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# Organoids in clinical applications: advantages and risks

**Juzi Xia, Di Sun, Jiaming Sun**

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Organoids, clinical translation, disease modeling, personalized medicine, regenerative medicine, drug screening, engineering control, safety evaluation

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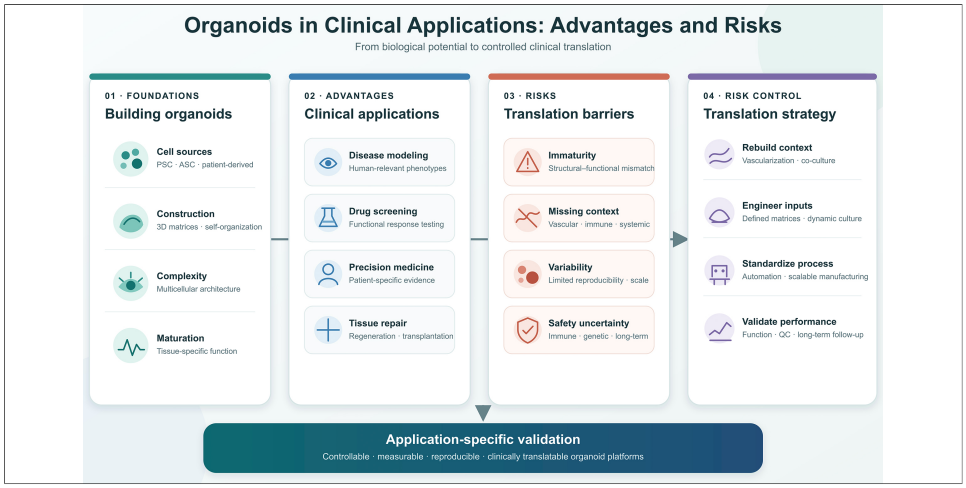


## Abstract

Organoids are self-organizing three-dimensional *in vitro* models generated from stem cells or patient-derived cells within defined culture systems, capable of recapitulating key aspects of human organs, including cellular composition, spatial architecture and functional characteristics. They have emerged as important platforms connecting basic research, drug development, precision medicine and regenerative therapy. This review focuses on the advantages and risks of organoids in clinical applications, summarizing their technical foundations, including cell sources, construction strategies, structural complexity and functional maturation, and discussing their applications in disease modeling, drug screening, personalized therapy and tissue repair. Owing to their high human relevance and three-dimensional modeling capacity, organoids can more faithfully reflect disease initiation and progression, improve therapeutic evaluation, and support precision and regenerative medicine. However, organoids are not complete replicas of native organs, and their clinical translation is limited by insufficient model validity, developmental immaturity, lack of vascular and immune microenvironments, inadequate engineering controllability and reproducibility, as well as safety concerns related to immune rejection, genetic instability and long-term outcomes. Current efforts aim to enhance clinical applicability through vascularization, microenvironment reconstruction, dynamic culture, defined matrices, automated manufacturing, quality control and safety evaluation. Future

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Department of Plastic Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China.

**Correspondence to:** Prof. Jiaming Sun, Department of Plastic Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China. E-mail: 2004xh0801@hust.edu.cn

development should establish integrated assessment frameworks encompassing molecular, structural and functional dimensions, enabling organoids to evolve from experimental models into controllable, measurable and clinically translatable platforms. This transition requires clearly defined application scenarios, standardized evaluation criteria and systematic understanding of model boundaries to ensure reliable interpretation and responsible clinical implementation.

## INTRODUCTION

Studies of human disease mechanisms, drug responses, and tissue repair strategies have traditionally relied on two-dimensional cell cultures, animal models, and clinical specimens[1-3]. However, these approaches are limited by insufficient tissue complexity, species differences, and restricted experimental accessibility. With the increasing demand for precision medicine, regenerative therapies, and human-relevant drug evaluation systems, new *in vitro* models that integrate biological relevance, structural complexity, and experimental controllability are required[1,2,4]. Organoids have emerged as important platforms bridging basic research, drug development, and clinical translation[2,3,5,6].

Organoids are typically derived from pluripotent stem cells, adult stem cells, progenitor cells or patient-derived tissue cells. Through self-organization, directed induction and microenvironmental regulation in three-dimensional culture systems, they can, to some extent, recapitulate the cellular composition, spatial organization and partial functional features of the corresponding organs[1,2,7,8]. According to their cellular sources and construction principles, organoids can be broadly classified into pluripotent stem cell-derived, adult stem cell-derived and patient-derived organoids[1,2,8,9]. These systems differ in developmental potential, degree of maturation, genetic background, construction efficiency and clinical applicability, which in turn define their distinct application boundaries in disease modeling, drug evaluation, personalized medicine and regenerative medicine[2,3,9,10].

From the perspective of clinical application, the major value of organoids lies in their ability to bridge human biology with clinically relevant questions across multiple levels[2,3,11]. Organoids provide human-relevant and experimentally manipulable systems for disease modeling, drug evaluation, personalized medicine, and regenerative therapy[1-3,9,12]. By preserving patient-specific characteristics and tissue-level organization, they offer opportunities to improve the predictive relevance of preclinical studies and support individualized therapeutic strategies[5,9,13]. However, the clinical value of organoids depends not only on their ability to reproduce tissue features, but also on whether their biological characteristics and functional outputs can be reliably interpreted within specific application contexts.

As research models, organoids should be considered complementary to, rather than replacements for, conventional two-dimensional cell cultures and animal models[1-4]. Two-dimensional cell systems offer high experimental controllability, low cost, ease of standardization, and compatibility with large-scale screening, but they generally fail to reproduce tissue architecture, multicellular organization, and complex cell-cell and cell-matrix interactions[1,2,4]. Animal models provide integrated whole-organism physiology and remain essential for evaluating systemic pharmacokinetics, metabolism, immune responses, toxicity, and long-term interactions among multiple organs; however, species differences may limit their ability to reproduce human-specific disease mechanisms and therapeutic responses[1-3]. Organoids occupy an intermediate position between these systems. They can preserve human genetic backgrounds, three-dimensional tissue organization, and selected tissue-level functions while remaining experimentally accessible and manipulable[2,3,5,6,9,12]. Nevertheless, they generally lack complete vascular, immune, neural, endocrine, and systemic interactions, and their self-organizing nature may introduce variability in cellular composition, maturation, and functional output[11,14-19]. Therefore, the suitability of each model depends on the scientific

question being addressed. Conventional cell models remain advantageous for reductionist mechanistic studies and high-throughput screening, animal models are indispensable for whole-organism and systemic evaluation, whereas organoids are particularly valuable for human-specific disease modeling, tissue-level functional studies, patient-specific drug-response testing, and selected translational applications[2,3,9,11-13]. These models should therefore be regarded as complementary components of a tiered experimental strategy rather than as interchangeable or hierarchically superior systems[1-4,11].

In this review, *clinical application* is used in a broad translational sense and does not imply that a given organoid approach has already entered routine clinical practice. To avoid conflating different levels of evidence, we distinguish among preclinical applications, clinical validation, early clinical investigation, and established clinical use. Preclinical applications refer to organoid-based studies performed *in vitro* or in animal models without direct demonstration of patient benefit. Clinical validation refers to studies that compare organoid-derived readouts with clinically relevant endpoints or patient responses. Early clinical investigation includes prospective clinical studies, feasibility studies, or first-in-human applications, whereas established clinical use requires reproducible clinical benefit, validated implementation procedures, and sufficient evidence for routine practice. Most current organoid applications remain within the preclinical or early translational stages, and their clinical relevance should therefore be interpreted according to the level of supporting evidence.

However, as organoid applications expand toward clinical translation, their limitations may become important sources of risk[3,11,14]. Organoids are not complete *in vitro* replicas of native organs, but rather selective reconstructions of cellular components, structural organization, and functional states[2,7,14]. Their clinical reliability is influenced by developmental maturity, cellular composition, functional integration, and the relationship between *in vitro* responses and actual clinical outcomes. In regenerative applications, additional concerns including genetic stability, immune compatibility, abnormal differentiation, and long-term integration must be carefully evaluated[11,15-17,20]. Moreover, because organoid formation relies on self-organization, variations in initial cellular conditions, culture environments, and extracellular signals may influence structural and functional outcomes, affecting reproducibility and standardization[14,15,18,19]. Therefore, the major challenges in clinical translation arise from the combined effects of biological limitations, model uncertainty, engineering variability, and safety concerns rather than from a single technical barrier[3,11,19].

To overcome these challenges, organoid research is increasingly shifting from empirical culture toward engineering-oriented development, emphasizing controllable construction, functional validation, and quality assessment[19,21,22]. Advances in vascularization, microenvironment regulation, dynamic culture, standardized manufacturing, and safety evaluation aim to improve reproducibility and clinical applicability[11,19,23-26]. Importantly, future development should not focus solely on increasing structural complexity, but should establish application-specific evaluation frameworks integrating molecular, structural, and functional criteria with appropriate risk-control strategies[11,19,22].

Based on these considerations, this review adopts an application-oriented and translational perspective to examine both the advantages and risks of organoid technologies. While recent comprehensive reviews have extensively summarized advances in organoid construction, organ-specific applications, and emerging clinical translation, the present review specifically focuses on the relationship between organoid capabilities, their corresponding translational risks, and the quality-control requirements needed for reliable clinical use. We therefore discuss disease modeling, drug screening, personalized medicine, and regenerative therapy together with four interconnected dimensions of translational challenge: biological limitations, model validity and risks of clinical extrapolation, engineering reproducibility and quality control, and clinical safety.

Rather than evaluating organoids primarily by morphological similarity or increasing structural complexity, we emphasize whether a given system can provide reproducible and clinically interpretable outputs within a clearly defined application context. Accordingly, this review further highlights the need for application-specific validation frameworks integrating molecular, structural, and functional criteria with appropriate quality-control and risk-management strategies[3,11,14,19,21,22,27-29].

## CURRENT ADVANCES IN ORGANOID TECHNOLOGY

Current advances in organoid technology mainly involve diversification of cellular sources, refinement of construction strategies, and improvement of structural and functional maturation, which together determine their biological capabilities and translational potential[1-3,7-10,21].

### Cell sources

Organoids can be broadly classified according to their cellular origin into pluripotent stem cell-derived, adult stem cell-derived, and patient-derived organoids. These systems differ in developmental potential, maturation state, genetic background, construction efficiency, and application suitability[1,2,8,9].

#### *Pluripotent stem cell-derived organoids*

Pluripotent stem cell (PSC)-derived organoids are typically generated from embryonic stem cells or induced pluripotent stem cells (iPSCs) through directed differentiation and three-dimensional self-organization. Their construction recapitulates key processes of embryonic development, including germ-layer specification, lineage commitment, progenitor formation, and tissue organization. Through stage-specific regulation of developmental signals, PSCs are guided toward ectodermal, mesodermal, or endodermal lineages and subsequently form organ-like structures under three-dimensional culture conditions[1,30-33]. Unlike ASC-derived organoids, PSC-derived systems reconstruct tissue formation through developmental programs rather than direct expansion of mature tissue cells.

PSC-derived organoid protocols generally follow a stepwise developmental strategy, in which exogenous signals guide cell fate and intrinsic self-organization drives three-dimensional patterning. Depending on the target tissue, different lineage induction routes are applied. Ectoderm-derived organoids, such as brain and retinal organoids, are generated through neural or sensory neuroepithelial specification. Brain organoids can form neuroepithelium-like structures, ventricle-like regions, and cortical-like organization, whereas retinal organoids can reproduce optic cup-like structures and generate photoreceptor-related populations[34-37]. Mesoderm-derived organoids include kidney and cardiac systems. Kidney organoids are generated through intermediate mesoderm and renal progenitor induction to form nephron-like structures containing podocyte-like and tubular components[33]. Cardiac organoids are produced through cardiac mesoderm differentiation and can exhibit organized cardiomyocyte structures and spontaneous contractile activity[38]. Endoderm-derived organoids include liver, gastric, intestinal, and lung systems generated through definitive endoderm and tissue-specific foregut or hindgut patterning. Liver organoids may incorporate endothelial and mesenchymal components to enhance tissue organization[39], whereas gastric[40], intestinal[41], and lung organoids[42] can reproduce distinct epithelial structures relevant to development and disease modeling.

The major advantage of PSC-derived organoids lies in their broad developmental potential, enabling reconstruction of multiple organ lineages and complex multicellular architectures. These systems are particularly valuable for studying human development, genetic disorders, and disease mechanisms. However, their reliance on prolonged differentiation and self-organization also introduces variability in differentiation efficiency, regional specification, and maturation. Many PSC-derived organoids remain in fetal-like or immature states, limiting their ability to fully represent adult tissues and emphasizing the need to interpret their functions according to developmental stage[15,16,43,44].

### *Adult stem cell-derived organoids*

Adult stem cell (ASC)-derived organoids are mainly established from adult tissue stem cells or progenitor cells, and their construction relies on the reconstruction of adult tissue stem cell niches *in vitro*. Unlike PSC-derived organoids, which are generated through pluripotency-driven lineage specification and developmental programs, ASC-derived organoids start from cells with predefined tissue identity and retain intrinsic self-renewal and tissue-specific differentiation capacities. Therefore, these systems do not aim to recapitulate embryonic organogenesis, but rather reproduce adult tissue maintenance, regeneration, and repair processes by providing appropriate microenvironmental conditions[45-48].

In general, ASC-derived organoids are generated by isolating stem cells, progenitor cells, glandular fragments, or epithelial clusters from adult tissues, followed by embedding in extracellular matrix-based systems such as Matrigel, collagen, or synthetic hydrogels. These matrices provide structural support for cell adhesion, polarity establishment, and three-dimensional organization. In combination with tissue-specific niche factors, including Wnt, R-spondin, epidermal growth factor (EGF), Noggin, fibroblast growth factor (FGF), or hepatocyte growth factor (HGF), ASC-derived cells can maintain proliferation, self-renewal, and lineage differentiation, gradually forming glandular, cystic, luminal, or budding structures[45-48]. Thus, the formation of ASC-derived organoids depends primarily on maintaining ASC activity and reconstructing tissue-specific niches rather than reactivating developmental programs.

The digestive system represents one of the most established fields for ASC-derived organoid development due to its high epithelial renewal capacity. Intestinal organoids generated from $Lgr5^+$ intestinal crypt stem cells can form crypt-like budding structures and differentiate into multiple epithelial lineages, including enterocytes, goblet cells, Paneth cells, and enteroendocrine cells under defined niche conditions[46]. Gastric organoids derived from gastric stem or progenitor cells can similarly generate gland-like epithelial structures through regulation of tissue-specific signaling environments[49]. Beyond the gastrointestinal tract, ASC-derived organoids have also been developed from hepatobiliary, pancreatic, and lung tissues. Liver and bile duct-derived organoids can maintain biliary epithelial or hepatocyte-like characteristics, whereas pancreatic duct-derived organoids preserve ductal epithelial features[47,50,51]. Lung organoids generated from adult epithelial progenitors, such as bronchial basal cells or alveolar type II cells, can reproduce airway- or alveolar-like epithelial structures[52,53].

Compared with PSC-derived systems, ASC-derived organoids exhibit stronger tissue specificity, more direct construction routes, and relatively stable maintenance of adult epithelial lineages. However, their differentiation potential is constrained by the original tissue source, and they generally lack complex multicellular components such as vasculature, nerves, immune cells, and stromal compartments without additional engineering strategies[46-48,50]. Therefore, the major advantage of ASC-derived organoids lies in their ability to preserve and amplify adult tissue regenerative programs *in vitro*, rather than fully reconstruct integrated organ-level systems.

### *Patient-derived organoids*

Patient-derived organoids (PDOs) are established from primary tissues obtained from patients, including surgical specimens, needle or endoscopic biopsies, fluid-derived cells, and tumor tissues. Similar to ASC-derived organoids, PDO construction relies on the expansion and self-organization of primary cells within three-dimensional matrices under defined culture conditions. However, because their starting materials are directly derived from individual patients, PDOs place particular emphasis on preserving patient-specific genetic backgrounds, disease-associated alterations, histopathological characteristics, and partial cellular heterogeneity[9,13,54]. Therefore, PDOs represent individualized organoid systems that directly connect patient-derived biology with clinical evaluation.

The establishment of PDOs generally involves tissue collection, mechanical or enzymatic dissociation, enrichment of target cells or glandular fragments, three-dimensional matrix embedding, and tissue-specific expansion. Surgical specimens usually provide sufficient tissue sources, whereas biopsy-derived samples require optimized isolation and culture approaches due to limited cell numbers. The isolated cells or tissue fragments are commonly embedded in extracellular matrix-based systems such as Matrigel or hydrogels, with supplementation of tissue-specific factors, including Wnt, R-spondin, EGF, Noggin, FGF, HGF, A83-01, or nicotinamide, to support survival, proliferation, and organoid formation[3,11,18]. Compared with PSC-derived organoids, PDOs generally bypass prolonged developmental differentiation processes and instead preserve characteristics of the original patient tissue.

Tumor-derived PDOs are among the most widely studied applications. Digestive system tumor PDOs, including gastric, esophageal, colorectal, liver, cholangiocarcinoma, and pancreatic cancer models, are typically established from surgical tissues or biopsy samples by expanding tumor epithelial clusters or glandular structures under optimized culture conditions[55-58]. Although these PDOs share common principles, their construction requirements vary depending on tissue origin and epithelial growth characteristics. For example, hepatobiliary and pancreatic tumor PDOs require culture conditions that support specific epithelial lineages, whereas gastrointestinal tumor PDOs often focus on maintaining glandular tumor architecture and patient-specific growth patterns. Non-digestive tumor PDOs, including prostate, ovarian, bladder, and lung cancer PDOs, can also be generated from surgical specimens, metastatic tissues, biopsies, ascites, or pleural effusion-derived cells. These systems aim to preserve tumor-specific phenotypes and provide individualized platforms for studying tumor biology, therapeutic responses, and resistance mechanisms[59-62].

Beyond oncology, PDOs have also been developed from patients with non-malignant diseases. Intestinal PDOs derived from inflammatory bowel disease patients, airway PDOs from respiratory disease samples, and endometrial PDOs from patient biopsies can maintain disease-associated epithelial characteristics and reproduce aspects of tissue-specific dysfunction[52,63-65]. The common construction principle of non-tumor PDOs is to isolate patient-derived epithelial stem or progenitor populations and maintain their expansion and organization under tissue-specific three-dimensional culture conditions.

Overall, PDO generation involves patient tissue acquisition, primary cell or tissue fragment isolation, matrix-based culture, expansion, and preservation of disease-associated features. Their major advantage lies in retaining individual patient variation and clinically relevant phenotypes, making them valuable for precision medicine and therapeutic evaluation. However, PDO establishment and reproducibility remain strongly influenced by sample quality, tissue heterogeneity, initial cell composition, culture conditions, and *in vitro* selection pressure, resulting in substantial variability between patients and disease types[3,11,18].

Overall, PSCs, ASCs, and PDOs represent three basic logics of organoid construction. PSC-derived systems generate organ-like structures by modeling developmental processes; ASC-derived systems maintain tissue-specific structures by reconstructing ASC niches; and PDOs preserve individualized disease features through *in vitro* expansion of patient-derived tissues. Clarifying cell source and construction logic is fundamental for understanding the structural features, functional levels, and clinical applicability of different organoid systems. Representative organoid systems, their cellular sources, induction strategies, structural features, and functional characteristics are summarized in Table 1.

## Construction strategies

Organoid construction is a process in which cell fate determination, morphogenesis, and tissue assembly are regulated within a three-dimensional environment. With advances in developmental biology and bioengineering, technical routes have expanded from self-organizing culture driven mainly by intrinsic

**Table 1. Representative organoid systems, their cellular sources, induction strategies and functional characteristics**

| Organoid | Source type | Cell source | Key induction signals/factors | Structural features | Functional features | Reference |
|---|---|---|---|---|---|---|
| Intestinal | PSC | hESC/hiPSC → hindgut endoderm | Activin A, FGF4, Wnt3a | Intestinal epithelial structure | Nutrient absorption, barrier function | [41] |
| | ASC | Mouse small intestinal Lgr5⁺ stem cells | Wnt3a, R-spondin, EGF, Noggin | Crypt-villus axis structure | Epithelial renewal, barrier maintenance, ion transport | [46] |
| | PDO | Human colorectal tumor tissue | Wnt, EGF, Noggin | Tumor-like structure | Drug sensitivity, retention of genetic characteristics | [13] |
| | | Intestinal mucosa/crypts from patients with inflammatory bowel disease | Wnt, R-spondin, EGF, Noggin | Colonic epithelial-like structure | Epithelial barrier, inflammation-related phenotypes | [65] |
| Liver | PSC | hESC/hiPSC → hepatic progenitor cells | Activin A, BMP4, FGF, HGF | Liver bud/hepatic plate-like structure | Albumin secretion, CYP enzyme activity | [39] |
| | ASC | Human EpCAM⁺ hepatobiliary progenitor cells/cholangiocytes | EGF, HGF, FGF, R-spondin | Bile duct/liver-like structure | Biliary epithelial features, partial hepatocyte-like function | [51] |
| | PDO | Human Primary liver tumor tissue | EGF, HGF, FGF | Tumor-like structure | Drug response, tumor heterogeneity | [57] |
| Bile duct | ASC | Human cholangiocytes/biliary progenitor cells | EGF, HGF, FGF, R-spondin, Wnt | Bile duct-like luminal structure | Bile transport, barrier function | [66] |
| | PDO | Human cholangiocarcinoma tissue/biliary tumor samples | EGF, HGF, FGF, R-spondin, Noggin | Bile duct tumor-like structure | Tumor phenotype, drug response | [58] |
| Stomach | PSC | hESC/hiPSC → foregut endoderm | Activin A, FGF, RA, BMP | Gastric-like structure | Epithelial differentiation | [40] |
| | ASC | Mouse Gastric gland Lgr5⁺ stem cells | Wnt, EGF, Noggin | Gastric gland-like structure | Epithelial renewal, secretory function | [49] |
| | PDO | Human gastric cancer tissue/gastric cancer biopsy samples | Wnt, R-spondin, EGF, Noggin, FGF10 | Gastric cancer-like glandular structure | Retention of tumor heterogeneity and molecular features | [55] |
| Esophagus | ASC | Human esophageal basal cells/esophageal epithelial progenitors | EGF, Noggin, R-spondin, Wnt | Esophageal squamous epithelial-like structure | Epithelial differentiation, barrier-related features | [67] |
| | PDO | Human Barrett's esophagus/esophageal adenocarcinoma tissue | EGF, Noggin, R-spondin, A83-01, FGF | Glandular/tumor-like structure | Retention of diseased epithelial and histological features | [56] |
| Pancreas | ASC | Human/Mouse pancreatic ductal cells | EGF, FGF10, R-spondin | Duct-like structure | Epithelial maintenance | [68] |
| | PDO | Human/Mouse pancreatic tumor tissue | Wnt, EGF, FGF | Tumor-like structure | Drug response, preservation of molecular characteristics | [68] |
| Islet | PSC | hESC/hiPSC → PDX1⁺ pancreatic progenitor cells | Activin A, CHIR, FGF10, RA, NGN3, MAFA | Islet-like cell clusters | GSIS, $Ca^{2+}$ dynamics | [69] |
| | ASC | Adult tissue-derived cells (e.g., adipose tissue) | Stepwise pancreatic induction system | Islet-like structure | Glucose-responsive insulin secretion | [70] |
| Thyroid | PSC | hESC → thyroid progenitor cells | BMP4, FGF2, TSH, NKX2-1, PAX8 | Thyroid follicle-like structure | Thyroglobulin expression, iodine uptake, thyroid hormone synthesis | [71] |
| Salivary gland | PSC | mESC → oral ectoderm/salivary gland progenitors | FGF7, FGF10, BMP, SHH | Salivary gland-like branching structure | Acinar-like differentiation, secretion-related features | [72] |
| | ASC | Mouse salivary gland stem/progenitor cells | EGF, FGF7, FGF10, RA, Wnt | Salivary gland acinar/duct-like structure | Secretion-related function, glandular regenerative potential | [73] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Heart | PSC | hESC/hiPSC → cardiac lineage | Biphasic Wnt modulation, BMP4, FGF2 | Cardiac tissue-like structure | Spontaneous contraction, electrical conduction, $Ca^{2+}$ transients | [38] |
| Kidney | PSC | hESC/hiPSC → intermediate mesoderm | CHIR99021, FGF9, BMP7 | Nephron-like structures (podocytes/tubules) | Tubular transport function, nephrotoxicity response | [33] |
| | PDO | Human renal cell carcinoma tissue | EGF, FGF, R-spondin, A83-01 | Renal cancer-like structure | Retention of histological and molecular features, drug and CAR-T responses | [74] |
| Brain | PSC | hESC/hiPSC → neuroectoderm | Dual SMAD inhibition, FGF, Wnt | Cortical-like layered structure | Spontaneous electrical activity, synaptic networks | [34] |
| | PDO | Human glioblastoma/glioma tissue | EGF, FGF | Tumor-like structure | Proliferation, invasiveness, drug response | [75] |
| Alveolus/Airway | PSC | hiPSC → $SFTPC^+$ alveolar stem cells (AT2) | CHIR99021, FGF10, KGF, DAPT (CFKD) | Alveolar organoids harboring $SFTPC^+$ alveolar stem cells | Secretory function | [42] |
| | ASC | Human bronchial basal cells ($KRT5^+$) | FGF7, FGF10, Wnt | Airway epithelial structure | Ciliary movement, secretory function | [52] |
| | ASC | Mouse AT2 cells | Wnt, FGF, BMP | Alveolar-like structure | Surfactant secretion | [53] |
| | PDO | Human primary lung cancer tissue/paired non-tumor airway tissue | EGF, FGF, Noggin, R-spondin, A83-01 | Lung cancer-like organoid structure | Tumor phenotype, drug response | [76] |
| Retina | PSC | hESC/hiPSC → neural retinal progenitor cells | IGF-1, DKK1, Noggin | Optic cup-like structure | Photoreceptor function | [77] |
| Skin/hair follicle | PSC | hESC/hiPSC (epidermis + neural crest) | BMP, Wnt, FGF | Epidermal-dermal structure with hair follicles | Barrier formation, hair follicle generation | [78] |
| | ASC | Mouse Hair follicle stem cells combined with dermal papilla cells | Wnt, BMP, FGF, SHH | Hair follicle-like structure | Hair follicle regeneration potential | [79] |
| Blood vessel | PSC | hESC/hiPSC → endothelial/pericyte cells | CHIR99021, BMP4, VEGF, FGF2 | Vascular network structure | Vascular permeability, perfusion capacity | [80] |
| Prostate | ASC | Human/mouse prostate epithelial stem cells | EGF, Noggin, R-spondin, DHT | Gland-like structure | Androgen response | [81] |
| | PDO | Human prostate cancer biopsy tissue/metastatic lesions/circulating tumor cells | EGF, Noggin, R-spondin, DHT | Tumor-like structure | Molecular subtype representation, drug response | [60] |
| Bladder | ASC | Human/mouse normal urothelial cells | EGF, FGF, Noggin, R-spondin | Urothelial-like structure | Urothelial differentiation | [82] |
| | PDO | Human bladder cancer tissue | EGF, FGF, Noggin, R-spondin | Bladder cancer-like organoid structure | Retention of histopathological and molecular features, drug response | [62,82] |
| Ovary | PDO | Human ovarian cancer surgical tissue/ascites/metastatic lesions | EGF, FGF, Noggin, R-spondin, A83-01 | Ovarian cancer-like glandular/cystic structure | Retention of histological and genomic features | [59] |
| Endo-metrium | ASC/PDO | Human endometrial glandular cells/patient endometrial tissue | EGF, FGF10, HGF, Noggin, R-spondin, hormonal regulation | Endometrial gland-like structure | Hormone response, secretion-like changes | [64] |
| Breast | ASC | Human normal mammary epithelial cells/mammary progenitor cells | EGF, FGF, neuregulin, R-spondin | Mammary acinus-like structure | Luminal cell/myoepithelial differentiation | [83] |
| | PDO | Human breast cancer tissue | EGF, FGF7, FGF10, heregulin, Noggin, R-spondin | Breast cancer-like structure | Retention of molecular subtype and drug response | [54] |

This table summarizes representative organoid systems according to tissue type, cellular source, key induction conditions, structural

characteristics, and functional readouts. The examples illustrate how differences in cell origin and construction strategy influence organoid architecture, function, and potential application. ASC: Adult stem cell; AT2: alveolar type II; BMP: bone morphogenetic protein; CAR-T: chimeric antigen receptor T cell; CYP: cytochrome P450; DHT: dihydrotestosterone; DKK1: Dickkopf-related protein 1; EGF: epidermal growth factor; EpCAM: epithelial cell adhesion molecule; FGF: fibroblast growth factor; GSIS: glucose-stimulated insulin secretion; hESC: human embryonic stem cell; HGF: hepatocyte growth factor; hiPSC: human induced pluripotent stem cell; IGF-1: insulin-like growth factor 1; KGF: keratinocyte growth factor; KRT5: keratin 5; Lgr5: leucine-rich repeat-containing G protein-coupled receptor 5; MAFA: MAF bZIP transcription factor A; mESC: mouse embryonic stem cell; NGN3: neurogenin 3; NKX2-1: NK2 homeobox 1; PAX8: paired box 8; PDX1: pancreatic and duodenal homeobox 1; PDO: patient-derived organoid; PSC: pluripotent stem cell; RA: retinoic acid; SHH: sonic hedgehog; SFTPC: surfactant protein C; TSH: thyroid-stimulating hormone; VEGF: vascular endothelial growth factor.

cellular programs to coordinated regulation of inductive signals, extracellular matrix, culture dynamics, and spatial assembly[7,10,19,21]. In terms of the objects of technical control, current construction strategies mainly include developmental signaling regulation, matrix environment design, dynamic culture, and multicellular assembly.

Developmental signaling regulation determines the lineage direction and tissue patterning of organoids. PSC-derived organoids usually follow the sequence of embryonic organogenesis by sequentially modulating pathways such as Wnt, bone morphogenetic protein (BMP), FGF, Activin/Nodal, sonic hedgehog (SHH), and retinoic acid, thereby guiding cells through germ-layer specification, organ progenitor formation, and tissue-specific differentiation. This process is not a simple superposition of multiple factors, but depends on signal intensity, timing, and order of combination. For example, McCracken *et al.* established human PSC-derived gastric organoids by stage-specific modulation of foregut patterning signals[40]. In comparison, ASC-derived organoids mainly maintain established lineages by reconstructing tissue niches, and their culture systems are usually adjusted around factors such as Wnt, R-spondin, EGF, and Noggin[46]. Therefore, although different cell sources can all form three-dimensional structures, their signaling regulation focuses respectively on reconstruction of developmental programs and maintenance of adult tissue renewal.

The extracellular matrix provides adhesion sites, mechanical support, and spatial boundaries for organoids, and directly participates in the establishment of cell polarity and morphogenesis. Matrigel is widely used in organoid culture because it contains laminin, collagen, and multiple matrix components, but its composition and mechanical properties are difficult to precisely regulate. Gjorevski *et al.* developed polyethylene glycol hydrogels with tunable stiffness, degradability, and adhesive ligands, and found that intestinal stem cell expansion and subsequent differentiation require different matrix properties. This finding indicates that organoid culture matrices may need to be dynamically adjusted during tissue formation[24]. On this basis, collagen, fibrin, gelatin derivatives, and synthetic hydrogels have gradually been used to establish more chemically defined three-dimensional environments. Microwell arrays and geometric patterning can also define the number, size, and shape of initial cell aggregates, thereby regulating subsequent lumen formation, tissue axis formation, and regional distribution.

Changes in culture mode further enhance the regulation of material transport and physical stimulation. Static culture mainly relies on diffusion for the exchange of oxygen, nutrients, and metabolic products, whereas rotating culture, stirred bioreactors, and perfusion systems introduce continuous flow and increase the dynamism of the culture environment. Microfluidic platforms can also generate controllable concentration gradients, shear stress, and interface conditions around organoids to simulate blood flow, luminal fluid flow, or material exchange between different tissue regions. Compared with simply increasing culture medium volume, these methods allow quantitative control of flow rate, direction, and duration of stimulation.

Multicellular assembly improves organoid tissue composition by regulating the proportions, timing of addition, and spatial positioning of different lineage cells. Takebe *et al.* cocultured PSC-derived hepatic endodermal cells with endothelial cells and mesenchymal cells, using interactions among different cell types

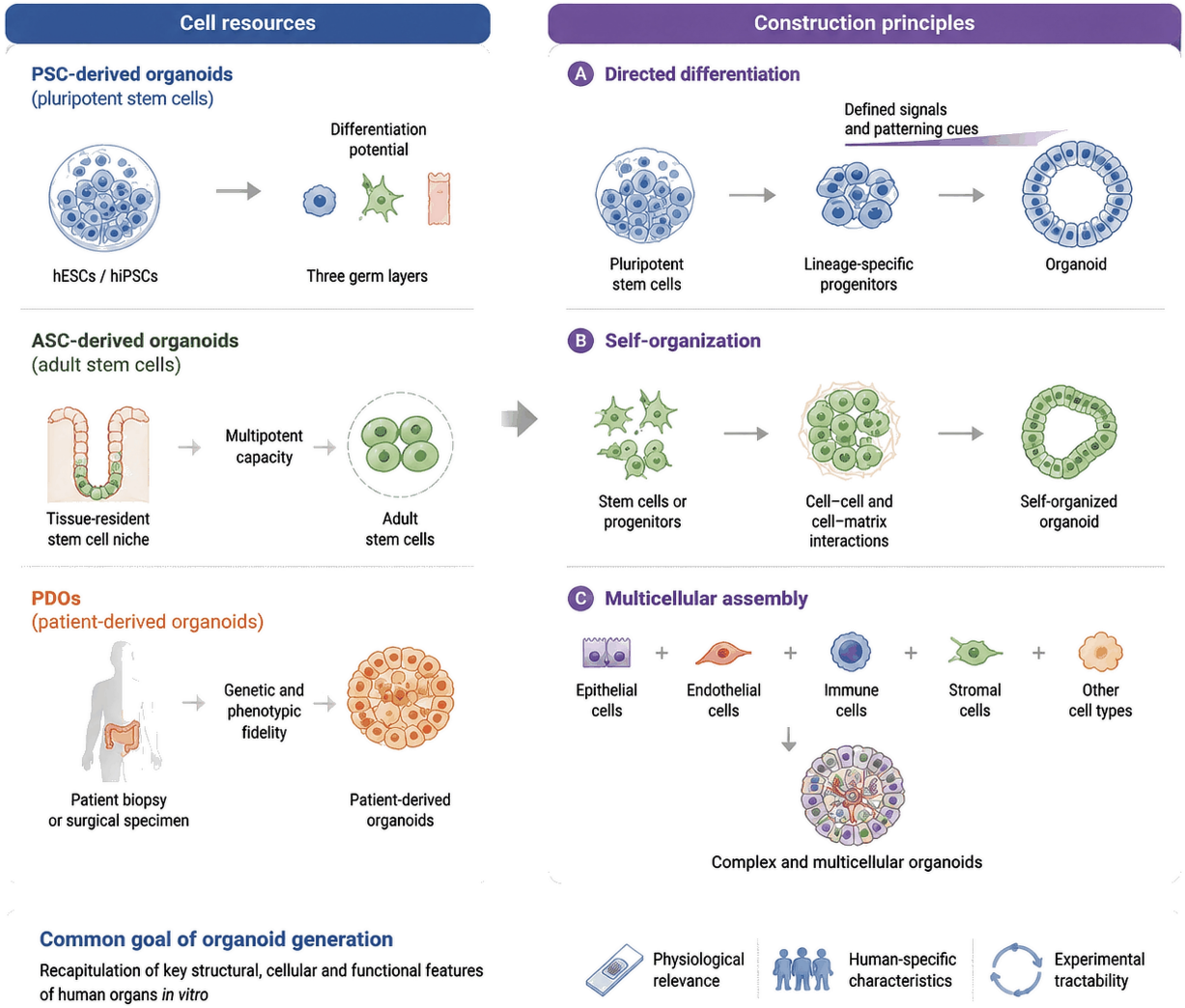


**Figure 1.** Overview of organoid generation and engineering strategies. (A) Directed differentiation guides pluripotent stem cells through defined developmental signals and progenitor states toward organoid formation; (B) Self-organization enables tissue-resident stem or progenitor cells to form three-dimensional structures through cell-cell and cell-matrix interactions; (C) Multicellular assembly integrates distinct cell types or tissue modules to reconstruct more complex tissue architecture and intercellular interactions. The figure also summarizes the major cellular sources of organoids, including PSC-derived, ASC-derived, and PDOs, together with engineering and microenvironmental strategies used to regulate organoid construction. ASC: Adult stem cell; hESC: human embryonic stem cell; hiPSC: human induced pluripotent stem cell; PDO: patient-derived organoid; PSC: pluripotent stem cell.

to form liver bud-like structures[39]. In addition to simultaneous coculture, different cells or tissue modules can also be induced separately and then assembled through fusion, contact culture, or bioprinting. This approach allows different lineages to complete early differentiation under their own suitable conditions and subsequently establish tissue interfaces. Bioprinting further provides technical means to control cell deposition sites, tissue geometry, and the distribution of perfusion channels, shifting organoid construction from random aggregation toward tissue manufacturing with predefined spatial information[26].

Overall, current organoid construction has formed a technical system that combines biological induction and engineering control, including developmental signaling, extracellular-matrix regulation, dynamic culture, and multicellular assembly [Figure 1].

## Structural complexity of organoids

The evolution of organoid construction strategies has enabled these systems to progressively develop tissue features at multiple structural levels. Organoid complexity is reflected not only by increased cellular diversity but also by the establishment of cell polarity, spatial compartmentalization, tissue interfaces, and inter-regional connections. Early organoids mainly reconstructed local tissue units, whereas advanced systems have developed regionalized structures, multilineage interactions, cross-module connections, and higher-order developmental patterns[2,3,7,21].

Early organoids were mainly characterized by spherical, cystic, or single-lumen structures, with structural organization centered on cell polarity, luminal orientation, and local tissue patterning. Intestinal organoids established by Sato *et al.* formed central lumens and crypt-like budding structures, with spatial separation of proliferative and differentiated regions[46]. Retinal organoids derived from human embryonic stem cells demonstrated optic cup-like morphogenesis and layered neural retinal organization, while cortical organoids developed axial polarity and inside-out neuronal layering resembling aspects of cortical development[44,77,84]. These studies demonstrated that three-dimensional culture systems could progress beyond simple cell aggregates toward organized tissue units with reproducible spatial characteristics.

A further advancement in structural complexity is the emergence of regionalized organoids containing distinct spatial identities within a single system. Regional organization depends not only on cell-type specification but also on local signaling environments, tissue boundaries, and spatial arrangement of progenitor populations. Brain organoids generated by Lancaster *et al.* developed multiple neuroepithelial regions and ventricle-like structures, whereas region-specific induction strategies enabled the generation of forebrain-, midbrain-, and hypothalamic-like organoid systems[34,85,86]. The establishment of regionally distinct structures provides a more informative framework for investigating cell migration, developmental interactions, and tissue-specific organization.

Beyond regionalization within individual organoids, organoid fusion and multicellular assembly have extended structural complexity toward interactions between different tissue modules. These approaches typically involve separately generating tissue components with distinct identities and subsequently combining them to study migration, projection, and interface formation. Dorsal-ventral forebrain assembloids demonstrated interneuron migration between different neural regions, while thalamic-cortical fusion models revealed bidirectional axonal connections between specialized brain tissues[87,88]. Cross-lineage assembloids further expanded this concept by integrating neural, muscle, or epithelial components. For example, cortical-spinal cord-muscle assembloids reproduced neuromuscular interfaces, whereas neural crest-intestinal organoid systems enabled formation of enteric neurons and glial populations within intestinal tissues[89,90]. These models illustrate that structural complexity depends not simply on increasing cell numbers, but on establishing functional spatial relationships between distinct tissue components.

Gastruloids represent another structural organization strategy that differs from organ-specific organoids. By regulating initial pluripotent cell aggregation and developmental signaling, gastruloids can undergo symmetry breaking, axis elongation, and multi-germ-layer patterning. Studies by van den Brink *et al.* and Beccari *et al.* demonstrated that gastruloids could generate spatial relationships among neural, mesodermal, and endodermal regions, providing models for early embryonic organization and body-axis formation[91,92].

Overall, organoid structural complexity has progressed from basic polarity and lumen formation to repeated tissue units, regional organization, cross-lineage interfaces, and multi-tissue connections. Organ-specific organoids primarily reconstruct spatial features of individual tissues, assembloids emphasize interactions between different tissue modules, and gastruloids model early developmental patterning across germ layers.

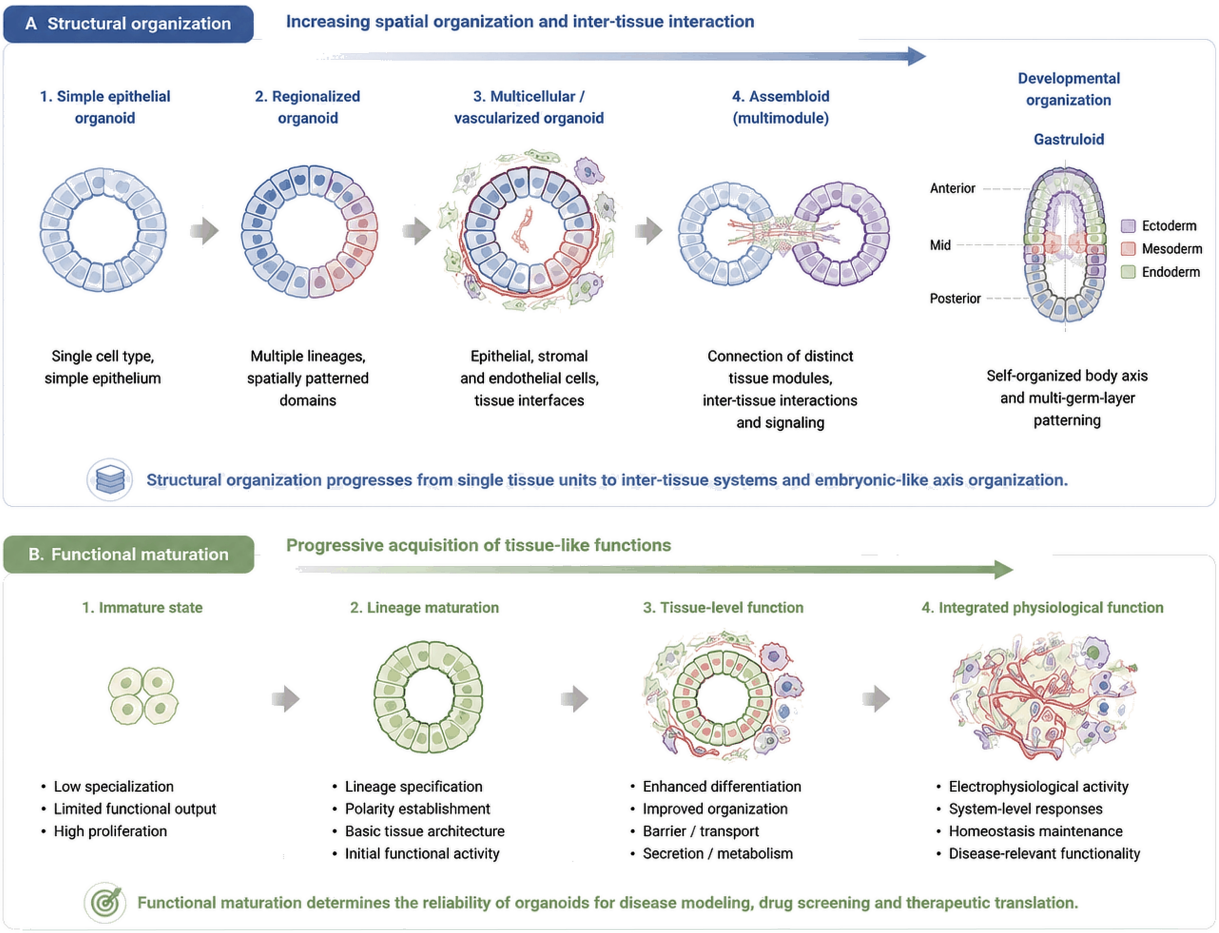


**Figure 2.** (A) Structural organization and functional maturation of organoids. Structural organization progresses from simple epithelial organoids toward regionalized, multicellular or vascularized organoids, assembloids, and higher-order developmental systems, reflecting increasing spatial organization and inter-tissue interactions; (B) Functional maturation represents the progressive acquisition of tissue-specific functions, from early lineage commitment and basic cellular activity toward more coordinated and mature tissue-level functional outputs. Structural complexity and functional maturation are related but not equivalent and may progress at different rates depending on cell source, culture conditions, and developmental context.

Therefore, structural complexity should not be evaluated solely by organoid size, cell number, or morphological sophistication, but by whether the model establishes spatial hierarchies and tissue relationships relevant to the biological process being studied. The progressive relationship between structural organization and functional maturation is summarized in Figure 2.

## Functional maturation of organoids

Functional maturation refers to the process by which cells and tissue units within organoids progress from acquiring basic lineage-specific functions to developing sustained, coordinated, and tissue-specific functional states. In early organoid formation, cells mainly complete lineage determination and initial differentiation, and the functions they display are usually limited to basic activities of specific cell types. With prolonged culture, further differentiation of cellular subtypes, and continuous remodeling of cell-cell connections, functional outputs can gradually evolve from transient and discrete cellular responses into more stable tissue-like activity[15,16,43,44]. Therefore, functional maturation is not the sudden emergence of a particular tissue-specific function, but a continuous developmental change in cellular state, functional strength, and tissue-level coordination.

Long-term culture has revealed a clear temporal dependence of organoid function. Taking neural organoids as an example, early neurons mainly show scattered spontaneous firing. Subsequently, as the ratio of excitatory to inhibitory neurons changes, synaptic connections increase, and local networks are established, electrical activity gradually strengthens and shows a trend toward synchronization. Trujillo *et al.* found that, over several months of culture, neural network activity in human cortical organoids progressed from sparse firing to periodic oscillatory activity, with temporal changes that partially resembled early human brain network development[93]. Fair *et al.* further observed that brain organoids developed action potential features, synaptic transmission, and sustained changes in network excitability with prolonged culture, indicating that the functional state of neural organoids continues to evolve after basic neural structures are formed[94]. Recent long-term culture and single-cell epigenomic studies have further characterized developmental progression and cell-fate regulation in human neural organoids[95,96].

Similar staged maturation is also observed in sensory organoids. Retinal organoids initially generate retinal progenitor cells and early differentiated neural cells, and later gradually develop photoreceptor inner and outer segment-related structures, synaptic ribbons, and neurotransmitter release capacity. Through long-term culture, Wahlin *et al.* observed photoreceptor outer segment-like structures, synaptic ribbons, and membrane electrical activity related to photoreceptor transmission, indicating that retinal organoid function can develop progressively as cellular structures and synaptic apparatus mature[97]. Compared with early cells that only possess photoreceptor lineage identity, these changes indicate further acquisition of functional features related to light capture and neural signal transmission.

Functional maturation also involves a transition from autonomous activity of individual cell populations to coordination among different cellular subtypes. Mature tissue function usually depends on interactions among parenchymal cells, supporting cells, and local regulatory cells. For example, neural network activity depends not only on the excitability of neurons themselves, but also on the balance between excitatory and inhibitory neurons, synaptic connectivity, and glial support. Retinal light responses likewise require photoreceptors, bipolar cells, and ganglion cells to form a continuous information transmission pathway. Therefore, the essence of organoid functional development from cell-autonomous function to tissue-level function is the transition of different cellular subtypes from separate emergence to coordinated activity in time and space.

In composite organoids, functional maturation may further be reflected in the establishment of stable connections between different regions or tissue modules. A single tissue module usually presents only local function, whereas organoid fusion and multilineage assembly allow neural impulses, mechanical activity, or secretory signals to be transmitted across tissue interfaces. For example, in the cortical-spinal cord-skeletal muscle assembloid constructed by Andersen *et al.*, cortical and spinal cord-like neural tissues established connections with muscle modules through axonal projections, ultimately forming a continuous functional chain in which neural activity drove muscle contraction[89]. At this stage, functional maturation is no longer confined to a single tissue module, but is manifested by directional input, conduction, and output relationships among multiple modules. This development represents a further extension of organoid function from local tissue activity toward cross-regional integration.

*In vivo* transplantation studies also indicate that some organoids can continue to undergo functional development after receiving host circulation and tissue signals. Mansour *et al.* transplanted human brain organoids into the mouse brain and observed host vascular ingrowth into the organoids, accompanied by increased neuronal dendritic complexity, axonal extension, and enhanced neural activity[98]. This suggests that the functional state formed by organoids *in vitro* is not a fixed endpoint; cellular and tissue functions can continue to develop in a more complete physiological environment. However, what this example illustrates

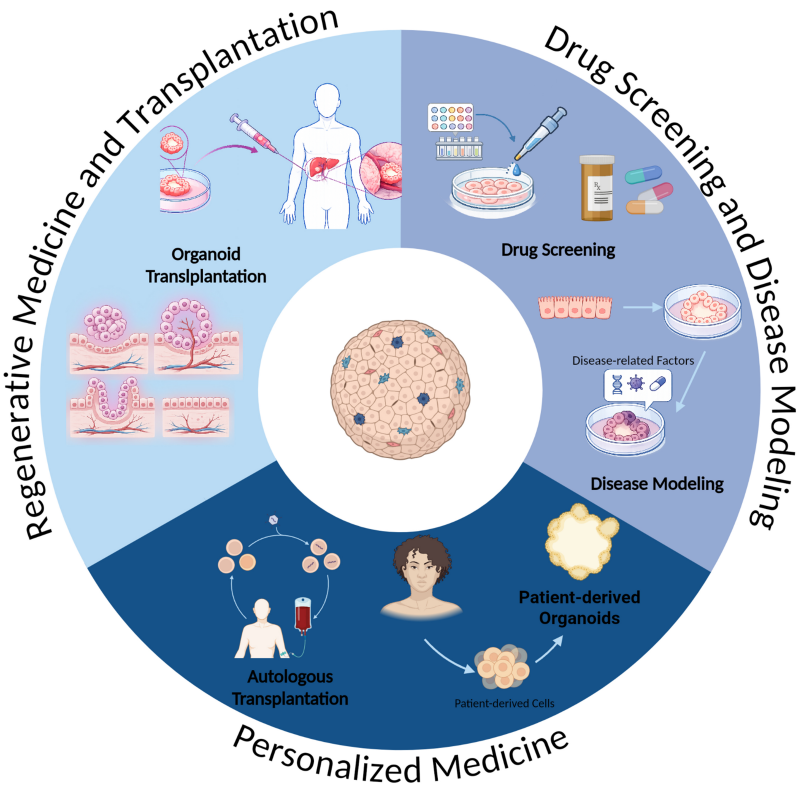


**Figure 3.** Progress in clinical applications of organoids. The figure summarizes the major translational application domains of organoids, including disease modeling and drug screening, personalized medicine, and regenerative medicine and transplantation. These applications represent different levels of translational maturity and require distinct forms of functional validation, clinical evidence, and quality control. Created in BioRender. Xia, J. (2026) https://BioRender.com/42nr7y9.

here is the plasticity of functional maturation, whereas post-transplantation safety, host integration, and clinical applicability are discussed in later sections.

Overall, functional maturation can be summarized as a progressive transition from basic lineage-specific activity, to enhanced local tissue function, and finally to coordinated responses among multiple cell populations or tissue modules. Importantly, functional maturation is related to but not equivalent to structural complexity: structural organization provides the spatial basis for function, whereas mature function requires appropriate cellular composition, temporal stability, and coordinated interactions among tissue components.

Together, differences in cell source, construction strategy, structural organization, and functional maturation define the capabilities and application boundaries of current organoid systems[2,3,7,10,19,21].

## MAJOR ADVANTAGES OF ORGANOIDS IN CLINICAL APPLICATIONS

Organoids provide human-relevant, three-dimensional, and experimentally tractable platforms for disease modeling, drug evaluation, personalized medicine, and regenerative applications. Their major advantages and application-specific value are discussed below [Figure 3][2,3,11].

### Disease modeling and drug screening

Disease modeling and drug screening are among the most established applications of organoids. Compared with conventional two-dimensional cultures and animal models, organoids can provide human tissue-relevant architecture and functional phenotypes while remaining amenable to genetic, pharmacological, and

microenvironmental manipulation[1,2,6,11]. However, this increased tissue relevance does not eliminate the need for simpler cell systems or whole-animal models, because organoids do not fully reproduce systemic physiology, pharmacokinetics, immune regulation, or multi-organ interactions.

In disease modeling, the core advantage of organoids lies in their ability to reconstruct human tissue-specific pathological processes. With advances in culture systems, gene editing, and single-cell analysis, their applications have expanded from organogenesis, tissue differentiation, and infection models to genetic diseases, infectious diseases, tumor evolution, drug tolerance, and tissue injury repair. For example, airway and lung organoids have been used to investigate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection mechanisms, viral host tropism, tissue injury responses, and antiviral drug screening, providing models closer to the native tissue context for studies of human respiratory infections[99-101]. In tumor research, patient-derived tumor organoids can retain key tumor mutations, molecular subtypes, and partial cellular heterogeneity, and can be used to analyze tumor initiation and progression, clonal evolution, therapeutic resistance, and recurrence-related mechanisms[102-104]. In addition, isogenic organoid models generated by clustered regularly interspaced short palindromic repeats (CRISPR) and other gene-editing technologies can introduce or correct specific mutations within the same genetic background, thereby distinguishing the effects of pathogenic mutations from individual genetic background differences and improving the causal interpretability of disease mechanism studies[9,105]. A kidney organoid-on-chip study further demonstrated that fluid flow reveals disease-associated mechanosensing pathways and drug-responsive cystic phenotypes that are incompletely represented in static culture[106].

The establishment of disease models also provides a more reliable biological basis for drug screening. Compared with conventional two-dimensional cell screening, organoids can assess not only simple endpoints such as cell viability or proliferation inhibition, but also multidimensional readouts, including tissue architecture, differentiation status, barrier function, metabolic activity, electrophysiological activity, secretory function, and changes in cellular composition. These features enable drug effects to be evaluated at a level closer to tissue physiology. For example, in cystic fibrosis research, patient-derived intestinal organoids can directly evaluate restoration of cystic fibrosis transmembrane conductance regulator (CFTR) function through the forskolin-induced swelling assay and can be used to screen and predict the efficacy of CFTR modulators; the resulting readouts show good concordance with clinical responses in patients[107,108]. In drug safety evaluation, liver organoids can be used to study drug-induced liver injury, whereas cardiac organoids can assess the cardiac safety of candidate drugs through contraction frequency, electrophysiological activity, and rhythm changes[109,110].

In precision drug screening, PDOs have important value. PDOs are usually derived from surgical specimens, biopsy tissues, or tumor tissues from patients, and can relatively well preserve the genetic background, mutational spectrum, cellular heterogeneity, and partial disease-related phenotypes of the original tissue. They are therefore suitable for patient-relevant drug sensitivity assessment and studies of resistance mechanisms[13,111]. With the development of organoid biobanks and high-throughput screening systems, PDOs from different cancer types, molecular subtypes, and treatment backgrounds can be systematically collected and expanded, enabling parallel testing of multiple drugs and drug combinations[112-115]. These platforms can also integrate genomic, transcriptomic, and clinical information to establish genotype-phenotype-drug response relationships. When combined with CRISPR screening, single-cell sequencing, spatial omics, and artificial intelligence-based analysis, they further expand the applications of organoids in target discovery and mechanism-driven drug development[116,117].

Overall, organoids provide tissue-relevant platforms for disease modeling and drug evaluation, but their results should be interpreted alongside complementary cell, animal, omics, and clinical data[18,118-120].

## Personalized medicine

Individualized medicine aims to develop more precise diagnostic and therapeutic strategies based on differences in patient genetic background, disease characteristics, and treatment response. Conventional precision medicine mainly relies on genetic testing, molecular subtyping, and pathological assessment, but these types of information do not always accurately predict real therapeutic responses. Organoid technology can expand patient-derived cells into *in vitro* models with certain tissue architecture and functional phenotypes, thereby converting individual variation into observable, manipulable, and quantifiable functional evidence[5,6,9,59]. Thus, organoids can be used not only for drug screening, but also as patient-derived functional validation platforms linking molecular features to clinical responses.

In individualized cancer therapy, PDOs currently represent one of the most evidence-supported systems. PDOs are usually established from patient surgical specimens, needle biopsy tissues, or metastatic lesion samples, and can preserve key mutations, molecular subtypes, tissue-of-origin features, and partial cellular heterogeneity of the original tumor *in vitro*[13,54,111]. For example, Sachs *et al.* established a PDO biobank covering different breast cancer subtypes and demonstrated that these organoids reflected the histological, genomic, and drug response features of the original tumors[54]. Tiriac *et al.* combined genomic, transcriptomic, and drug-response profiles in pancreatic cancer PDOs and demonstrated their value for pharmacotyping[102]. Vlachogiannis *et al.* reported concordance between PDO drug responses and clinical outcomes in metastatic gastrointestinal cancers[13]. Ooft *et al.* prospectively evaluated PDO-based chemotherapy response prediction in metastatic colorectal cancer and demonstrated particularly useful negative predictive value for selected regimens[121]. Yao *et al.* demonstrated that responses of patient-derived rectal cancer organoids to chemoradiotherapy correlated with clinical responses in locally advanced rectal cancer[103]. These studies indicate that organoids can serve as co-clinical models, transforming tissue responses during patient treatment into detectable *in vitro* signals and thereby assisting treatment selection, efficacy prediction, and resistance tracking. More recently, Martini *et al.* combined colorectal cancer organoid drug screening with genotyping to inform exploratory treatment decisions, supporting clinical feasibility while highlighting the need for larger validation studies[122].

Beyond oncology, the individualized value of organoids is also emerging in genetic diseases and gene repair strategies. Taking cystic fibrosis as an example, patient-derived intestinal organoids can be used not only to evaluate CFTR channel function, but also as functional validation platforms for gene repair. Schwank *et al.* used a CRISPR-associated protein 9 (Cas9)-based genome-editing system to repair the CFTR locus in intestinal stem cell organoids derived from patients with cystic fibrosis, and demonstrated that the repaired allele could be expressed and restore function, providing proof of concept for gene correction in patient-derived cells for monogenic diseases[123]. The significance of this type of study is that organoids can assess, within a patient's own genetic background, whether a mutation produces a detectable tissue-level functional defect and whether function is restored after gene repair or therapeutic intervention. For rare diseases, hereditary developmental abnormalities, or tissue-specific functional disorders, patient-derived or iPSC-derived organoids can also provide expandable systems when clinical samples are limited, supporting mechanism analysis, therapeutic strategy validation, and pre-evaluation before potential cell therapy[6,9,59].

Overall, organoids extend personalized medicine beyond molecular stratification by providing patient-specific functional evidence. Their clinical use, however, depends on timely model establishment, stable preservation of relevant phenotypes, and validated associations between *in vitro* readouts and patient outcomes[6,103,118,119]. Accordingly, PDO drug-response testing should currently be regarded primarily as a clinically validated or investigational decision-support approach in selected settings, rather than as an established standard of care. However, the predictive performance of PDO-based pharmacological testing depends on how well the model preserves clinically relevant tumor-stromal, immune, vascular, and

extracellular-matrix interactions; this limitation is discussed in detail in Section "Absence of key microenvironmental components".

### Regenerative medicine and transplantation

Regenerative medicine and transplantation represent the most therapeutically oriented applications of organoids, aiming to restore tissue structure and function rather than merely replace individual cells, although most applications remain preclinical or at an early clinical-investigation stage. Their pre-organize three-dimensional architecture and tissue-specific cellular composition may provide advantages over single-cell transplantation in selected settings[10,20,66,124].

In epithelial injury and luminal structure repair, ASC-derived organoids have shown considerable application potential. Intestinal organoids can form crypt-like structures *in vitro* and maintain epithelial proliferation, differentiation and renewal capacity. In multiple intestinal injury models, they can engraft, expand and contribute to mucosal repair[125]. Human intestinal organoids have further been used to promote mucosal barrier reconstruction and to explore preclinical strategies involving gene correction-autologous transplantation in inherited intestinal diseases[123,125]. Similarly, cholangiocyte organoids can not only provide a source of biliary epithelial cells, but also form lumen-like structures with biliary epithelial characteristics and barrier function. Studies by Sampaziotis *et al.* showed that human cholangiocyte organoids could repair damaged biliary epithelium in an *ex vivo* liver perfusion system and improve bile duct structure and barrier function. Subsequent exploratory human applications have provided preliminary evidence regarding feasibility, safety, and potential therapeutic benefit in selected biliary conditions[66]. These studies indicate that the regenerative value of organoids lies not only in their ability to form tissue-like structures *in vitro*, but also in whether they can participate in the repair of real tissue interfaces under conditions approaching clinical settings.

Beyond epithelial and luminal structure repair, organoids can also serve as important sources for the replacement of specific functional cells. Taking the retina as an example, PSC-derived retinal organoids can undergo differentiation processes resembling *in vivo* development and generate photoreceptor precursors and retina-like tissue structures, thereby providing a cellular source for replacement therapy in degenerative retinal diseases. Multiple studies have shown that organoid-derived photoreceptor precursors or retinal tissue sheets can achieve a certain degree of survival, integration and improvement of visual function in animal models, and some related clinical trials have also entered early stages[35-37]. Compared with the direct induction of a single cell type, retinal organoids can provide cell populations at different developmental stages, offering more options for optimizing the state of transplanted cells.

Overall, the advantages of organoids in regenerative medicine are mainly reflected in three aspects. First, they can provide relatively stable, manipulable and tissue-specific cellular sources through stem cell expansion. Second, they possess certain spatial organization and local functional features, making them more structurally relevant to tissue repair than single-cell transplantation. Third, PDOs or autologous organoids after gene correction offer possibilities for personalized regenerative therapy. Importantly, organoids used as experimental models should be distinguished from organoids intended to serve as implantable therapeutic products. Experimental organoid models are primarily evaluated according to whether they reproducibly reproduce the biological features and functional readouts required for a defined research question. By contrast, implantable organoid products must satisfy substantially more stringent manufacturing, quality, and safety requirements because the graft itself is introduced into the patient and may persist *in vivo* for prolonged periods. In addition to defined cellular composition and functional potency, such products require rigorous assessment of sterility, genomic stability, residual undifferentiated or abnormally differentiated cells, tumorigenic potential, immune compatibility, and long-term functional integration with

host tissues. Therefore, successful formation of tissue-like structures *in vitro* is insufficient to establish therapeutic suitability; clinically relevant evaluation must further demonstrate long-term survival, controlled differentiation, safety, and stable structural and functional integration after transplantation[11,17,20,39,126,127].

Successful transplantation therefore requires evaluation beyond graft survival alone. Functional integration should be assessed at several interconnected levels. Vascular integration is required to provide sustained oxygen and nutrient delivery and to support further tissue maturation, whereas neural or ductal connections may be essential for tissues whose physiological functions depend on signal transmission or anatomical continuity. Immune compatibility and adaptation to the local stromal and mechanical microenvironment further determine whether the graft can remain stable without persistent inflammation, rejection, or maladaptive remodeling. Importantly, structural engraftment does not necessarily indicate functional recovery; transplanted organoids should demonstrate sustained, tissue-appropriate functional outputs and coordinated interaction with the surrounding host tissue over time. Accordingly, the therapeutic evaluation of organoid transplantation should include long-term assessment of graft survival, vascularization, host-graft connectivity, immune responses, cellular composition, genomic stability, and functional performance rather than relying primarily on short-term engraftment or morphological similarity[17,20,39,126].

As different organoid applications have progressed to substantially different stages of clinical translation, their clinical relevance should not be interpreted uniformly. Some applications, such as PDO-based drug-response testing, have undergone patient-level clinical validation in selected settings, whereas many disease models and regenerative approaches remain predominantly preclinical or at an early stage of clinical investigation. To provide a clearer overview of these differences, the clinical maturity, representative evidence, major limitations, and translational status of selected organoid applications are summarized in Table 2.

## RISKS AND CHALLENGES IN THE CLINICAL APPLICATION OF ORGANOIDS

Although organoids have shown substantial value in disease modeling, drug screening, personalized medicine, and regenerative applications, their clinical translation remains constrained by multiple levels of uncertainty. The major translational risks can be grouped into four categories: biological limitations, model validity and clinical extrapolation risks, engineering and quality-control challenges, and clinical safety and long-term risks[18,66,128]. This section discusses these four dimensions and their implications for clinical translation [Figures 4-7].

### Biological limitations

Although organoids can recapitulate human tissue structure and function to a certain extent, their further clinical translation is still limited by multiple biological factors. Among them, insufficient developmental maturation, absence of key microenvironmental components, and difficulty in integrating multilineage structures are currently the major bottlenecks[11,17,120].

#### *Insufficient maturation*

Insufficient developmental maturation represents a common limitation of many developmentally generated organoids, although the degree of maturation depends on cell source and culture conditions. Brain organoids can exhibit immature or stress-altered cell states, while prolonged culture can yield later developmental signatures[15,16,95]. Liu *et al.* reported fetal-like metabolic limitations in existing hepatocyte organoid approaches and improved maturation using optimized culture conditions[129]. Transplanted PSC-derived human intestinal organoids undergo maturation resembling stages of fetal intestinal development[130]. Single-cell multiomic analysis identified epigenetic immaturity in kidney organoids relative to adult human kidney[131].

**Table 2. Clinical maturity and translational status of representative organoid applications**

| Organ/tissue | Organoid source | Intended application | Validation level/Current translational status | Clinical evidence | Principal limitations | Reference |
|---|---|---|---|---|---|---|
| Cystic fibrosis - intestine | Patient-derived intestinal organoids | Prediction of CFTR modulator response | Clinical validation | Organoid functional responses have shown concordance with patient treatment responses and have been incorporated into prospective clinical evaluation strategies | Assay standardization; mutation-specific applicability; incomplete representation of systemic physiology | [107,108] |
| Colorectal/rectal cancer | Tumor PDOs | Drug-response prediction and treatment selection | Clinical validation | PDO drug responses have been compared with treatment outcomes in multiple patient cohorts | Tumor heterogeneity; sampling bias; loss of stromal/immune components; turnaround time | [13,103,121] |
| Pancreatic cancer | Tumor PDOs | Pharmacotyping and individualized treatment selection | Clinical investigation | Patient-derived models have been evaluated for feasibility and correlation with clinical treatment response | Rapid disease progression; variable establishment success; stromal dependence; time constraints | [102,113] |
| Ovarian cancer | Tumor PDOs | Drug-response prediction and personalized therapy | Clinical investigation | PDO-based treatment-response studies, including more complex co-culture approaches, have entered clinical evaluation | Tumor heterogeneity; incomplete tumor microenvironment; assay standardization | [59,111] |
| Liver | PSC-/ASC-derived organoids | Disease modeling and hepatotoxicity testing | Preclinical validation | Functional and toxicity-related responses have been demonstrated mainly *in vitro* | Developmental immaturity; incomplete metabolism; lack of vascular and systemic regulation | [110] |
| Cardiac tissue | PSC derived cardiac organoids | Disease modeling and cardiotoxicity testing | Preclinical validation | Contractile, electrophysiological, and calcium-related functional readouts have been demonstrated *in vitro* | Immature cardiomyocyte phenotype; incomplete mechanical and systemic regulation | [38,109] |
| Brain | PSC derived brain organoids | Neurological disease modeling and experimental transplantation | Experimental/preclinical | Disease phenotypes have been reproduced *in vitro*, while transplantation studies remain predominantly animal-based | Developmental immaturity; insufficient vascularization; variability; uncertain long-term safety | [34,98] |
| Kidney | PSC derived kidney organoids | Kidney disease modeling and nephrotoxicity testing | Experimental/preclinical | Nephron-like structures and disease-related phenotypes have been demonstrated *in vitro* | Incomplete vascular perfusion, filtration, and tubular integration | [33] |
| Retina | PSC derived retinal organoids/organoid-derived retinal cells | Cell replacement for retinal degeneration | Early clinical investigation | Preclinical transplantation has demonstrated survival and partial functional integration, with related approaches entering early-stage human studies | Functional integration; immune compatibility; tumorigenic risk; long-term safety | [36,37] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Bile duct | Cholangiocyte organoids | Biliary epithelial repair | Preclinical validation | Cholangiocyte organoid engraftment and biliary epithelial repair have been demonstrated in animal models and *ex vivo* perfused human livers, providing preclinical proof of concept without establishing clinical safety or efficacy in patients | Limited human evidence; uncertain long-term engraftment and durability; manufacturing consistency | [66] |
| Lung cancer | Tumor PDOs | Drug-response and resistance prediction | Preclinical/early translational | Patient-derived lung cancer organoids have reproduced major tumor characteristics and demonstrated differential responses to anticancer agents *in vitro*, supporting their potential for patient-specific therapeutic screening | Sampling limitations; loss of stromal/immune components; variable establishment efficiency | [76] |
| Intestine | ASC-/patient-derived intestinal organoids | Epithelial repair and regenerative transplantation | Preclinical/early translational | Engraftment and mucosal repair have been demonstrated predominantly in preclinical models | Vascularization; long-term integration; scale-up; manufacturing consistency; safety | [123,125] |

This table summarizes representative organoid applications at different stages of clinical translation. Clinical maturity and translational status reflect the current level of supporting evidence, from preclinical validation to clinical validation and early clinical investigation, and do not necessarily indicate routine clinical use. The examples shown are representative rather than exhaustive. ASC: Adult stem cell; CFTR: cystic fibrosis transmembrane conductance regulator; PDO: patient-derived organoid; PSC: pluripotent stem cell.

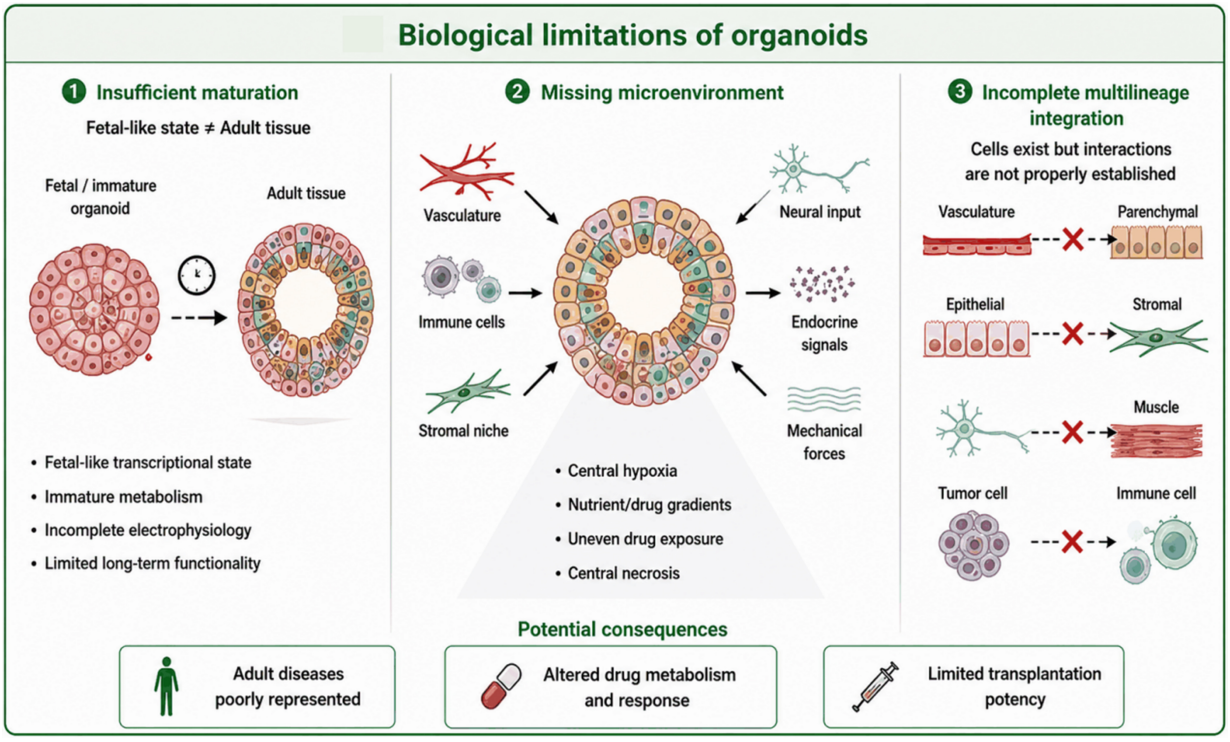


**Figure 4.** Biological limitations restricting the physiological relevance of organoids. The figure summarizes three major biological limitations of current organoid systems: insufficient developmental maturation, incomplete reconstruction of key microenvironmental components, and limited multilineage integration. These deficiencies may restrict tissue-level function, physiological regulation, and the reliability of disease modeling, drug evaluation, and regenerative applications.

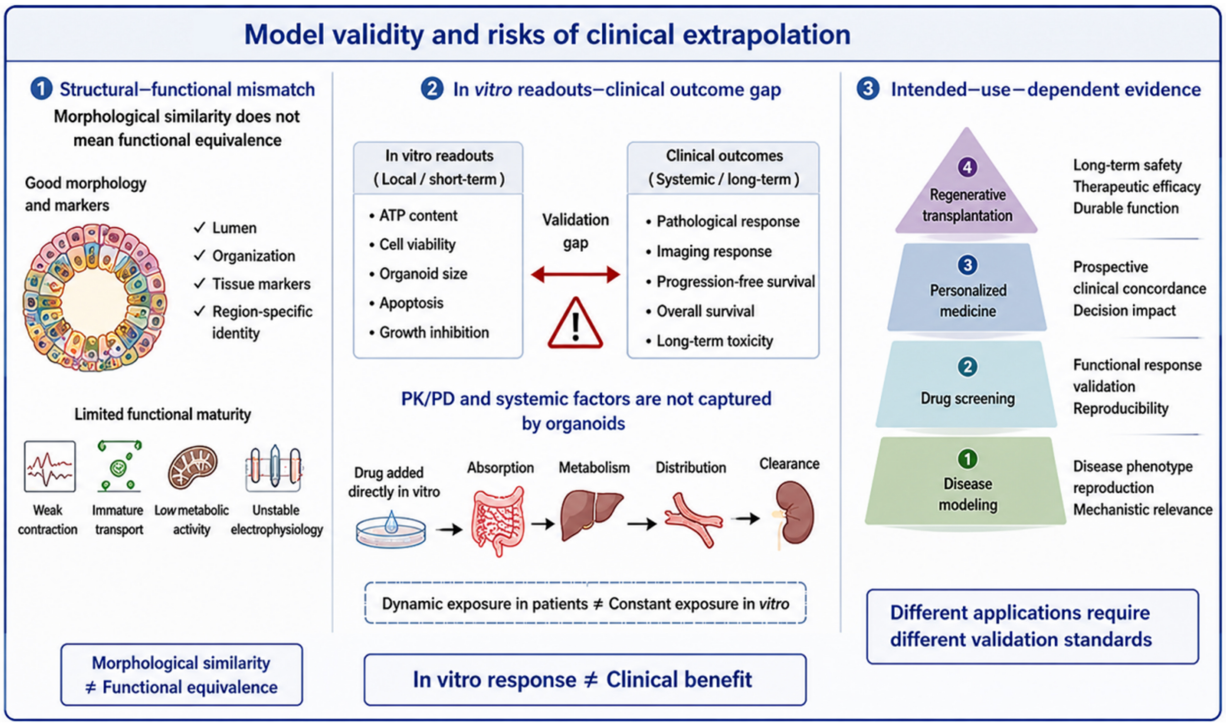


**Figure 5.** Model validity and risks of clinical extrapolation. The figure summarizes the principal factors limiting clinical interpretation of organoid-derived readouts, including structural-functional mismatch, incomplete correspondence between *in vitro* responses and clinical outcomes, and differences in validation requirements among intended applications. Reliable translation therefore requires intended-use-specific functional validation rather than reliance on morphology or marker expression alone. ATP: Adenosine triphosphate; PK/PD: pharmacokinetics/pharmacodynamics.

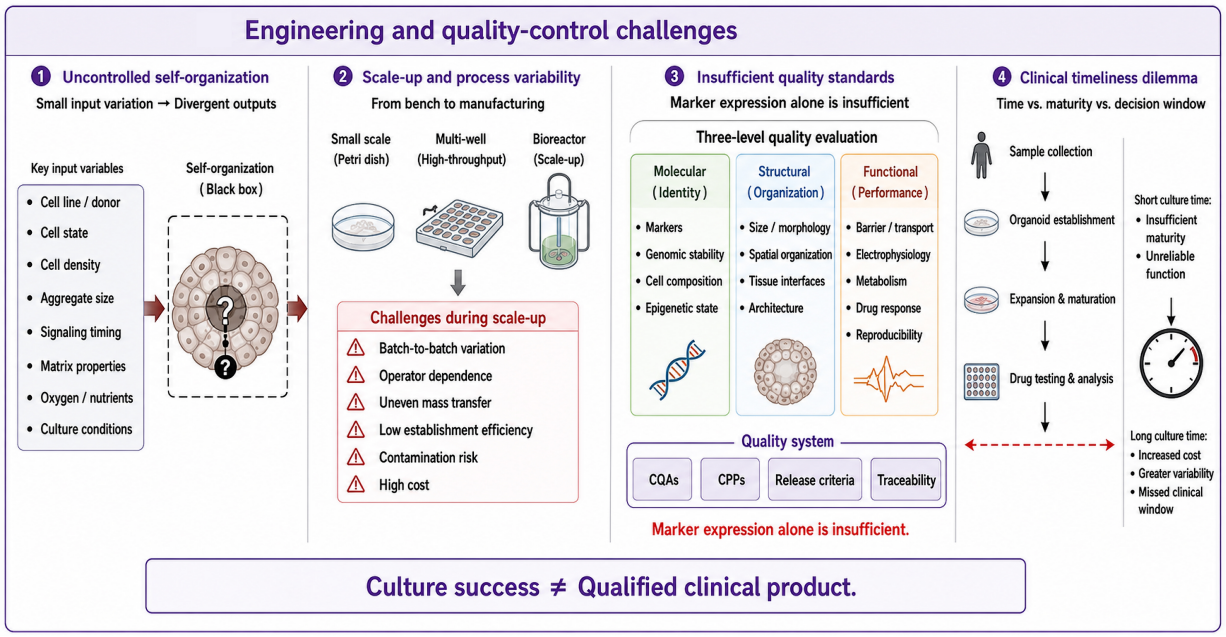


**Figure 6.** Engineering challenges affecting organoid reproducibility and clinical translation. The figure integrates major engineering challenges, including insufficient control of self-organization, batch-to-batch variability, limited standardization and quality-control systems, and long production cycles. These factors influence process consistency, critical quality attributes, scalability, and the reproducibility of functional outputs across organoid preparations. CPPs: Critical process parameters; CQAs: critical quality attributes.

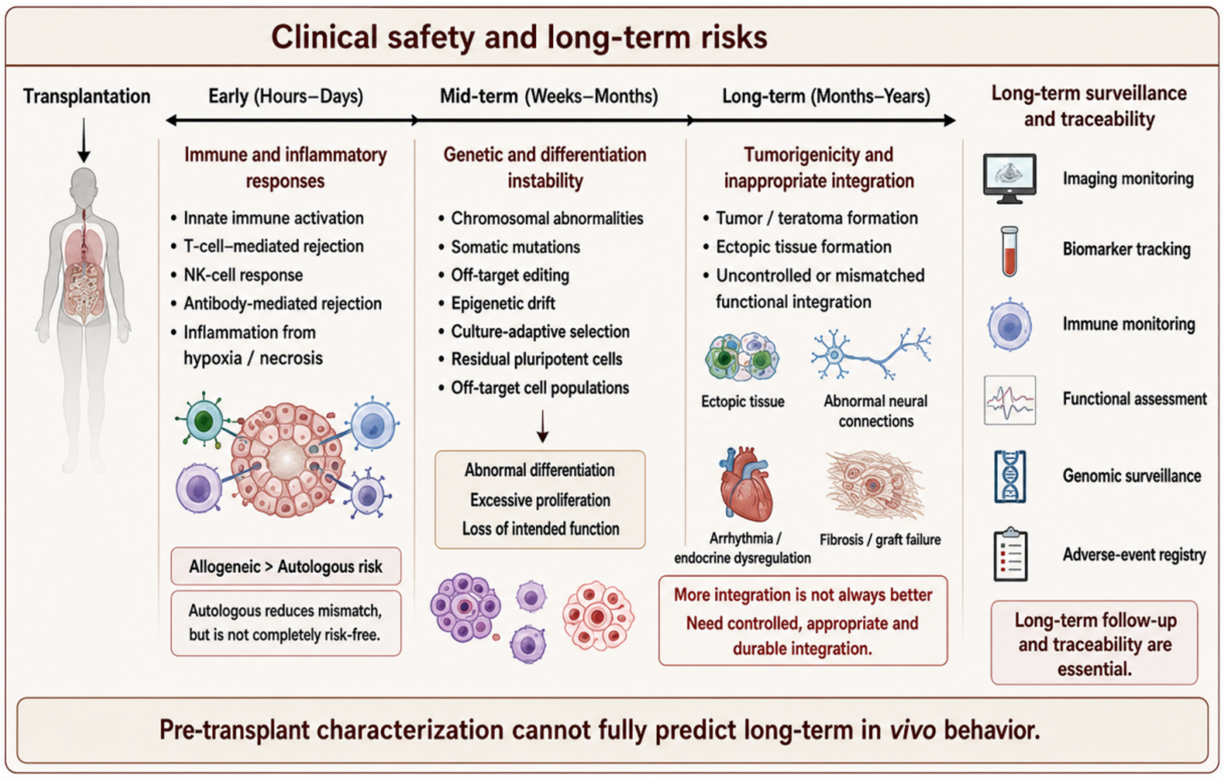


**Figure 7.** Clinical safety and immune issues associated with organoid transplantation. The figure summarizes major safety considerations for implantable organoid products, including immune rejection, tumorigenic and genomic risks, and incomplete or abnormal integration with host tissues. These risks are dynamic after transplantation and therefore require assessment of both pre-transplant product quality and long-term *in vivo* behavior. NK: Natural killer.

Multi-chamber cardioids reproduce major embryonic heart compartments rather than demonstrating adult myocardial equivalence[132]. Recent neurovascular intestinal organoids and proximal-biased kidney organoids have shown improvements in selected functions, illustrating that maturation remains model- and condition-dependent[133,134]. These findings should not be generalized to all adult tissue-derived organoids. However, immature states do not necessarily reduce the value of organoids. For studies focusing on early organogenesis, lineage specification, and congenital developmental disorders, developmental immaturity may preserve stage-specific cellular characteristics and molecular programs[33,34,38]. In contrast, when organoids are applied to adult disease modeling, chronic disease simulation, drug metabolism evaluation, or regenerative transplantation, insufficient maturation may limit their ability to accurately represent adult tissue physiology and clinical responses[11,135].

The consequences of incomplete maturation vary among organoid systems. Brain organoids can generate neuroepithelial structures, ventricle-like regions, neuronal populations, and spontaneous electrical activity, but they generally lack mature neural features such as fully developed network organization, glial maturation, myelination, vascular support, and immune regulation[15,16,34]. Therefore, they are highly valuable for studying human neurodevelopment but may incompletely reproduce adult neurodegenerative disorders or long-term neurotoxicity. Liver organoids and liver bud-like structures can express hepatic markers and exhibit partial metabolic activity, but their enzyme profiles, bile transport capacity, detoxification functions, and long-term toxicity responses often remain different from adult liver tissue, limiting their application in predicting adult drug-induced liver injury[39,110].

Similar limitations exist in other organs. Cardiac organoids can display spontaneous contraction, calcium activity, and electrical properties, but cardiomyocytes often remain in immature metabolic states with incomplete sarcomere organization, mitochondrial maturation, and mechanical adaptation[38,109]. Therefore, contraction alone does not indicate adult-level electromechanical function. Kidney organoids can generate nephron-like structures containing podocyte-like and tubular components, but mature renal physiology requires vascular perfusion, filtration barriers, fluid flow, and tubule-interstitium interactions, which are difficult to reproduce *in vitro*[17,33]. Intestinal and lung organoids also demonstrate partial tissue-specific functions but lack important systemic regulators. Intestinal maturation depends on microbiota, immune regulation, enteric nervous input, and mechanical stimulation, whereas lung maturation requires alveolar-capillary interactions, air-liquid interfaces, and respiratory mechanics[41,46,50,52,53]. Thus, incomplete maturation reflects a broader gap between organoids and mature tissues in developmental stage, cellular state, spatial organization, and systemic regulation.

The causes of insufficient maturation are multifactorial. First, *in vivo* organ development depends on dynamic and stage-specific changes in signaling pathways, whereas *in vitro* systems usually rely on relatively fixed induction protocols that cannot fully reproduce continuous developmental transitions[1,30-32]. Second, adult tissue maturation requires integration of vascular, neural, immune, endocrine, extracellular matrix, and mechanical signals, many of which are absent in conventional organoid cultures[17,23]. Third, limitations in oxygen, nutrient, and metabolite transport create spatially heterogeneous microenvironments within larger organoids, resulting in regional differences in cell state, stress responses, and functional maturation[34,136]. Fourth, long-term culture may introduce selection pressure, allowing cells adapted to *in vitro* proliferation conditions to become enriched while certain mature tissue states are gradually lost[16,18]. Finally, many organs require prolonged physiological stimulation for maturation, such as mechanical loading in cardiac tissues, air-liquid interfaces in lung tissues, fluid flow in kidney systems, and microbiota-associated signals in intestinal tissues[24,25,135].

Therefore, expression of tissue markers, formation of organ-like structures, or short-term functional activity cannot alone demonstrate complete tissue maturation. The major risk of insufficient maturation is not that organoids fail to mature completely, but that their developmental characteristics may be overlooked and their findings may be directly extrapolated to adult tissues, chronic diseases, or clinical applications. For developmental studies, immaturity may represent an advantage; however, for adult disease modeling, drug evaluation, personalized medicine, and regenerative therapy, incomplete maturation may lead to inaccurate interpretation of disease phenotypes, therapeutic responses, and functional potential. Consequently, organoid maturation should be evaluated through integrated assessment of molecular states, cellular composition, spatial organization, functional performance, and application-specific requirements rather than relying solely on individual markers or morphological similarity[11,15,16].

### *Absence of key microenvironmental components*

The absence of key microenvironmental components represents another major biological limitation restricting the ability of organoids to reproduce *in vivo* tissue states. Native organs do not consist of isolated epithelial or parenchymal cells, but are maintained within dynamic microenvironments composed of blood vessels, immune cells, stromal populations, nerves, endocrine signals, and extracellular matrix (ECM). These components regulate tissue organization, cell fate decisions, functional maturation, and repair processes through paracrine signaling, cell-cell interactions, mechanical stimulation, and metabolic exchange[17,137]. Therefore, microenvironmental deficiency is not simply the absence of specific cell types, but the disruption of regulatory networks required for maintaining tissue function, thereby affecting disease modeling, drug responses, and clinical interpretation.

Among these deficiencies, the lack of vascularization is one of the most prominent limitations across organoid systems. *In vivo*, blood vessels function not only as transport systems but also as regulators of tissue organization, signaling communication, and mechanical environments[23,80]. Without perfusable vasculature, organoids rely mainly on passive diffusion for oxygen and nutrient supply. As organoid size increases, gradients of oxygen, nutrients, metabolites, and drugs can develop between peripheral and central regions, resulting in hypoxia, cellular stress, abnormal metabolism, or necrosis[34,136]. These heterogeneous environments can generate regional differences in maturation and increase variability between organoids. In drug evaluation, the absence of vascular delivery also alters exposure patterns because compounds diffuse from the outside inward rather than being distributed through circulation and tissue perfusion as *in vivo*.

Furthermore, endothelial cells and vascular-associated cells contribute directly to tissue development and maturation through interactions with parenchymal and stem/progenitor cells. Signaling pathways involving vascular endothelial growth factor (VEGF), angiopoietin, Notch, and Wnt participate in vascular development, niche maintenance, and tissue repair[23,80]. Therefore, even when organoids contain target cell types and tissue-like structures, the absence of endothelial interactions, pericyte support, and flow-associated signals may limit functional maturation. For example, brain organoids lacking vascular support often show hypoxia-related limitations in neuronal survival, glial maturation, and network stabilization. Kidney organoids may generate nephron-like structures but cannot fully reproduce filtration and tubular transport without vascularized glomerular environments and fluid stimulation. Islet-like organoids without vascular networks may also fail to reproduce glucose sensing and endocrine regulation under physiological conditions[17,33,69]. In regenerative applications, insufficient vascular integration may further impair graft survival because larger organoids can experience hypoxic injury before establishing connections with host circulation[17,20].

Beyond vascular components, the absence of immune and stromal elements substantially limits the ability of organoids to model complex diseases. Many organoids are primarily composed of epithelial, parenchymal, or tumor cells, while lacking fibroblasts, immune populations, endothelial cells, pericytes, and native ECM components[16,138]. This limitation is particularly evident in tumor PDOs. Although PDOs can preserve tumor-specific genetic alterations and some histological characteristics, tumor-associated fibroblasts, immune cells, vascular cells, and matrix components are often reduced during expansion[118,139,140]. Because tumor progression, immune escape, invasion, and therapeutic resistance depend strongly on tumor-immune-stromal interactions, epithelial tumor organoids alone may have limited predictive value for immunotherapy, anti-angiogenic treatment, and stromal-targeting therapies[141-143]. This limitation is particularly important when tumor PDOs are used for pharmacological prediction. Although epithelial PDOs may retain tumor-cell-intrinsic genetic and phenotypic features, loss of stromal, immune, vascular, and extracellular-matrix components can alter drug penetration, paracrine signaling, immune responses, and treatment resistance. Consequently, epithelial-only PDOs may incompletely reproduce responses to therapies that depend on tumor-stroma, tumor-immune, vascular, or extracellular-matrix interactions. Accordingly, the predictive validity of PDO-based drug testing should be interpreted in relation to both the mechanism of action of the therapy and the extent to which relevant microenvironmental components are preserved or reconstructed in the model[118,139-145]. Similar limitations occur in inflammatory and fibrotic diseases, where immune recruitment, cytokine signaling, fibroblast activation, and ECM remodeling are central components of disease progression[65,146-149].

Neural, endocrine, and mechanical signals also represent essential components of tissue regulation. Many organ functions depend not only on local cellular differentiation but also on external regulatory inputs. Intestinal function, for example, requires interactions among epithelial cells, enteric nerves, smooth muscle, microbiota, and immune components, whereas islet function depends on vascular regulation, neural signals,

multiple endocrine cell types, and systemic glucose feedback. Lung maturation similarly requires air-liquid interfaces, cyclic mechanical stimulation, and alveolar-capillary interactions[24,25,135]. Therefore, organoids lacking these regulatory environments may reproduce local cellular behaviors but remain unable to fully model dynamic physiological functions.

The ECM represents another critical microenvironmental component influencing organoid organization and function. Matrix composition and mechanical properties regulate epithelial polarity, branching morphogenesis, lumen formation, and stem cell maintenance in intestinal, gastric, hepatic, pancreatic, mammary, and salivary gland organoids[8,24,40,46,68,72,73,83]. In tumor and fibrosis models, matrix stiffness, collagen deposition, and ECM remodeling further influence invasion, epithelial-mesenchymal transition, fibroblast activation, and drug diffusion[142,144,145,147-149]. Therefore, ECM mismatch does not merely affect scaffold selection, but can alter cellular states, tissue architecture, and disease-associated phenotypes.

Importantly, the extent of microenvironmental reconstruction required depends on the intended application. For studies focusing on cell-autonomous mechanisms, epithelial differentiation, or specific genetic effects, simplified organoid systems may provide sufficient information. However, applications involving immunotherapy, chronic inflammation, fibrosis, infection, tissue repair, and regenerative transplantation require reconstruction of key components such as vasculature, immune cells, stroma, nerves, and ECM[11,17]. Therefore, the major consequence of microenvironmental deficiency is not simply reduced complexity, but restricted applicability and uncertain interpretation of organoid models. Future clinical translation requires defining application-specific microenvironmental requirements and establishing corresponding evaluation criteria to improve predictive reliability.

### *Difficulty in multilineage integration*

As organoid technology progresses from single epithelial or parenchymal models toward more complex tissue systems, the integration of multiple cellular lineages has become a major challenge for improving model complexity and physiological relevance. Native organs are composed of cells originating from different germ layers or developmental programs, and their functions depend not only on the presence of multiple cell types but also on precisely coordinated interactions established through developmental timing, spatial organization, and signaling environments[89,90]. For example, intestinal function requires interactions among endoderm-derived epithelial cells, mesoderm-derived smooth muscle, vascular and stromal components, and neural crest-derived enteric neurons. Similarly, skin and hair follicle formation depends on coordinated interactions among epidermal cells, dermal fibroblasts, dermal papilla cells, blood vessels, nerves, and immune components[78,79,89]. Therefore, multilineage integration requires not simply increasing cell diversity, but reproducing the developmental coordination and functional relationships among different lineages.

A major limitation of multilineage integration is the mismatch of developmental timing between different cell populations. *In vivo*, different lineages emerge and mature according to specific developmental sequences, and disruption of this timing can affect tissue organization. For example, intestinal development involves coordinated epithelial differentiation, stromal maturation, smooth muscle formation, and neural crest migration. Although Workman *et al.* combined human PSC-derived neural crest cells with intestinal organoids to generate models containing enteric nervous system components, successful integration still depends on precise control of cell addition timing, cell ratios, and maturation conditions[90]. Thus, multilineage construction requires synchronized regulation of multiple developmental programs rather than simple combination of independently generated cell populations.

Another challenge arises from incompatible culture requirements among different lineages. Conditions that support epithelial stem cell expansion, such as Wnt/R-spondin and EGF signaling, may not be optimal for maintaining neuronal maturation, immune activity, or stromal stability[16,18]. In tumor organoid systems, maintaining tumor epithelial cells together with fibroblasts, immune cells, and vascular components requires balancing expansion of tumor populations with preservation of microenvironmental functions. Similarly, immune cells require specific cytokine stimulation for functional maintenance, whereas stromal cells are highly sensitive to ECM composition, matrix stiffness, and transforming growth factor beta (TGF-β) signaling. Consequently, multilineage integration requires coordinated control of different cellular requirements across time and culture conditions.

Spatial organization represents another critical limitation. In native tissues, different cell types are arranged into specific interfaces rather than randomly distributed. Examples include the endothelial-basement membrane-podocyte structure of the glomerular filtration barrier, the alveolar epithelial-capillary interface required for gas exchange, and neuromuscular junctions formed between motor neurons and skeletal muscle fibers. If multiple cell types are simply mixed within organoids, lineage markers may be detected, but physiologically relevant tissue interfaces and functional interactions may not be established. Because organoid self-organization remains partly stochastic, precise control of cell proportion, spatial distribution, boundary formation, and long-term maintenance of multilineage structures remains difficult[19,89].

The ultimate challenge of multilineage integration is the establishment of stable functional coupling between different cell populations. Advanced systems such as assembloids have attempted to overcome this limitation by connecting specialized tissue modules. Forebrain assembloids developed by Birey *et al.* reproduced interneuron migration between dorsal and ventral neural regions, providing models for studying interregional neural interactions, although long-term network maturation and reproducibility remain challenging[87]. Gut-neural organoid systems incorporating neural crest-derived cells can model interactions between enteric neurons and intestinal tissues, but whether they fully reproduce peristalsis, neurotransmitter regulation, and long-term innervation remains unclear[90]. Similarly, neuromuscular organoids containing functional neuromuscular junctions and contractile activity demonstrate the potential of cross-lineage integration, although their maturation and mechanical environment remain different from native systems[150].

From a clinical translation perspective, insufficient multilineage integration affects the reliability of disease modeling, drug evaluation, and regenerative applications. Many pathological processes, including inflammation, fibrosis, tumor progression, immune responses, and tissue repair, are driven by interactions among epithelial, immune, stromal, vascular, and neural components rather than individual cell types. Without stable lineage integration, organoids may fail to accurately reproduce disease mechanisms or therapeutic responses. In regenerative medicine, incomplete integration with host vasculature, nerves, immune components, and mechanical environments may compromise long-term survival and functional reconstruction after transplantation[11,17,20]. Therefore, the fundamental challenge of multilineage integration is not merely increasing cellular complexity, but achieving precise control of developmental timing, spatial organization, and functional coupling among multiple lineages.

Overall, these biological limitations restrict the extent to which organoids reproduce *in vivo* development, maturation, and homeostasis. Their significance therefore depends on the biological requirements of the intended application rather than on structural complexity alone[11].

## Model validity and risks of clinical extrapolation

The biological limitations described above ultimately define the validity boundaries of organoids as preclinical and translational models. Clinical interpretation therefore requires intended-use-specific validation of whether organoid-derived functional readouts reliably reflect the relevant physiological,

pathological, or clinical endpoints[3,11,19,22]. The major dimensions of model-validity and clinical-extrapolation risk are summarized in Figure 5.

### *Structural-functional mismatch*

A fundamental issue in evaluating organoid model validity is that structural similarity does not necessarily indicate functional equivalence. Organoids can generate luminal, tubular, layered, or regionalized structures and express tissue-specific markers under three-dimensional culture conditions, demonstrating partial reconstruction of organogenesis or tissue maintenance[3,7,11,14,19,120]. However, organ function is not determined solely by cell composition and morphology, but depends on dynamic interactions among cellular identity, spatial organization, material exchange, signal transmission, and multicellular coordination. Therefore, morphological resemblance, marker expression, or isolated functional outputs indicate only partial reconstruction of organ characteristics and cannot be directly interpreted as equivalent organ-level function.

This inconsistency mainly results from the selective reconstruction of developmental programs during organoid formation. Although *in vitro* systems can guide lineage differentiation and promote tissue organization through defined signals, they usually fail to reproduce the continuous circulation, neural and endocrine regulation, mechanical stimulation, and long-range communication present *in vivo*. Consequently, organoids may develop recognizable structures while lacking the integrated regulatory networks required for complete physiological function. Structural similarity therefore reflects the presence of relevant components, whereas functional validity requires appropriate spatial relationships, dynamic regulation, and coordinated activity among these components.

Kidney organoids provide a representative example of this distinction. PSC-derived kidney organoids can generate podocyte-like cells, proximal and distal tubular structures, and express nephron-associated markers[33]. However, conventional static culture conditions often lack perfusable vascular networks, continuous tubular connections, and physiological fluid flow. As a result, the presence of nephron-like structures or transporter expression does not demonstrate complete filtration, reabsorption, and excretion functions. Perfusion-based approaches can improve vascularization and structural maturation, further indicating that kidney function depends on integration of multiple components rather than isolated structural formation[33,135].

Similarly, morphological features and molecular markers may not fully represent cellular identity or developmental state. Brain organoids can generate neuroepithelial structures, ventricular-like cavities, neuronal populations, and cortical-like organization[34,43]. However, transcriptomic studies have revealed persistent stress-related states, including hypoxia, glycolytic activation, and endoplasmic reticulum stress, which may influence neuronal maturation and regional specification[16]. Therefore, expression of neural markers alone does not confirm that organoid cells fully correspond to their *in vivo* counterparts. Evaluation of brain organoid validity requires integration of molecular profiles, spatial organization, lineage relationships, and electrophysiological properties rather than reliance on morphology or individual markers.

The same principle applies to functional outputs. Stem cell-derived islet-like organoids can generate insulin-producing β-like cells expressing markers such as insulin, pancreatic and duodenal homeobox 1 (PDX1) and NK6 homeobox 1 (NKX6-1), and may respond to glucose stimulation[69]. However, mature islet function requires coordinated processes including glucose sensing, metabolic coupling, calcium signaling, insulin secretion dynamics, and interactions among α, β, and δ cells. Therefore, insulin expression or a single stimulation-induced response represents only part of the functional pathway and cannot demonstrate complete glucose homeostasis regulation. Functional assessment should include dynamic responses, stimulus-secretion relationships, repeated stimulation stability, and intercellular coordination.

Beyond biological limitations, structural-functional inconsistency also reflects a mismatch between evaluation scales. Histological staining, imaging, and single-cell sequencing mainly describe cellular composition and spatial states at specific time points, whereas organ functions are continuous, directional, and dynamically regulated processes. For example, kidney function requires sustained filtration and transport, neural function depends on network-level information processing, and endocrine function relies on feedback-controlled secretion. Thus, endpoint markers or isolated responses may incorrectly equate cell presence with maturation, local activity with tissue function, or transient responses with physiological regulation[3,19,22,120].

Therefore, organoid validity should be evaluated through a hierarchical framework integrating molecular identity, spatial organization, functional connectivity, and dynamic outputs. For disease modeling, evaluation should focus on whether organoids preserve disease-relevant cell states and tissue interactions. For drug testing, the relationship among drug targets, biological responses, and functional readouts should be clearly established. For regenerative applications, stable integration and physiological regulation after transplantation must be demonstrated. Overall, structural reconstruction provides the foundation for organoid function, but reliable clinical interpretation requires consistency between molecular, structural, and functional characteristics within the intended application context.

### *Limited predictive value of in vitro readouts*

The presence of corresponding structural and functional phenotypes in organoids does not mean that their *in vitro* readouts can directly predict patient clinical outcomes. Structural-functional consistency reflects the internal biological validity of the model, whereas prediction of efficacy, toxicity, resistance, and prognosis belongs to external validity. Only when organoid readouts establish stable, reproducible, and independently validated relationships with prespecified clinical endpoints can experimental phenotypes be translated into clinical predictive evidence[3,6,11,13,103,121].

The main difference between *in vitro* readouts and clinical outcomes lies in the biological levels they represent. Organoid drug-sensitivity assays commonly use adenosine triphosphate (ATP) content, cell viability, organoid area, apoptosis proportion, or short-term growth inhibition rate to evaluate drug effects, primarily reflecting the direct action of drugs on tumor cell survival and proliferation. Clinical efficacy, by contrast, involves imaging response, pathological response, progression-free survival, or overall survival, and is also affected by regrowth of residual cells, expansion of resistant clones, and duration of therapeutic effect. Therefore, reduced viability *in vitro* only demonstrates direct inhibition of organoids by a drug and cannot be interpreted, without validation, as durable clinical benefit for patients[6,11,102]. Ooft *et al.* found in a metastatic colorectal cancer organoid drug-sensitivity study that PDOs had value in negative prediction for some chemotherapy regimens, but their predictive performance varied across treatment regimens[121]. This suggests that organoid readouts are not universally applicable drug sensitivity indicators, but rather reflect relationships among specific experimental conditions, drug exposure modes, and clinical treatment regimens.

Inconsistency between *in vivo* and *in vitro* drug exposure conditions can further alter this correspondence. Taking irinotecan as an example, after clinical administration, the drug must be converted by carboxylesterases into the active metabolite SN-38, whose activity is significantly higher than that of the parent compound; subsequent inactivation and clearance are also affected by glucuronidation and other metabolic processes. A single tumor organoid system usually cannot fully reconstruct these systemic metabolic processes. Responses obtained by directly adding irinotecan may be influenced by the organoid's own metabolic capacity and may not represent active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38)

exposure formed in the patient's body. To reduce this bias, some colorectal cancer organoid drug-sensitivity assays directly use SN-38 to evaluate irinotecan-related sensitivity, but constant SN-38 concentrations in culture medium still differ from the time-varying free drug exposure in patients[121]. Therefore, the drug form, concentration, and treatment duration used *in vitro* should be set according to the active form and clinically achievable exposure; otherwise, marked inhibition produced by high-concentration continuous treatment may not be achievable under real dosing conditions[6,11,151].

Therefore, clinical translation of organoid *in vitro* readouts should consider both evidence level and intended use. Concordance between *in vitro* results and clinical outcomes in retrospective cases only indicates preliminary correlation. Predictive evidence requires prespecified protocols and decision thresholds to predict subsequent outcomes in independent cohorts. Clinical utility can be demonstrated only when test results improve treatment selection and provide incremental information beyond existing indicators. Overall, organoid readouts are functional mappings of specific components of disease processes, not direct substitutes for clinical outcomes. Only when drug mechanism of action, *in vitro* functional endpoints, and prespecified clinical outcomes form an independently validated continuous evidence chain can organoid models obtain clinical interpretive boundaries appropriate to their intended use[6,11,103,121].

## Engineering challenges

Clinical translation is also constrained by insufficient engineering controllability. Major challenges include variability in self-organization, difficulty in scalable manufacturing, incomplete standardization and quality-control systems, and long production cycles[11,14,15,19,22]. The major engineering challenges affecting reproducibility, scalability, and quality control are summarized in Figure 6.

### *Insufficient controllability of self-organization*

Organoid formation involves multiple stages, including cell proliferation, lineage differentiation, migration, and spatial rearrangement, and its outcomes are highly sensitive to initial conditions and culture timing. Differences in cell-line differentiation tendencies, cell aggregate size, growth factor exposure windows, and local oxygen and nutrient gradients can all alter final tissue architecture. Brain organoids provide a typical example. Early non-directed brain organoids often showed differences in regional composition and structure, whereas Velasco *et al.* restricted the initial cellular state and dorsal forebrain induction program and found that approximately 95% of organoids formed highly similar cortical-related cell types[15]. This indicates that narrowing developmental trajectories and strictly controlling early conditions can reduce the stochasticity of self-organization. However, reproducibility remains a property of specific cell lines, culture protocols, and evaluation scales, and cannot be extrapolated from a single morphological indicator to other systems. Engineering control therefore needs to cover the entire process, including cell source, aggregate size, medium changes, stirring, and mass transport, rather than merely selecting organoids with acceptable appearance at the endpoint. More recently, controlling initial tissue geometry enabled more reproducible intestinal organoid patterning and crypt formation[152].

### *Difficulty in scalable manufacturing*

Scalable manufacturing of organoids is not simply a matter of increasing culture numbers; it requires maintaining consistency in cellular composition, spatial structure, and functional output while increasing yield. Current organoid culture commonly relies on manual operations and self-organization. Subtle differences in initial cellular state, aggregate size, signaling exposure time, and local culture conditions may be amplified during culture, ultimately leading to batch-to-batch differences in morphology, lineage proportions, and maturation. Especially in PSC-derived organoids, even with the same protocol, different cell lines or batches may follow different differentiation trajectories, indicating that procedural consistency does not equal biological consistency[15,18,19,128,153].

Uncertainty in culture matrices is another major obstacle. Animal-derived matrices such as Matrigel have complex compositions, and their biochemical composition, mechanical properties, and growth factor content are difficult to define completely and may vary between batches. The matrix not only provides three-dimensional support, but also directly regulates cell proliferation, differentiation, and tissue morphology. Gjorevski *et al.* found that intestinal stem cell expansion requires relatively high matrix stiffness, whereas subsequent organoid formation and differentiation depend on matrix softening, indicating that different culture stages require different material properties. Thus, scalable production must control not only the amount of matrix, but also dynamic properties such as stiffness, degradability, and ligand presentation, which increases the difficulty of cross-batch replication and process transfer[19,24,120].

Scaling up culture also changes the mass transport and mechanical environment surrounding organoids. As organoid number, culture volume, and tissue size increase, restricted diffusion of oxygen and nutrients, accumulation of metabolic waste, and changes in fluid shear may lead to central hypoxia, cellular stress, or even necrosis. Studies of brain organoids have shown that cellular stress affects not only survival, but also cellular identity and developmental programs. Therefore, culture conditions that are effective in microwell plates may not yield the same tissue composition and functional state after transfer to rotating culture or bioreactors[16,86,135,136].

Automation can reduce pipetting and medium-change errors, but it mainly improves operational consistency rather than necessarily improving biological consistency of the product. The automated kidney organoid platform established by Czerniecki *et al.* demonstrated the feasibility of high-throughput culture and phenotypic analysis. However, if differences already exist in initial cellular state, early differentiation efficiency, or aggregate formation, automation may simply reproduce these biases at high throughput[154]. Therefore, the core obstacle to scalable organoid manufacturing is not a lack of equipment, but the absence of stable process windows that can maintain consistency across cell batches, material batches, and culture scales. If comparability of key structural and functional attributes before and after scale-up cannot be demonstrated, increased yield cannot be equated with stable manufacturing. A permeable-membrane culture platform has also improved kidney organoid uniformity and selected maturation readouts, providing a recent example of engineering strategies for scalable culture[155].

### *Lack of standardization and quality-control systems*

Incomplete standardization and quality-control systems are major obstacles to the transition of organoids from research models to drug evaluation and clinical application. At present, different laboratories vary substantially in cell source, medium formulation, matrix system, induction timing, and passaging method. Even when constructing the same type of organoid, different cellular compositions, maturation states, and functional phenotypes may be obtained. Therefore, identical protocol names do not necessarily mean that final products are comparable, and cross-laboratory reproducibility and multicenter validation remain difficult[3,18,153,156].

A deeper issue is that organoid quality is difficult to define using a single indicator. Diameter, roundness, lumen formation rate, and expression of specific markers are convenient to measure, but mainly reflect appearance or cellular identity and cannot directly demonstrate expected function. For example, expression of albumin and hepatocyte nuclear factor 4 alpha (HNF4α) in liver organoids does not mean that mature drug metabolism and bile transport capacity have been established. Spontaneous contraction in cardiac organoids does not prove that electrical conduction and mechanical output have reached adult myocardial levels. Organoids with similar morphology may have different cellular subpopulation proportions and drug responses, whereas samples with different morphology may produce similar functional outputs. This inconsistency among morphology, composition, and function makes marker positivity or structural similarity insufficient as a direct basis for quality qualification[19,22,120,156].

The lack of clear links between critical quality attributes and critical process parameters also limits traceability of quality problems. In particular, the relationship between reproducibility and key quality-related variables remains insufficiently defined. Starting-cell composition and state, passage number, matrix composition, initial aggregate size and final organoid size, cellular composition, functional potency, and genomic stability can all vary across preparations and contribute to batch-to-batch differences. However, which of these variables should be regarded as critical quality attributes, how strongly they determine intended-use-related performance, and what degree of variation is acceptable remain incompletely established. Consequently, reproducibility is often assessed using morphological consistency or selected marker expression rather than through a defined set of quality attributes linked to biological function and clinical performance[11,19,22,153,156,157].

In addition, different clinical uses impose different quality requirements on organoids. Disease models need to stably reproduce target pathological phenotypes. Drug screening systems place greater emphasis on reproducibility, assay dynamic range, and the ability to distinguish drug responses. Individualized treatment models also need to demonstrate correlations between *in vitro* results and patient clinical responses. Transplantable organoids must further evaluate cell purity, genetic stability, residual undifferentiated cells, and functional potency. Therefore, there is no single quality standard based only on morphology and general markers that can apply to all organoid systems[11,103,107,108,157].

Overall, the core difficulty in organoid quality control is not a lack of detection methods, but the absence of clear knowledge about which indicators truly determine model validity or clinical performance. Without a continuous evidence framework from cellular identity and tissue architecture to intended-use-related function, simply adding imaging, omics, or marker-based assays may generate large amounts of descriptive data without producing executable quality criteria.

### *Long production cycles*

Long production cycles and limited effective yield are important factors restricting engineering applications of organoids. PSC-derived organoids usually need to sequentially undergo germ-layer induction, organ-specific progenitor formation, three-dimensional self-organization, and functional maturation, with complete culture processes often lasting weeks to months. Brain organoids, cardiac organoids, and related systems often require long-term culture to obtain relatively stable cellular composition and functional phenotypes, making them difficult to apply in scenarios that demand timely results, such as clinical decision-making, rapid drug screening, and acute toxicity evaluation[43,44,93,109].

Prolonged culture periods also increase the cumulative effects of process deviation. As culture time increases, initial cellular state, matrix changes, nutrient exchange, and operational errors may continuously influence tissue development, leading to differences in size, cellular composition, and maturation across batches. Although some samples may form three-dimensional structures, they may lack intended-use-related function because of insufficient differentiation, central necrosis, or low proportions of target cells. Therefore, morphological culture success is not equivalent to obtaining an effective product for subsequent research or clinical evaluation[16,18,136,153].

Time constraints are particularly prominent in individualized applications of PDOs. Sample acquisition, model establishment, expansion, and drug testing must be completed within a limited therapeutic window. However, patient samples differ substantially in establishment success rate and growth speed, which may prevent test results from being available in time for clinical decision-making[6,11,103,121]. Therefore, long

production cycles not only increase material and labor costs, but also reduce the feasibility of organoids for rapid response and scalable application. The core tension is that shortening the culture period may compromise tissue maturity and functional stability, whereas extending culture further increases cost, variability, and risk of failure.

Overall, these engineering limitations reduce comparability across batches, platforms, and production scales, thereby weakening the reliability of functional and translational readouts[11,19].

## Clinical safety and immune issues

As organoids transition from experimental *in vitro* models to implantable therapeutic products for transplantation and tissue repair, the requirements for safety evaluation increase substantially, shifting from model fidelity and short-term cell behavior toward product-level sterility, genomic stability, tumorigenic risk, immune compatibility, and long-term *in vivo* function[11,17,20]. The major safety and immune-related issues associated with organoid translation are summarized in Figure 7.

### *Immune rejection*

Allogeneic organoids carry human leukocyte antigens and other cell-surface antigens that differ from those of the recipient, potentially triggering immune attack mediated by T cells, natural killer cells, and antibodies[147,158]. Compared with single-cell transplantation, organoids usually contain cells at multiple differentiation states, and their antigen expression and immunostimulatory capacity are not fully uniform. As a result, local survival and local rejection may coexist. In addition, hypoxia, necrosis, and cell damage during transplantation may release damage-associated molecules and further activate innate immune responses.

Autologous iPSC- or ASC-derived organoids can reduce donor-recipient antigen mismatch, but this does not mean complete immune tolerance. Reprogramming, long-term culture, and gene editing may alter antigen expression, and abnormally differentiated or immature cells may display immune phenotypes different from those of the original tissue[158-160]. Deuse *et al.* generated hypoimmunogenic PSCs by intervening in major histocompatibility complex-related pathways, demonstrating that genetic engineering can reduce allogeneic immune recognition. However, excessive reduction in immune visibility may also weaken host immune surveillance against abnormal cells[158]. Therefore, there is a potential trade-off between immune evasion and tumor surveillance, and short-term graft survival alone cannot serve as sufficient evidence of immune safety.

### *Potential tumorigenic risk*

The major risks of PSC-derived organoids arise from residual undifferentiated cells and abnormally differentiated cells. Even if undifferentiated cells account for only a small proportion, they may continue to proliferate in a suitable *in vivo* environment and form teratomas or other abnormal tissues[161,162]. This risk depends not only on pre-transplantation cell purity, but also on transplantation dose, cell aggregation state, transplantation site, and host immune status. Therefore, a single negative *in vitro* test cannot fully exclude long-term risk.

Long-term culture may also produce selective clonal expansion. The *in vitro* environment continuously selects cell subpopulations with stronger proliferative capacity and culture adaptability, and some of these subpopulations may carry chromosomal abnormalities, driver mutations, or epigenetic drift. Blokzijl *et al.* found that ASC-derived organoids can accumulate somatic mutations during long-term expansion, indicating that adult-derived systems should not be considered inherently safe[163]. For organoids derived

from cancer patients, the oncogenic mutations they carry determine that they are mainly suitable for modeling and drug screening, rather than direct transplantation[9]. Therefore, tumor risk assessment should simultaneously consider residual pluripotency, abnormal clonal expansion, genetic stability, and post-transplantation proliferative behavior, rather than relying only on karyotyping or a small number of tumor markers.

### *Difficulty in transplantation integration*

Functional recovery after organoid transplantation depends on whether organoids can establish stable vascular, neural, and structural connections with the host. Without timely vascularization, graft interiors are prone to hypoxia and necrosis. Even if transplanted tissue survives, if it cannot couple with host ducts, neural networks, or local mechanical environments, it may form isolated tissue without effective function[17,138].

Studies of cholangiocyte organoids by Sampaziotis *et al.* showed that human cholangiocyte organoids can engraft in injured bile ducts and participate in epithelial repair, indicating that for luminal epithelium with relatively clear structure, organoids can achieve functional integration at appropriate anatomical interfaces[66]. However, such results cannot be directly extrapolated to organs that depend heavily on fine vascular and neural connections, such as the brain, heart, or kidney. Brain organoid transplantation studies have shown that host vascular ingrowth can improve tissue survival and maturation, but may also alter the original developmental trajectory, causing the cellular composition and spatial architecture established during *in vitro* culture to be further remodeled *in vivo*[98,127].

The host microenvironment may also amplify internal heterogeneity within organoids. Different cell populations respond differently to hypoxia, inflammatory factors, mechanical stress, and local growth signals, which may lead to overexpansion of certain lineages, abnormal migration, or functional imbalance[16,20]. Therefore, *in vivo* integration is both a necessary condition for therapeutic efficacy and a new source of risk. Clinical evaluation should not only assess whether grafts survive, but also continuously monitor their spatial boundaries, cellular composition, functional output, and long-term effects on neighboring tissues.

Risk reduction therefore requires coordinated control before and after transplantation. Before transplantation, organoid products should be evaluated for cellular composition, residual undifferentiated cells, differentiation stability, genomic abnormalities, and tumorigenic potential. Strategies that promote prevascularization or enhance rapid vascular connection with the host may reduce early hypoxic injury, whereas appropriate selection of transplantation sites and anatomical interfaces may improve structural and functional coupling[20]. Immune compatibility should be considered together with long-term immune surveillance, because excessive immune suppression or immune-evasive engineering may improve graft survival while potentially reducing the clearance of abnormal cells[17,98,158]. After transplantation, longitudinal monitoring of graft size, cellular composition, spatial boundaries, functional output, and host-tissue responses is required to detect delayed overgrowth, ectopic differentiation, or integration imbalance[127,161-163].

Overall, organoid safety after transplantation is dynamic and cannot be determined by pre-transplant characterization alone. Long-term monitoring of immune responses, graft composition, proliferation, integration, and functional behavior is therefore required[11,17,147,158].

## STRATEGIES FOR RISK CONTROL

Risk control should move from isolated technical optimization toward integrated management of model construction, functional validation, manufacturing consistency, and long-term monitoring[11,19]. For transplantable organoids in particular, risk control should span the entire therapeutic process, including

pre-transplant product characterization, optimization of host-graft integration, and long-term post-transplant monitoring.

First, strategies addressing insufficient maturation and microenvironmental deficiency mainly focus on vascularized construction, multicellular integration, dynamic perfusion, *in vivo* maturation, and cross-tissue assembly. Takebe *et al.* incorporated endothelial and mesenchymal cells into liver bud systems, enabling vascular-like network formation and improved survival after transplantation[39]. Cakir *et al.* induced vascular-related programs in brain organoids and observed reduced hypoxia and cellular stress with improved neuronal function[23]. Mansour *et al.* further demonstrated that transplanted brain organoids receiving host vascular support exhibited increased neuronal complexity, axonal extension, and electrophysiological maturation, indicating that vascularization provides not only nutrient exchange but also developmental regulation[98]. More recently, microfluidic perfusion systems have shifted vascularization from static vessel formation toward dynamic transport regulation. Quintard *et al.* established a vascularized organoid-on-chip system integrating vascular organoids, islet spheroids, and mesenchymal components within perfusable networks, enabling material exchange between vascular and parenchymal compartments[164]. Such approaches suggest that vascular evaluation should extend beyond endothelial marker expression toward functional assessment of perfusion, barrier properties, and transport efficiency. However, engineered vascular systems still cannot fully reproduce immune, neural, and endocrine regulation, while *in vivo* maturation remains dependent on host environment and transplantation conditions. Therefore, vascular strategies should be evaluated according to whether they restore the transport and regulatory functions required for specific applications rather than simply vessel-like morphology[17,135,137,165].

For complex tissue interactions that cannot be achieved by single organoids, assembloids and gastruloids provide alternative construction strategies. These systems should be considered tools for reconstructing specific biological interfaces rather than simply increasing structural complexity. Multimodule models may introduce challenges including asynchronous maturation, unstable interfaces, and abnormal lineage interactions; therefore, their design should focus on the minimal combination of components required to reproduce the intended biological function[87,89,91,92,166].

Second, controlling model validity requires a layered evaluation framework based on intended application. Morphological similarity, marker expression, or isolated functional activity only demonstrate partial tissue characteristics and cannot directly prove accurate disease modeling or clinical prediction. Organoid validation should integrate molecular identity, spatial organization, and functional output, while confirming that these features are biologically connected within the target physiological or pathological context[3,11,14,19]. Different applications require different validation criteria. Developmental models should evaluate lineage progression and spatial patterning; disease models should demonstrate reproducible disease-associated phenotypes and responses to intervention; drug screening systems require stable dose-response relationships, assay windows, controls, and cross-batch reproducibility. For PDOs intended for clinical decision support, correlations between *in vitro* responses and patient outcomes require validation in independent cohorts. Studies by Vlachogiannis, Ooft, and Yao comparing gastrointestinal tumor organoid responses with clinical treatment outcomes provided preliminary evidence for predictive potential[13,103,121]. However, these relationships remain dependent on tumor type, drug class, sampling strategy, and evaluation criteria, and cannot be generalized without further validation. Therefore, clinical translation requires predefined performance indicators, failure boundaries, blinded testing, multicenter replication, and comparison with existing diagnostic approaches[6,11,153].

Third, reducing batch variation and improving scalability require transformation of organoid culture into an engineered manufacturing process with defined inputs, process parameters, and quality attributes. Conventional dependence on animal-derived matrices such as Matrigel introduces variability that limits

reproducibility and clinical-grade production. Gjorevski *et al.* developed synthetic hydrogels with controlled biochemical and mechanical properties, demonstrating that defined matrices can support intestinal organoid formation while reducing uncertainty associated with biological materials[24,167,168]. Control of initial aggregation and automated production can further improve consistency. Brandenberg *et al.* developed a high-throughput automated organoid culture platform based on stem-cell aggregation in microcavity arrays, whereas Czerniecki *et al.* established an automated high-throughput platform for kidney organoid differentiation and multidimensional phenotypic analysis. Automated liquid handling, high-content imaging, and standardized aggregate formation provide additional opportunities for scalable culture and quantitative phenotypic analysis[154,169].

Engineering approaches such as three-dimensional bioprinting further enable direct control of tissue architecture. Kolesky *et al.* generated multicellular printed tissues containing perfusable channels[170], Skylar-Scott *et al.* developed high-cell-density biofabrication strategies with embedded vascular channels[171], and Grigoryan *et al.* established multivascular networks within biocompatible hydrogels[172]. These studies demonstrate that predefined spatial organization can reduce dependence on uncontrolled self-organization. However, engineering control should not eliminate biological self-organization, which remains essential for physiological relevance. Quality control should therefore focus on variables affecting intended outputs, including starting cell state, aggregate size, matrix properties, oxygen transfer, and culture conditions, rather than morphology alone[11,19,22,128]. Different applications require different critical quality attributes: disease models and screening platforms should prioritize phenotype stability and reproducibility, whereas transplantable products require evaluation of composition, potency, residual undifferentiated cells, genetic stability, and sterility[153,156]. A practical translational quality-control framework should therefore link critical process parameters to application-specific critical quality attributes. Starting-cell composition and state, passage number, matrix composition, aggregate and organoid size, and culture conditions should be monitored as major input or process variables, whereas cellular composition, phenotype stability, functional potency, and genomic stability should be evaluated as product-related quality attributes. Batch-to-batch reproducibility can then be assessed by determining whether these predefined attributes remain within acceptable ranges across independent preparations. Importantly, the required attributes and acceptance criteria should be defined according to intended use rather than applied uniformly across all organoid systems[11,19,22,153,156,157].

Finally, clinical translation requires systematic evaluation of immune compatibility, genetic stability, and long-term safety. Patient-specific organoid approaches offer potential opportunities for regenerative medicine, although their clinical translation remains challenging due to difficulties in establishing, expanding, and scaling up clinical-grade organoid cultures[27]. Universal allogeneic strategies are being explored through immune engineering approaches, including hypoimmunogenic PSCs, human leukocyte antigen (HLA) modification, and immune-regulatory strategies[158-160]. However, reduced immunogenicity may also weaken immune surveillance against abnormal proliferation or malignant transformation. Therefore, immune-engineering strategies should be accompanied by stringent tumorigenicity assessment and long-term graft monitoring[157,158,161]. PSC-derived organoids require particular attention to residual undifferentiated cells, chromosomal abnormalities, oncogenic mutations, and genetic or epigenetic drift during long-term culture[161-163,173]. For complex systems such as assembloids and gastruloids, safety evaluation should also consider post-transplantation changes in cellular composition and interactions with host vascular, immune, and mechanical environments. The International Society for Stem Cell Research (ISSCR) guidelines emphasize that successful translation requires integrated consideration of cell source, manufacturing standards, potency and toxicity testing, ethical oversight, and long-term follow-up[157].

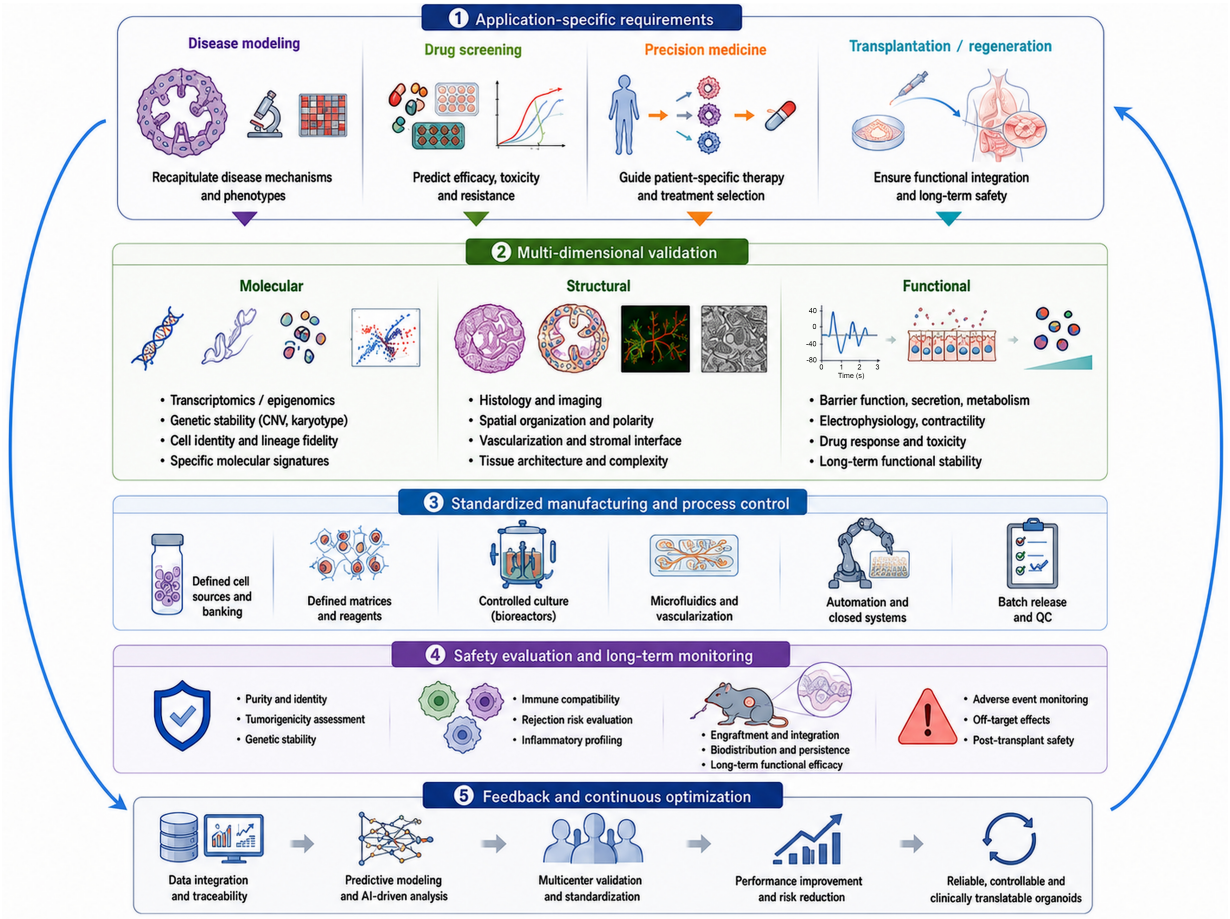


**Figure 8.** Framework for clinical translation and quality control of organoids. The figure integrates the major requirements for organoid translation, including model construction, intended-use-specific functional validation, engineering and manufacturing control, critical quality attributes, safety assessment, and long-term clinical monitoring. Translational readiness should therefore be evaluated according to the requirements of a defined application rather than by a universal standard applicable to all organoid systems. AI: Artificial intelligence; CNV: copy number variation; QC: quality control.

Overall, these strategies aim to make organoid performance measurable, reproducible, and traceable within clearly defined application contexts. An integrated framework linking model validation, engineering control, quality assurance, safety evaluation, and clinical translation is summarized in Figure 8.

## CONCLUSIONS AND PERSPECTIVES

Organoids provide human-relevant and experimentally tractable platforms that bridge conventional cell models, animal models, and clinical specimens, with applications spanning disease modeling, drug evaluation, personalized medicine, and regenerative therapy[1-4]. However, their translational value should not be judged by structural complexity or morphological similarity alone. Organoids selectively reproduce specific biological dimensions of native tissues, and the reliability of their outputs depends on developmental maturity, microenvironmental completeness, engineering reproducibility, and the clinical question being addressed[11,14,19,21,22].

Importantly, different organoid applications require different levels of validation and quality assurance. Disease models should reproducibly recapitulate disease-relevant phenotypes; drug-screening platforms require robust assay performance and validated relationships between *in vitro* responses and clinically relevant endpoints; PDOs intended for treatment support require prospective clinical validation; and implantable organoid products require substantially higher standards for manufacturing consistency, sterility, genomic stability, tumorigenic risk, immune compatibility, functional potency, and long-term host

integration[6,11,20,103,121,157]. Therefore, current organoid systems should generally be regarded as application-specific translational tools rather than uniformly clinically ready technologies.

Future progress should focus less on defining a universal threshold of organoid complexity or clinical readiness and more on establishing intended-use-specific validation frameworks that integrate molecular identity, structural organization, functional performance, critical quality attributes, evidence level, and safety requirements. The key question is therefore not whether organoids are clinically ready as a technology class, but whether a particular organoid system has been sufficiently validated for a clearly defined clinical purpose[27-29].

## DECLARATIONS

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### Authors' contributions

Data collection and manuscript writing: Xia J
Manuscript review: Sun D, Sun J

### Availability of data and materials

Not applicable.

### AI and AI-assisted tools statement

During the preparation of this manuscript, the AI tool ChatGPT (GPT-5.6, released 2026-07-09) was used solely for language editing. The tool did not influence the study design, data collection, analysis, interpretation, or the scientific content of the work. All authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

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### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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