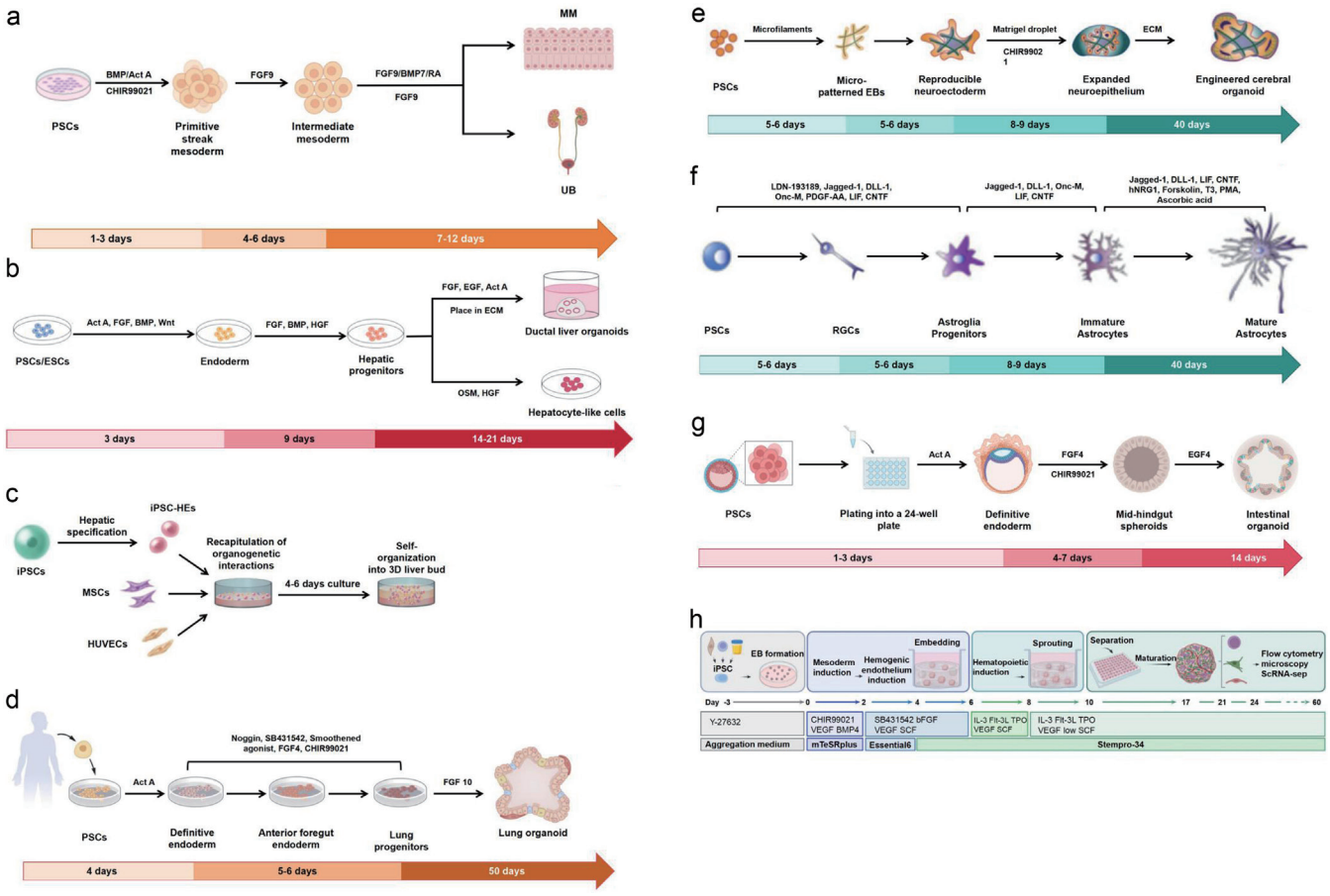

Fig. 2. Schematic diagram of the construction of representative organoid models.[52,53,46,54–59] (a) Schematic diagram of kidney organoids constructed by BMP/FGF and CHIR99021-FGF pathways. BMP/FGF pathway: PSCs differentiate into PSM via BMP4/Act A, then into IM via FGF9. IM further differentiates into MM and UB via FGF9/BMP7/RA. CHIR99021-FGF pathway: PSCs differentiate into PSM via Wnt agonist CHIR99021, then into IM via FGF9, and subsequently into MM and UB. (b) Schematic diagram of constructing liver organoids by monotypic cell culture method. PSCs differentiate into endoderm cells via Act A, FGF, BMP, and Wnt signaling. Endoderm cells become liver progenitor cells when induced by BMP, FGF, and HGF. Liver progenitor cells respond to OSM signals to form hepatocyte-like cells. Alternatively, culturing liver progenitor cells in ECM with FGF, EGF, and Act A modulation generates duct-like organoids. (c) Schematic diagram of constructing liver organoids by polytypic cell co-culture. iPSCs are specified toward hepatic lineages to generate iPSC-HEs. Subsequently, iPSC-HEs, MSCs, and HUVECs are co-cultured to recapitulate intercellular interactions during organogenesis. Cells influence each other via secreted factors, mimicking signal exchange in *in vivo* liver development. After four to six days of culture, a 3D hepatic bud is formed. (d) Schematic diagram of constructing lung organoids by the carrier-based scaffolding system method. Human-derived PSCs are differentiated into DE via Activin A treatment. Subsequently, DE cells are further induced into FE and then LPCs using a cocktail of factors including Noggin, SB431542, Smoothened agonist, FGF4, and CHIR99021. Finally, LPCs are cultured with FGF10 for 50 days to generate lung organoids. (e) Schematic diagram of brain organoids constructed by the non-guided method. Starting from PSCs, these cells interact with microfilaments to form micropatterned EBs. The embryoid bodies then develop into reproducible neuroectoderm. Combined with Matrigel droplets and CHIR99021 supplementation, the neural epithelium expands. Finally, under the influence of ECM, these cells differentiate and self-organize over 40 days to form engineered brain organoids. (f) Schematic diagram of constructing brain organoids by guided methods. PSCs differentiate into RGCs via LDN-193189, Jagged-1, and DLL-1. Subsequently, RGCs transition to astroglia progenitors under Jagged-1, DLL-1, and Onc-M. These progenitors develop into immature astrocytes with Jagged-1, DLL-1, and LIF, and finally mature into mature astrocytes over 40 days with the same factors. (g) Schematic diagram of intestinal organogenesis. PSCs are seeded onto Matrigel-coated 24-well plates. Upon reaching 80–95% confluence, cells are treated with Activin A for three days to form definitive endoderm. Subsequently, endodermal cells are treated with posterior gut induction medium containing FGF4 and CHIR99021 to induce mid/hindgut spheroids. These spheroids are then embedded in Matrigel and cultured in intestinal growth medium supplemented with EGF to generate HIOs. (h) Schematic diagram of bone marrow organoids. iPSCs form EBs in Y-27632 medium. Mesoderm is induced with mTeSR Plus medium + BMP4, CHIR99021, and VEGF. On day 2, medium is switched to Essential 6 + VEGF. On day 4, EBs are collected and overlaid with StemPro-34 SFM + IL-3 and cytokines. Cytokines are switched on days 6 and 8. On day 10, sprouting EBs are harvested, transferred to 96-well plates, and self-assemble into spherical organoids. Act A, activin A; BMP, bone morphogenetic protein; CNTF, ciliary neurotrophic factor; DE, definitive endoderm; DLL-1, delta-like ligand 1; EB, embryoid body; ECM, extracellular matrix; EGF, epidermal growth factor; ESCs, embryonic stem cells; FE, foregut endoderm; FGF, fibroblast growth factor; Flt-3L, fms-like tyrosine kinase 3 ligand; HE, hematopoietic stem cells; HGF, human growth factor; HIOs, human intestinal organoids; hNRG1, human Neuregulin-1; HUVECs, human umbilical vein endothelial cells; IL-3, interleukin-3; IM, intermediate mesoderm; iPSC, induced pluripotent stem cells; iPSC-HEs, iPSC-derived hepatocyte-like cells; LIF, leukemia inhibitory factor; LPCs, lung progenitor cells; MM, metanephric mesenchyme; MSCs, mesenchymal stem cells; Onc-M, oncostatin M; OSM, oncostatin M; PDGF-AA, platelet-derived growth factor-AA; PMA, phorbol-12-myristate-13-acetate; PSCs, pluripotent stem cells; PSM, primitive streak mesoderm; RA, retinoic acid; RGCs, radial glial cells; SCF, stem cell factor; scRNA, single-cell RNA; TPO, thrombopoietin; UB, ureteric bud; VEGF, vascular endothelial growth factor. *Some images were created with BioRender.*

Table 3. Applications of organoids in disease modeling

Organs	Sources	Disease models	Applications	References
Kidney	RCC tumor samples	RCC	To determine effective treatments for individual RCC patients	89
	HiPSCs	Cisplatin-induced acute kidney injury (AKI) model	Validation of the feasibility of using human kidney organoids to mimic AKI from chemotherapeutic agents *in vitro*	94
Intestinal	ASCs	Intestinal organoids infected with norovirus	Uncovering the replication regulation mechanism of norovirus	90
		Colorectal cancer organoids	Revealing the regulatory role of DKK2 (Wnt signaling pathway antagonist) in colorectal cancer	95
Brain	HiPSCs	Brain organoids infected with the Zika virus	Studying the effects of Zika virus infection during neurogenesis and growth	91
Heart	HiPSCs	MI organoid	Organotypic features that summarize the short-term injury state after acute MI	92
Liver	HiPSCs	Polycystic liver disease and CF organoid	Reproducing the effects of the therapeutic compounds Verapamil, Octreotide, and VX809	93
Lung	Lung tissue from adult C57B/L6 mice	Small cell lung cancer (SCLC) model	Revealing the role of KMT2C gene in SCLC	96

ASCs, adult stem cells; CF, cystic fibrosis; DKK2, dickkopf-related protein 2; HiPSCs, human induced pluripotent stem cells; MI, myocardial infarction; RCC, renal cell carcinoma.

paring a buffer, and formulating a culture medium.[79] This medium is supplemented with growth factors such as Wnt, R-spondin1, and Noggin. The addition of insulin-like growth factor 1 and FGF-2 facilitates long-term passaging and multilineage differentiation.[80] Renewal and differentiation processes in intestinal organoids are regulated by the Wnt and Notch signaling pathways.[81] Administration of CHIR99021 (a Wnt activator) and valproic acid (a Notch activator) has a synergistic effect that promotes self-renewal.[82] Compared to intestinal stem cell-derived intestinal organoids, PSC-derived organoids retain more pronounced fetal-like characteristics.[82] Furthermore, co-culture of PSC-derived intestinal organoids with endothelial cells promotes maturation.[83]

Bone organoid

Bone organoids are *in vitro* tissue models prepared using stem or progenitor cells, featuring a 3D, miniaturized, and simplified structure. They mimic the structure, function, and cellular complexity of natural bone tissue, including crucial aspects such as mineralization and angiogenesis (Fig. 2h).[84,85] PSCs can be reprogrammed from adult cells to restore pluripotency,[86] enabling the formation of specific cell types and their self-assembly into 3D structures that mimic the bone marrow microenvironment, allowing development of osteochondral organoids.[43,86,87]

Researchers have constructed bone organoids with self-mineralization ability and pluripotent differentiation potential by integrating bone mesenchymal stem cells with bone matrix-mimicking bioinks. Bone mesenchymal stem cells-embedded gelatin methacrylate microspheres were prepared and subjected to chondrogenic induction, resulting in bone callus organoids.[44] In bone tissue engineering, bone organoids have various applications: they can be used to fabricate composite scaffolds that repair large weight-bearing bone defects, enhance osteogenesis and angiogenesis, and mimic natural bone tissue.[44] Additionally, bone organoids serve as models for drug screening and evaluation in disease research and drug development, helping researchers study disease mechanisms.

Organoid applications in biomedicine

Organoids are pivotal in drug discovery and development. By emulating the complexity of the human environment and establishing *in vitro* models, organoids facilitate the rapid screening of pharmaceuticals. Moreover, they offer more authentic and dependable frameworks for toxicity assessment. Beyond their role in drug discovery, organoids hold immense potential in disease modeling. They provide substantial support for the thorough examination of disease mechanisms, thereby catalyzing novel advancements in medical research and clinical practice.

Disease modeling

Organoid technology generates 3D tissues *in vitro* that accurately reproduce the structure, function, molecular features, genomic alterations, expression profiles, and TME of primary tumors (Table 3).[88–96] Kazama *et al*.[89] developed patient-derived tumor organoids from renal cell carcinoma tumor samples. They demonstrated that renal cell carcinoma tumor organoids can replicate the histological features of clinical tumors *in vitro* while preserving their genomic integrity during long-term culture.[89] Organoid technology can also reveal viral pathogenesis and host cell responses. In a study on norovirus, researchers cultivated norovirus strains using intestinal organoids and found that bile was a pivotal factor in strain-dependent norovirus replication.[90] Zika virus is a flavivirus transmitted through mosquitoes and sexually.[97,98] In a seminal study, Patricia P. Garcez and colleagues exposed hiPSC-derived brain organoids to the Zika virus.[91] Published in the journal Nature, the study revealed that Zika virus-induced neuronal cell death occurs in the early stages of brain development. Furthermore, organoid technology plays a pivotal role in studying the pathogenesis and clinical characteristics of complex multi-system diseases. Richards' team developed an *in vitro* 3D model of post-myocardial infarction myocardial tissue.[92] This model was created using a combination of an oxygen diffusion gradient and chronic adrenergic stimulation to mimic the human heart after myocardial infarction. The model closely resembles transcriptome data from both an animal model

Table 4. Applications of organoids in drug screening

Organoids	Sources	Screening drugs	References
CRC organoids	Patient-derived colonic tissue	Fedratinib, trametinib, bortezomib	99
BTC organoids	Patient-derived biliary tissue	Amorolfine, fenticonazole	100
Pancreatic ductal adenocarcinoma organoids	Cell lines of pancreatic ductal adenocarcinoma	irbesartan	101
Lung organoids	hPSCs in patients with new crowns	Imatinib, articaine hydrochloride	102
Cervical cancer organoids	Patient-derived cervical tumor tissue	Bortezomib, MLN2238, MLN9708, carfilzomib, panobinostat, romidepsin, homoharringtonine	103
Brain organoids	Alzheimer's disease patients' iPSCs	Flibanserin, ripasudil, everolimus	104
Kidney organoids	hiPSCs	GFB-887	105
Breast cancer organoids	Patient-derived xenografts	Erbaxane, fluorouracil	106

BTC, biliary tract carcinoma; CRC, colorectal cancer; hiPSCs, human induced pluripotent stem cells; hPSCs, human pluripotent stem cells; iPSCs, induced pluripotent stem cells.

of myocardial infarction and human ischemic cardiomyopathy tissue following acute infarction. Fotios Sampaziotis and colleagues successfully constructed disease models of polycystic liver disease and cystic fibrosis (CF)-associated liver disease using patient-derived iPSCs.[93] They found that the experimental CF drug VX809 rescued the disease phenotype of CF cholangiopathy *in vitro*. Organoid disease modeling offers significant potential to enhance our understanding of organ development, maintain homeostasis, and identify disease mechanisms, thereby paving the way for innovative advancements in diagnostic and therapeutic strategies.

Drug screening

Organoids provide a more accurate model of physiological conditions for drug development, making them valuable tools for testing drug efficacy and safety, as well as screening potential drug candidates (Table 4).[99–106] Recent reports show that patient-derived cancer organoids have great accuracy in predicting cancer patients' responses to various drugs.[99,107,108] These organoids not only provide closer models of real physiological conditions but also serve to test drug efficacy and safety and screen drug candidates.[99,107,108] Mao and colleagues developed an organ-based drug screening system to evaluate the anticancer effects of 34 drugs on human colorectal cancer.[99] Through this screening, they identified fedratinib, trametinib, and bortezomib as anticancer agents with notable efficacy.[99] Saito *et al*.[100] developed a drug compound library to inhibit organoids derived from biliary tract carcinomas. Their findings demonstrated that the antifungal drugs amorolfine and fenticonazole significantly inhibited the growth of biliary tract carcinoma-derived organoids with minimal toxicity to normal biliary epithelial cells.[100] The development of organoid-based high-throughput screening (HTS) marked a significant advancement in drug screening applications.[101] Zhou and colleagues established a biobank of human pancreatic ductal adenocarcinoma organoid models.[101] They discovered that irbesartan reversed resistance in these cells via the Hippo/YAP1/c-Jun/stemness/iron metabolism axis, as observed through HTS.[101] Han *et al*.[102] performed HTS of Food and Drug Administration (FDA)-approved drugs using SARS-CoV-2-infected lung organoids and identified potential clinical candidates, including imatinib and articaine hydrochloride. Organoids have thus provided a novel technological tool for large-scale drug screening, highlighting their ability to maintain histological characteristics long-term.

Biobank

A biobank is defined as a comprehensive repository of biospecimen entities, bioinformatic specimen phenotypic data, and specimen research information.[109,110] The primary purpose of a biobank is to support scientific research, clinical diagnostics, and drug discovery and development.[109,110] Additionally, biobanks facilitate the long-term preservation of biospecimens.[109,110] In recent years, biobanks of organoids originating from various types have been established, enabling the preservation of histological characteristics and increasingly used in disease modeling (Table 5).[39,111–116] These include intestinal,[111] breast cancer,[112] lung,[117] ovarian,[113] kidney,[118] liver,[114] and other organoids. Van de Wetering's team was the first to establish a living colorectal cancer organoid biobank, highlighting their potential in individual patient-level genomic and functional studies through organoid culture platforms.[111] Fadi Jacob and colleagues established a biobank composed of glioblastoma organoids consisting of 70 biological samples from a variety of patients.[115] These glioblastoma organoids reflect substantial genomic alterations associated with glioblastoma pathogenesis and maintain numerous critical characteristics of their respective parental tumors.[115] This biobank offers substantial support for future research in glioblastoma biology and the assessment of therapeutic approaches.[115] Minsuh Kim and colleagues have established a biobank of LCOs, derived from 95 subtypes of lung cancer and covering more than 56% of lung cancer patients, providing significant data to support drug screening, genomic research, and immunotherapy-related studies.[39]

Precision medicine

Van de Wetering's team was the first to establish a living colorectal cancer organoid biobank, demonstrating its potential for genomic and functional studies at the individual patient level.[119] Consequently, selecting a more targeted and individualized treatment regimen remains a significant challenge in improving efficacy. Organoid models derived from patients' autologous cells have emerged as a promising solution to address this critical gap.[119] These models offer a unique opportunity to assess the efficacy and potential toxicity of multiple drugs in patients, thereby facilitating the development of personalized intervention strategies.[119] Berkers' team quantified the response to CF transmembrane conductance regulator modulators *in vitro* by evaluating *in vitro* forsko-

Table 5. Application of the organoid biobank

Organoids	Sources	Names	Applications	References
Intestinal organoids	Patient-derived colorectal cancer tissue	Biobank of living colorectal cancer organoids	Demonstrating that organoid culture platforms can be used for genomic and functional studies at the individual patient level	111
GBOs	Patient-derived glioblastoma tissue	Biobank of GBOs	Reflects major genomic changes associated with glioblastoma pathogenesis	115
Lung organoids	Patient-derived lung cancer tissue	Biobank of LCOs	To summarize the histological and genetic features of lung cancer subtypes while maintaining genomic heterogeneity with the original patient genomes	39
Breast cancer organoids	Patient-derived breast cancer tissue	Biobank of breast cancer organoids	Genetic characterization of breast cancer organoids confirmed and the development of drugs targeting breast cancer characteristics	112,116
Ovarian organoids	Patient-derived ovarian cancer tissue	Biobank of high-grade serous ovarian cancer (HGSC)	Addressing the limited availability of fresh tumor material for HGSC organoid studies	113
Liver organoids	Primary liver cancer tissue	Biobank of liver cancer organoids	Reproducing the proliferative and metabolic subtypes of hepatocellular carcinoma and the worst/best prognostic subtypes of intrahepatic cholangiocarcinoma	114

GBOs, glioblastoma organoids; LCOs, lung cancer organoids.

lin-induced swelling in rectal organoids from patients.[120] Their findings demonstrated that CF transmembrane conductance regulator modulation correlates with two independent indicators of *in vivo* treatment response (percent predicted forced expiratory volume in 1 second and sputum cell count).[120] Furthermore, the team showed that biobanked stem cell resources can be correlated with *in vivo* therapeutic response.[120] This suggests that the biobank's stem cell resources can be utilized to personalize treatments for individual patients.[120] Organoid technology has been particularly used in precision medicine for tumors. PDOs have been demonstrated to preserve the gene expression and histopathological characteristics of tumor tissues. Furthermore, they accurately mimic the tumor's response to drugs in the patient's body.[8] Using organoids derived from a patient's tissue, HTS can help clinicians understand the patient's disease characteristics, drug response, and therapeutic efficacy, enabling the development of personalized treatment plans.[8] Ji Shuyi and colleagues established a biobank housing patient-derived hepatocellular carcinoma organoids. They demonstrated the synergistic inhibitory effect of tesirolimus combined with lenvatinib in both organoid models and patient-derived xenograft experiments.[114] This research highlights the potential for personalized treatment strategies based on individual tumor characteristics.[114]

Toxicity assessment

Stem cells exhibit varying sensitivities to exogenous chemicals depending on whether they are undergoing proliferation or differentiation.[121] Conducting toxicological studies based on organoid formation processes helps evaluate the developmental toxicity of chemicals, identify the sensitive periods during which chemical action occurs, and pinpoint molecular targets related to toxicity pathways.[121] This approach facilitates a deeper understanding of how external substances affect cells at different stages (Table 6).[121–128] Leite's team developed a functional human liver organoid capable of

Table 6. Application of organoids in toxicity assessment

Organs	Sources	Applications	References
Liver	HepaRG cells and primary human HSCs	APAP induces activation of HSCs	122
	Human liver tissue	CPZ and TNF-α synergistically induce intrahepatic bile duct injury	125
Ovarian	Female germ-line stem cell (FGSC)	Salinomycin damages ovarian organoids by inducing apoptosis	123
Kidney	PSCs	Tenofovir, aristolochic acid, and cisplatin induce proximal renal tubular injury	124
	iPSCs	Accelerated tacrolimus nephrotoxicity by rapamycin (autophagy inducer) treatment	127
Lung	iPSCs	Two drugs, GNE7915 and amiodarone, were studied, and amiodarone was found to cause a more severe lung injury phenotype	128
Brain	ESCs	Co-exposure of As and Pb interferes with neural and retinal development and activates proteins associated with carcinogenesis	126

APAP, acetaminophen; As, arsenic; CPZ, chlorpromazine; ESCs, embryonic stem cells; HSCs, hepatic stellate cells; HSCs, hepatic stellate cells; iPSCs, induced pluripotent stem cells; Pb, plumbum; PSCs, pluripotent stem cells; TNF-α, tumor necrosis factor-alpha.

identifying acetaminophen as a substance inducing hepatic fibrosis and activating hepatic stellate cells.[122] This liver organoid represents the first model able to detect compound-induced hepatic stellate cell activation.[122] Li's team developed an ovarian organoid model derived from germline stem cells.[123] This model was utilized to ascertain that salinomycin impedes ovarian organoid formation and germ cell populations by inducing apoptosis.[123] Koichiro Susa and his team investigated the effects of three drugs—tenofovir, aristolochic acid, and cisplatin—on kidney cells.[124] They discovered that at low concentrations, these drugs caused injury in the proximal renal tubular area without damaging podocytes.[124] Using a specialized kidney organoid model, they evaluated the toxicity of these drugs.[124] In their study, Wang *et al*.[125] employed cholangiocellular organoids to model chlorpromazine-induced intrahepatic bile duct injury. Their findings showed that chlorpromazine itself did not induce an inflammatory response, but when combined with TNF-α, it resulted in a synergistic effect.[125] The application of organoids in the assessment of environmental pollutants, including heavy metals and pesticides, is a promising field of research. These organoids can replicate the effects of pollutants on human organs, thereby facilitating the evaluation of health risks posed by environmental toxins. Chen's team methodically examined the neurotoxicity of arsenic and lead by utilizing optic vesicle brain organoids.[126] Their findings revealed that concurrent exposure to arsenic and lead impeded neural and retinal development, while also triggering proteins linked with carcinogenesis in optic vesicle brain organoids.[126]

Application of organoids in TCM

Organoids have unique advantages. They reproduce organ morphology and function through a three-dimensional structure, simulate the in vivo microenvironment with multiple cell types, and allow real-time dynamic observation.[129] With these advantages, organoids integrate the holistic view of TCM and modern molecular biology across scales. They provide technical support for drug efficacy screening, target identification, and safety evaluation in the field of TCM.[129]

Screening of active components in TCM

TCM has diverse chemical components and complex interactions between these components. This makes the accurate identification and screening of its pharmacologically active substances a bottleneck in the modernization research of TCM. Organoid technology simulates the complex molecular network regulation process mediated by TCM components after they enter the human body, thus solving the problem of screening active components of TCM. Take the classic TCM formula Pien Tze Huang (PZH) as an example. Its main ingredients include Moschus (musk), Calculus Bovis (bezoar), Fel Serpentis (snake bile), and Radix Notoginseng (notoginseng root).[130,131] Researchers constructed a colorectal cancer organoids model to screen for active components in PZH. The results showed that ginsenoside F2 and ginsenoside Re, two components contained in PZH, can inhibit the growth of colorectal cancer organoids.[132] In the field of liver cancer research, the natural compound Omuralide A can directly target transketolase and activate p53 signaling, thereby inhibiting the growth of hepatocellular carcinoma organoids.[133]

Research on the pharmacological mechanism of TCM

TCM compound prescriptions contain a variety of active components, which exert their effects through multiple pathways and targets.[134] Organoid technology evaluates the effects of each component on specific cells or tissues via high-throughput screening, and thus becomes an important tool for studying the multi-target mechanism of action of TCM.[134] In the research on TCM against Alzheimer's disease, brain organoids are used. They confirm that ginseng polysaccharide (GP) can do two things: inhibit the accumulation of amyloid-β and downregulate neuroinflammation.[135] Amyloid-β is the main component of amyloid plaques. The mechanism of GP is related to promoting neuronal mitophagy. This provides support for GP to become a candidate drug for anti-Alzheimer's disease.[135] In anti-tumor research, researchers established a gastric cancer organoid model. They found that decursin can inhibit the growth of gastric organoids and regulate the expression of cathepsin C and autophagy-related proteins.[136] This study reveals a new mechanism by which decursin regulates cell growth and autophagy. It also indicates that decursin may become a potential anti-cancer drug that inhibits both cell growth and autophagy.[136]

Safety assessment of TCM

TCM has complex components. Natural toxic components (such as alkaloids and glycosides), improper processing or compatibility, and excessive use may all cause toxicity risks.[137] Therefore, scientific evaluation is needed. It can identify safe doses, toxic target organs and mechanisms of action. This provides a basis for medication in special populations.[137] In the research on TCM toxicity evaluation, organoid technology has shown unique value. For example, esculentoside A is the main toxic component of Phytolaccae Radix (pokeweed root).[138] Researchers conducted toxicity evaluation using kidney organoids. They confirmed two things: esculentoside A has nephrotoxicity. And its nephrotoxicity is related to epithelial-mesenchymal transition mediated by the STING signaling pathway.[138] In the research on the protective effects of TCM, Haiyang Chen and his team constructed a brain organoid model. They studied the intervention effect of Codonopsis pilosula polysaccharides (CPPs) on rotenone-induced neuronal toxicity.[139] The results showed two things: first, CPPs can effectively improve the cytotoxicity of brain organoids caused by rotenone; second, CPPs can correct the abnormal DNA methylation induced by rotenone.[139] This provides reliable experimental support for the research on the toxicity protection mechanism of TCM components.

Drugs need to be absorbed by the intestines and metabolized by the liver before acting on target organs. In vitro single-organ models cannot simulate the complex metabolic process of TCM in the human body. Traditional animal models also have this limitation.[140] In the future, the integration of organoids and TCM can be promoted in three aspects. First, optimize models. Strengthen the simulation of specific pathological organs (such as liver injury and intestinal barrier dysfunction), improve the functional maturity of intestinal and liver organoids, and replace animal models to reduce species interference.[134] Second, build "multi-organ organoid chips" through interdisciplinary cooperation. These chips can reproduce the complete action path of TCM—from entering the human body to exerting therapeutic effects.[134] Third, innovative applications. Assist in TCM syndrome differentiation and typing as well as individualized medication, and accelerate the translation of TCM.[134] Its value can also be explored in the research of traditional non-pharmacological therapies. These include analyzing the mechanism of acupuncture effects and conducting in vitro verification of yoga's regulation on the function of specific organs.

Frontier technologies of organoids

As a cutting-edge field in 3D cell culture, organoid technology

provides revolutionary tools for life science and medical research by mimicking organ development and physiological functions. Its rapid advancement stems from the interdisciplinary integration of gene editing, single-cell sequencing, 3D bioprinting, and artificial intelligence (AI).

Gene editing technology

Organoids simulate *in vitro* organ development and physiological functions, while gene editing technologies knock in, knock out, or modify specific genes to precisely explore gene function mechanisms in development, disease, and other processes.[141] For example, in testicular organoids, researchers knocked out outer dense fiber 2 and intraflagellar transport 88 genes to inhibit primary cilia formation.[142] Results showed reduced cilia numbers, shortened cilia lengths, and affected *in vitro* tubule morphogenesis.[142] Beyond labeling cell types to study migration, growth, and development, gene editing technologies can model diseases. For example, researchers introduced the LRRK2-G2019S mutation via gene editing to generate midbrain-specific organoids from Parkinson's disease patients.[143] These studies demonstrate the potential of combining genome engineering with organoid technology, enabling in-depth exploration of developmental processes in different organs and the roles of specific molecular pathways.

Single-cell RNA sequencing (scRNA-seq)

scRNA-seq enables high-throughput transcriptomic profiling of individual cells. It compares genetic information, reveals intercellular heterogeneity, and identifies distinct cell subpopulations to explore disease progression.[144] It is primarily used to analyze interactions between cell subpopulations and gene regulatory processes.[145] Zhao *et al*.[146] established seven hepatobiliary tumor organoids and used scRNA-seq to detect cancer stem cell markers. They found that cluster of differentiation 44-positive cell populations may induce drug resistance in hepatocellular carcinoma 272.[146] Additionally, while integrating scRNA-seq into routine clinical diagnosis and personalized medicine, most current scRNA-seq-based clinical studies remain exploratory, mainly focusing on re-evaluating and better understanding disease processes, as well as identifying diagnostic and therapeutic markers.[147]

3D printing technology

Bioprinting techniques use bioinks made of biocompatible nonliving materials and cells to build 3D constructs in a controlled manner and with micrometric resolution.[148] Compared to 2D cell cultures and animal models, 3D bioprinted organoid models better recapitulate native tissue architecture, intercellular interactions, and cell-ECM crosstalk.[149] In bone tissue engineering, researchers combined 3D printing with polycaprolactone scaffolds, parathyroid hormone-loaded mesoporous silica nanoparticles, and gelatin methacrylate/methacrylated silk fibroin composite hydrogels to create porous PM@GS/polycaprolactone scaffolds for repairing large load-bearing bone defects.[44] A study used 3D bioprinting to create 3D structures from iPSC-derived cortical neurons and glial cells, which correctly express neuronal and astrocyte markers.[148] This approach offers possibilities for generating more complex human neural 3D structures.[148] Additionally, 3D bioprinting can mimic the structure of neuromuscular tissue (NMT). The Jeong Sik Kong team developed a CNS-decellularized ECM bioink.[150] Using 3D printing, they created NMT with high contractility for long-term culture.[150] With the development of 3D bioprinting technology, it is expected to play a key role in disease research, drug development, tissue engineering, and regenerative medicine in the future.

AI technology

AI is a field of computer science that aims to develop computer systems capable of performing tasks that typically require human intelligence, such as visual perception, speech recognition, decision-making, and language translation.[151] The use of AI algorithms enables detailed analysis of complex organoid behavior, diverse cellular interactions, and dynamic responses to external stimuli.[152] Deep intelligent perception of images is an emerging technology in AI research, training on large datasets for image prediction, classification, and other complex image perception tasks.[153] Chen's team utilizes various 2D depth characteristics to approximate features in 3D medical images.[154] Researchers use an automatic detection method to detect cerebral microbleeds, employing a support vector machine classifier and magnetization rate-weighted imaging for this purpose.[154] The use of AI in analyzing organoid omic data helps researchers identify various cell types and track cell state changes, revealing natural processes of cellular growth and disease development.[155,156] This framework can identify conserved and specific developmental trajectories, as well as genes and functions expressed during development.[156] The integration of AI technology improves precision, objectivity, and the ability to handle complex data in organoid research, driving progress toward precision therapies with organoids.

Discussion

As a frontier in 3D cell culture, organoid technology provides revolutionary tools for life science and medical research by mimicking organ development and physiological functions. As a major research model for 3D cell culture in recent years, organoids have overcome the shortcomings of traditional 2D models in simulating cell growth, microenvironment, and genetic stability.[6] For instance, organoids retain properties of the original tumor tissue, including similar pathological features, gene expression patterns, and drug responses to the parental tumor.[157,158] Additionally, they can restore the ductal response in the fibrotic microenvironment.[157,158] PSC-derived organoids exhibit high stability at the chromosomal and structural levels, maintaining genetic integrity throughout months of culture.[65] Conventional mouse models fail to mimic human-specific microbial interactions, whereas organoids can create *in vitro* models closer to human physiology.[159] For example, while mouse models assess immune cell interactions and gut microbiota changes, they struggle to establish causality and cannot replicate human-specific microbial crosstalk.[159] In contrast, organoids construct physiologically relevant *in vitro* models to accurately study human microbial interactions, addressing the complexity of the gut microenvironment and challenges in real-time parameter evaluation. This enables more effective deduction of causal relationships between hosts and microbiota.[159] In salivary gland organoid culture, single-cell dissociation is avoided to maintain cellular diversity, as traditional methods may cause cell transformation through dissociation.[160] Organoid culture can also induce cell differentiation by adjusting culture conditions and adding specific substances (e.g., DAPT) to study cell fate changes, which are difficult to accurately model and investigate in mouse models.[160]

Although organoid technology bridges the gap between cell lines and *in vivo* models, current systems still face challenges. The difficulty of accurately replicating the natural TME, ethical concerns related to controversial issues, and the high costs of research impede the advancement of organoid technology.

The ECM is a dynamic structure that continuously reshapes and controls tissue homeostasis.[11] Its components act as ligands for

cell receptors like integrins, constantly interacting with epithelial cells to transmit signals that regulate adhesion, migration, proliferation, apoptosis, survival, or differentiation.[11] Additionally, cells continuously rebuild and reshape the ECM through synthesis, degradation, recombination, and chemical modification.[12] Therefore, different batches of ECM differ in spatial structure and cytokine composition, making it difficult to mimic the immune components and blood vessels in the natural TME.[161] If the lack of TME in PDOs prevents tumor cells from forming clones, it leads to discrepancies between *in vitro* and *in vivo* models.[162] The future inclusion of PDOs with immune components from lymph nodes and blood has the potential to create more comprehensive models.[163] To address this challenge, co-culture organoid models incorporating immune cells, vascular systems, and cancer-associated fibroblasts have been developed.[163] These models can mimic tumor infiltration and peripheral immune cell populations in the TME and co-culture with immune cells from peripheral blood mononuclear cells or lymph nodes to simulate tumor immune processes, including T cell proliferation, activation, and tumor cell recognition.[163] Additionally, decellularization technology can bridge the gap between 3D culture assays and *in vivo* tumor models.[164] By analyzing decellularized normal and colon tumor tissues, scientists confirmed that the composition and arrangement of the ECM remain largely intact after decellularization.[165] Combining PDO models with decellularization technology and co-culture assays in the future may provide a more powerful platform for cancer research and anticancer drug screening.[164]

There are also concerns that when PDOs are transplanted into animals for culturing, it may lead to the entry of animal genes into the human genome, posing a genetic risk.[166] PDOs also address the human rights of donors, which must be distinguished from tissue organs derived directly from the human body.[167] However, there are no binding principles or legal norms that define the rights and obligations of donors and biobanks.[167] In the future, different types of organoids will likely be ethically ranked and evaluated, while also accounting for diverse cultural and social contexts.[168] Furthermore, sophisticated ethical value frameworks can be developed and implemented in a timely manner, taking into account both scientific and technological advancements as well as shifts in ethical concepts.[168]

The cost of organoids is currently a significant limiting factor in their widespread use. Acquiring high-quality, high-viability stem cells or primary cells is challenging, and cell processing and preservation require meticulous handling. Emerging organoid models, such as 3D bioprinted organoids, microfluidic organoid models, and genetically engineered organoids, further complicate material procurement and cost-effectiveness.[169,170] Additionally, determining whether organoids have functions similar to *in vivo* organs and validating their drug response consistency with *in vivo* conditions require integrating multiple detection technologies and analytical methods, which demand high expertise and experience from technicians.[169,170] To address this challenge, biobanks and shared sample repository systems are being established to provide standardized patient-derived cells, effectively reducing sample acquisition difficulties and costs.[169,170] In the future, organoid technology is expected to integrate gene editing and data-sharing platforms, enabling researchers to optimize material selection strategies and reduce resource waste. This will accelerate their transition from laboratory research to clinical applications.

The limitations of this review include insufficient discussion of emerging technical details (e.g., 3D bioprinting parameters, single-cell spatial transcriptomics) in organoid culture, inadequate coverage of clinical regenerative medicine applications (e.g., organ transplantation immunity, personalized cell therapy) and tissue-specific organoids (ophthalmic/cardiovascular), and a lack of systematic cross-species comparisons between human and animal organoids in disease modeling. These gaps suggest opportunities to deepen technical insights, integrate cross-disciplinary applications, and update research perspectives.

Conclusions

As an emerging 3D cell culture system, organoid technology has demonstrated substantial potential in basic research and translational medicine by recapitulating *in vivo* organ structures and functions. Generated through methods like ALI culture, bioreactor systems, and vascularization strategies, organoids create representative models of kidneys, livers, lungs, and brains for multi-dimensional simulations of organ development, disease pathogenesis, and drug responses. By mimicking the *in vivo* microenvironment, this technology plays a pivotal role in biomedical research, facilitating HTS, establishing physiologically relevant toxicity assessment models, and advancing disease modeling and biobanking for precision medicine.

This review also explores emerging organoid technologies, such as 3D bioprinting for scalable model fabrication, microfluidic systems for dynamic microenvironment control, and genetically engineered organoids for gene-disease association studies. These innovations address traditional limitations in model consistency and complexity, opening new frontiers for mechanistic research and clinical applications, as well as offering novel technical support for accelerating the modernization and translational application of TCM.

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Conflict of interest

Dr. Hongtao Jin has been an Associate Editor of *Future Integrative Medicine* since November 2021. Dr. Hongtao Jin is the chairman of Beijing Union-Genius Pharmaceutical Technology Development Co., Ltd., and Dr. Wanfang Li and Dr. Jie Bao are employees of the company. The authors have no other conflict of interest to note.

Author contributions

Writing–review & editing (XS, HYJ, XYF, XYD, HTJ), writing–original draft (XS, TSL), validation (XS), data curation (XS, HYJ, XYD, WFL, JB, BSH), investigation (XS, XYF, XYD, TSL, HTJ), conceptualization (XS, HYJ, XYF, WFL, JB, BSH, HTJ), methodology (HYJ), supervision (XYF, WFL, JB, BSH, HTJ), resources (WFL, JX, BSH, HTJ), and funding acquisition (HTJ). All authors have approved the final version and publication of the manuscript.

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