REVIEW ARTICLE

The Impact of Organoid to Assembloid Technology in Biomedical Research

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Abstract

Organoids, three-dimensional, self-organized structures derived from pluripotent or adult stem cells, have revolutionized biomedical research by overcoming the inherent limitations of traditional 2D cultures and animal models. This review traces the evolution of organoid technology from initial studies to the contemporary "assembloid" phase, examining systemic inter-organ communication. The primary discovery of this review resides in the shift from descriptive modelling to a functional "Comparative Paradigm." A major perspective addressed is the transition from exclusively descriptive modelling to a functional 'Comparative Paradigm.' By merging developmental biology with bioengineering, a systematic framework is created to discover the most clinically relevant models, employing patient-derived 'avatars' to enhance personalized medicine and and high-throughput drug discovery. Ultimately, this review provides a systematic framework for identifying the most clinically applicable models by integrating developmental biology and bioengineering. The lack of vascularization, embryonic immaturity, and batch-to-batch repeatability issues remain major technical obstacles despite their potential. Finally, we explore potential future approaches in bioengineering, including the incorporation of 3D bioprinting, AI-driven imaging, and microfluidics (organ-on-a-chip). Organoid technology is a key component of next-generation medicine because it bridges the gap between "bench and bedside," providing previously unattainable insights into human biology and illness.

KEYWORDS: organoids, stem cells, disease modeling, bioengineering, personalized medicine, assembloids, regenerative medicine; organ-on-a-chip, translational manufacturing

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Introduction

The field of biomedical research is experiencing a major transformation, fueled by the rise of organoid technology. The term "organoid" has held various meanings throughout the history of biomedicine, many of which have since become obsolete. Today, an organoid is defined as a three-dimensional (3D) assembly containing multiple cell types arranged with realistic histology, at least at the microscale. (1) These structures can be produced from human or animal cells, using differentiated cells, pluripotent stem cells (PSCs), or tissue-specific adult stem cells (ASCs).(2,3)

The main driver for this technological advancement is the ongoing limitation of conventional research models. For many years, two-dimensional monolayer cultures were the prevailing model; however, they fail to replicate the complex cell-cell interactions and metabolic gradients

essential for organ function.(4) Likewise, although animal models have played a critical role in research, they are limited by inherent differences in gene expression across species. Organoid research initially sought to minimize or replace animal experimentation by eliminating the uncertainty of translating findings from animals to humans. (1,5) They offer a high-fidelity "human-first" platform that allows complex biological questions to be asked within a simplified system, isolated from the confounding variables of the body as a whole. These “mini organs”, now widely used in cancer research, genetic diseases, infectious diseases, and regenerative medicine, create new possibilities for drug discovery, large-scale drug screening, and precision medicine.(4,6)

Nonetheless, as the discipline advances, notable impediments have arisen that obstruct complete clinical translation. Presently, numerous models encounter the "oxygen diffusion limit," resulting in necrotic cores within bigger cultures (7), and frequently persist in a fetal or embryonic stage of maturation (8). Moreover, the absence of integrated immune systems and microbiome interactions, along with significant batch-to-batch variability, continues to pose a substantial challenge.(9,10) Addressing these constraints necessitates a transition to advanced bioengineering, wherein organ-on-a-chip integration, 3D bioprinting, and AI-enhanced live imaging are transforming organoids into dynamic, predictive systems.(11–15)

In contrast to prior reviews that mostly enumerate organoid kinds in isolation, this writing presents a "Translational Readiness" framework. We transcend the "single organ" paradigm to integrate the developing "assembloid" era, offering a strategic comparison matrix that assesses these models not merely as biological oddities, but as functional diagnostic "avatars”.(16) This review connects developmental biology and bioengineering, providing a cohesive framework for the progression from laboratory prototypes to advanced clinical applications. This review's literature was identified by a multi-database search encompassing PubMed, Web of Science, and Google Scholar, concentrating on peer-reviewed studies published from 2015 to 2026 to ensure a thorough and impartial synthesis. Terms such as 'organoid technology,' 'assembloids,' 'translational readiness,' and 'precision medicine' were employed. The selection criteria emphasized high-impact clinical trials, pivotal developmental biology research, and recent bioengineering innovations that offer validated frameworks for clinical translation, thus ensuring the incorporation of both fundamental principles and the latest technological advancements.

Brief Historical Background

The lineage of organoid technology is rooted in a century-long fascination with cellular self-organization, beginning with H.V. Wilson's 1907 revelation that dissociated sponge cells have the inherent knowledge necessary to autonomously reaggregate into functional organisms.(17) This seminal finding established the notion of differential adhesion, which subsequently influenced the development of mid-20th-century spheroids. Although these fundamental cell aggregates offered a first insight into 3D biology, they were constrained by an absence of self-renewal and restricted structural complexity. A definitive paradigm shift occurred in 2006 with the discovery of induced PSCs (iPSCs).(18,19) Researchers bypassed the ethical and potency constraints of embryonic and adult stem cells by utilizing a unique mix of transcription factors: octamer-binding transcription factor 4 (Oct4), SRY-box-containing gene 2 (Sox2), Krüppel-like factor 4 (Klf4), and cellular myelocytomatosis (c-Myc). This advancement enabled the development of "patient-in-a-dish" models that maintain a donor's exact genetic makeup. Thus, the capacity to examine disease presentation within three-dimensional tissue structures has formed the contemporary basis for precision medicine.(20)

The "stem cell revolution" was further accelerated by the discovery of crucial biochemical and physical signals necessary to guide pluripotency. A significant advancement was the advent of Matrigel, a basement membrane matrix that offered more than merely a structural support. Unlike previous 2D models, Matrigel provides essential physical cues due to its intricate mix of laminin, collagen IV, and entactin, which activate integrin-mediated "outside-in" signaling required for cell polarization.(21) This biophysical support, along with "niche" signaling elements like as Wnt, R-spondin, Noggin, and epidermal growth factor (EGF), facilitated the preservation of stemness and guided differentiation by emulating the *in vivo* environment. R-spondin 1 functions as a vital enabling factor by acting as a leucine-rich repeat-containing G-protein coupled receptor 5 (+) (Lgr5+) agonist, inhibiting the degradation of Wnt receptors to maintain the stem cell reservoir necessary for ongoing self-renewal.(22)

Despite these breakthroughs, cerebral organoids remained largely in a stochastic era.(21,23) Researchers relied on the intrinsic genetic mechanisms of stem cells to facilitate morphogenesis with limited external regulation, resulting in significant batch-to-batch variability and erratic anatomical configurations, exemplified by the arbitrary

emergence of neuronal rosettes in brain models.(24) The development of animal-derived matrices like as Matrigel marked another significant milestone; yet, it did not resolve the issues of matrix heterogeneity and insufficient mechanical tunability. Matrigel's relatively constant stiffness (Young's modulus ~450 Pa) cannot emulate the diverse elasticities of various human tissues, such as the markedly softer milieu of the brain or the more rigid structure of the liver.(24,25)

The field is now experiencing a major transition from stochastic to deterministic. Through the use of synthetic scaffolds, 3D bioprinting, and microfluidic "organ-on-a-chip" systems, researchers can precisely regulate the organoid's shape, size, and nutrient supply.(25) This transition from "nature-led" to "engineer-supported" growth is essential to ensure the resulting tissue is functional, uniform, and physiologically accurate, and for scaling organoids for high-throughput drug screening and clinical application.(26) In this approach, researchers provide a physical and biochemical "blueprint" that guides stem cells into specific shapes and functional arrangements. These scaffolds replicate the natural extracellular matrix (ECM) by supplying the structural support and mechanical cues necessary for cell maturation. In contrast to early techniques that depended on animal-derived matrices such as Matrigel, engineered scaffolds are typically fabricated from synthetic or highly purified natural materials, enabling precise control over stiffness, porosity, and chemical composition.(24,27)

Crucially, the matrix provides a continuous flow of information interpreted by cells through mechanotransduction. While Matrigel maintains a constant stiffness (~0.5 kPa), engineered hydrogels allow researchers to tailor the Young's Modulus to specific lineages: "soft" matrices (~0.1–1 kPa) r facilitate neurogenesis, whereas "stiff" matrices (~25–40 kPa) promote osteogenic differentiation.(28) Unlike elastic synthetic gels, human tissues distribute force gradually over time. Scaffolds with rapid stress relaxation facilitate cellular mechanical remodeling of their surroundings, hence driving essential symmetry-breaking processes like crypt sprouting in intestinal models.(24)

Ultimately, this evolution signifies a pivotal transition from passive observation of nature's inherent organization to intentional engineering of it. The integration of genetic reprogramming with precision bioengineering, together with real-time artificial intelligent (AI)-guided monitoring, has advanced the area from experimental curiosities to standardized, replicable platforms.(29) Nonetheless, a "physiological ceiling" persists. The principal unresolved challenges comprise the oxygen diffusion limit, requiring vascularization for tissues larger than 400 μm, and the continuation of fetal-stage growth. The forthcoming major progress in clinical readiness involves the shift from isolated silos to integrated, systemic assembloids that encompass immunological and microbial axes. Table 1 summarize evolution of organoid technology throughout the era.

Structural and Biological Components of Organoids

Organoid technology signifies a transition from inflexible, two-dimensional cell culture to a more organic biological design.(30) By integrating stem cell biology with biomaterials technology, researchers have shifted from imposing rigid cellular configurations to creating a supportive microenvironment, or "soil," that allows cells to adhere to their inherent blueprints.(31) In this intricate process of self-organization, chosen stem cells

Table 1. The evolutionary roadmap of organoid technology.

Year	Milestone	Impact & Significance	Limitations
1907	Sponge Reaggregation (17)	Proved Differential Adhesion; established that cells contain intrinsic self-organization data.	Lack of mammalian complexity; restricted to simple organisms.
2006	iPSC Discovery (Yamanaka Factors) (18)	Paradigm Shift: Created ethically clear, patient-specific "starter cells" for any tissue type.	Epigenetic Memory: Variability in differentiation potential across donor lines.
2009	Intestinal "Mini-Guts" (21,22)	Defined the Biochemical Niche (Wnt, R-spondin); proved ASCs can form self-renewing epithelium.	Stochastic Growth: Unpredictable morphology; reliance on animal-derived Matrigel.
2013	Cerebral Organoids (23)	Demonstrated complex brain regionalization (cortical polarity) from PSCs.	Oxygen Diffusion Limit: Lack of vascularization led to necrotic cores in larger tissues.
2016	Designer Matrices (24)	Identified Physical Cues (stiffness/stress relaxation) as drivers of symmetry breaking.	Complexity of synthetic chemistry limits high-throughput accessibility.
2020+	The "Assembloid" & OOC Era (25)	Shifted focus to Systemic Connectivity (e.g., gut-brain axis) and fluidic perfusion.	Scalability Gap: High technical barrier; lack of immune/microbiome integration.
Future	AI-Guided Bioprinting (29)	Transition to Deterministic Engineering; real-time quality control and "Digital Twins."	Data privacy hurdles and high computational costs for real-time analytics.

interact and reorganize into complex three-dimensional structures, directed by both autonomous mechanisms and meticulously timed biochemical signals.(32) This approach harmonizes the cell's intrinsic motivation with externally guided differentiation, enabling the cultivation of small models that reflect the spatial intricacies of human organs while elucidating the present limitations of the replicative capabilities *in vitro.*

Cell Sources: iPSCs *vs.* ASCs

The selection of cell source is a fundamental factor shaping an organoid's identity, complexity, and intended use. iPSCs and tissue-derived adult stem cells represent the two principal starting materials, each reflecting distinct biological assumptions and experimental trade-offs.(33) iPSCs exhibit remarkable developmental plasticity, maintaining the ability to differentiate into nearly any somatic cell lineage. This property allows the generation of organoids that mimic early organ development and complex interactions among multiple cell lineages, making iPSC-derived organoids especially valuable for investigating human development, congenital disorders, and genetically driven diseases.(34) Moreover, patient-specific iPSCs provide a powerful framework for personalized disease modeling by retaining each individual's genetic background.(35)

However, pluripotency also brings significant challenges. iPSC-derived organoids often exhibit considerable heterogeneity, extended differentiation periods, and inconsistent lineage specification. Factors such as residual epigenetic memory, variations in reprogramming efficiency, and line-to-line variability can significantly affect reproducibility. In addition, many iPSC-derived organoids remain developmentally immature, more closely resembling fetal than adult tissues, which restricts their applicability for modeling age-related diseases and mature organ functions.(36,37)

By contrast, ASCs obtained directly from tissues are lineage-restricted and intrinsically programmed to sustain and regenerate particular organs. Organoids generated intestinal, liver, or airway ASCs typically show faster organization, stable tissue structures, and more mature functional characteristics.(38,39) Because of these characteristics, organoids derived from ASCs are very useful for studying tissue regeneration, epithelial homeostasis, and disorders arising from adult tissue dysfunction.

Nonetheless, ASC-based systems are constrained by their limited ability to differentiate. They generally lack mesenchymal, vascular, neural, or immune components, which reduces overall tissue complexity.(40,41) In addition, obtaining high-quality primary tissue can be challenging, and prolonged culture may promote the selective growth of certain subpopulations.(37,42) So, choosing between iPSCs and ASCs reflects a strategic trade-off between developmental versatility and physiological relevance, rather than a simple ranking of model quality.(33,43)

iPSCs are the optimal selection for simulating congenital diseases, neurodevelopment, and multi-lineage complexity (*e.g.*, integration of nerves or blood arteries) due to their ability to serve as a "blank slate" that replicates embryonic development over an extended period.(44) In contrast, ASCs should be chosen when the objective is to investigate adult tissue homeostasis, infectious diseases, or expedited drug screening, as they are inherently designed to develop mature, functioning epithelia within 1–2 weeks.(45) A study on infant heart defects would require patient-specific iPSCs to accurately reflect early morphogenesis, while a clinician evaluating a patient's response to a specific chemotherapy would employ ASC-derived "tumoroids" due to their immediate physiological relevance and expedited results.(46,47)

The Microenvironment: ECM and Synthetic Hydrogels

Beyond the choice of cell source, the surrounding microenvironment plays a crucial role in supporting organoid formation and maturation. *In vivo*, stem cells reside in specialized niches where biochemical cues, mechanical forces, and spatial organization collectively govern cell fate decisions. Organoid cultures seek to recapitulate key aspects of this niche, with the ECM serving as a central regulatory component rather than a passive scaffold.(30,48)

Natural ECM-based matrices, particularly Matrigel, have been central to the advancement of organoid technology by providing a complex blend of proteins and growth factors that support spontaneous self-organization.(36,49) However, dependence on these natural matrices introduces substantial experimental variability. Batch-to-batch differences in composition and stiffness can alter differentiation outcomes, while their tumor-derived nature poses barriers for clinical translation.(50,51) To overcome these limitations, synthetic hydrogels and bioengineered scaffolds have been developed as alternatives that emphasize tunability and reproducibility.(12) By independently controlling parameters such as stiffness and degradability, researchers can systematically examine how physical cues shape organoid behavior.(6,52)

A clear illustration of this precision is the use of polyethylene glycol (PEG) hydrogels for cultivating intestinal organoids. In contrast to conventional self-

assembly, which frequently produces irregular shapes, bioengineered PEG scaffolds can be "programmed" to degrade at a rate that precisely matches organoid growth. This provides the consistent mechanical tension necessary to encourage the formation of functional crypt and villus structures, ensuring the high-level reproducibility required for large-scale drug testing.(27)

Likewise, 3D silk scaffolds have helped overcome major challenges in cerebral organoid research. Specifically, they reduce the formation of necrotic cores, where central cells die from limited nutrient and oxygen supply, by offering a porous, sponge-like structure that enhances diffusion. These scaffolds also served as mechanical templates, guiding iPSCs to distribute evenly and develop a more organized neuroepithelium than is typically achieved through spontaneous assembly alone.(53)

The key outcome of scaffold-assisted growth is the transformation of organoids from biological curiosities into reliable medical tools. When combined with microfluidic systems, these scaffolds allow researchers to recreate dynamic physiological conditions, such as blood flow or the mechanical motions of breathing lungs and beating hearts. This bioengineering strategy closes the gap between simple cell clusters and functional human organ models suitable for surgical planning, disease modeling, and personalized drug screening.(48,54) Selection of scaffold determines both physiological significance and experimental consistency. Matrigel is the benchmark due to its "instructive" environment rich in laminin and collagen; nonetheless, its tumor-derived origin leads to batch variability and biochemical complexity that may obscure distinct mechanical signals.

Alternatively, defined ECM (recombinant proteins) and synthetic hydrogels (*e.g.*, PEG) provide a reductionist methodology with enhanced tunability. PEG scaffolds can be engineered to degrade at a rate that aligns with organoid expansion, delivering the exact mechanical stress necessary for the formation of functioning crypt and villus structures. Hybrid gels address this disparity by functionalizing synthetic frameworks with bioactive motifs (*e.g.*, RGD peptides), merging structural consistency with critical biological signals. Ultimately, decellularized ECM (dECM) offers the most genuine tissue-specific biochemical profile, while it frequently exhibits deficiencies in structural homogeneity and scalability compared to synthetic alternatives.

Self-Organization *vs.* Directed Differentiation: Mimicking Developmental Logic

Organoids has a unique characteristic which is its reliance on self-organization, wherein cells autonomously generate organized tissues through localized interactions and feedback mechanisms.(32,55) This simulates embryonic development, wherein morphogen gradients and mechanical forces facilitate pattern creation.(56,57) Nonetheless, self-organization frequently does not yield distinct tissue identities or reliable regional patterning.(31)

The majority of methods utilize directed differentiation by the temporal administration of growth factors (*e.g.*, wingless-related integration site (WNT), bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and Notch) to mimic *in vivo* biochemical gradients and direct lineage specification.(58,59) The primary problem is in adjusting these interventions; excessive external control may inhibit physiologically significant emergent behaviors, whereas inadequate guidance heightens experimental variability. (60,61) Ultimately, organoids are hybrid systems in which developmental potential is elucidated by context-dependent guidance.(62) Figure 1 maps the critical choices and inherent compromises that define the creation of physiologically relevant human tissue models. The landscape illustrates how selections in cellular source (iPSCs *vs.* ASCs), scaffold composition (Natural ECM (Matrigel) *vs.* synthetic hydrogels), and morphogenetic strategy (stochastic self-organization *vs.* directed/bioprinted differentiation) directly impact the final organoid's reproducibility, maturity, and complexity. This scaffolded environment, utilizing either natural Matrigel or synthetic hydrogels, delivers crucial biochemical and physical signals to facilitate the evolution of basic cell clusters into functioning, self-organized organ models.(32)

Types of Organoids Developed to Date

By leveraging the self-organizing capacity of stem cells, organoid technology creates 3D models that recapitulate human physiology and disease mechanisms.(34,63) Recent progress in modeling neurological, gastrointestinal, and cardiovascular conditions has been further enhanced by integrating bioengineering tools, such as organ-on-chip systems and complex "assembloids".(64,65)

Gastrointestinal & Hepatic Organoids: The Gold Standard

At the moment, the most advanced versions for clinical use are intestinal organoids. As a "gold standard" benchmark, organoid responses in the Forskolin-Induced Swelling (FIS) assay directly predict a patient's clinical improvement on CFTR modulators.(66) As a result, the model is elevated

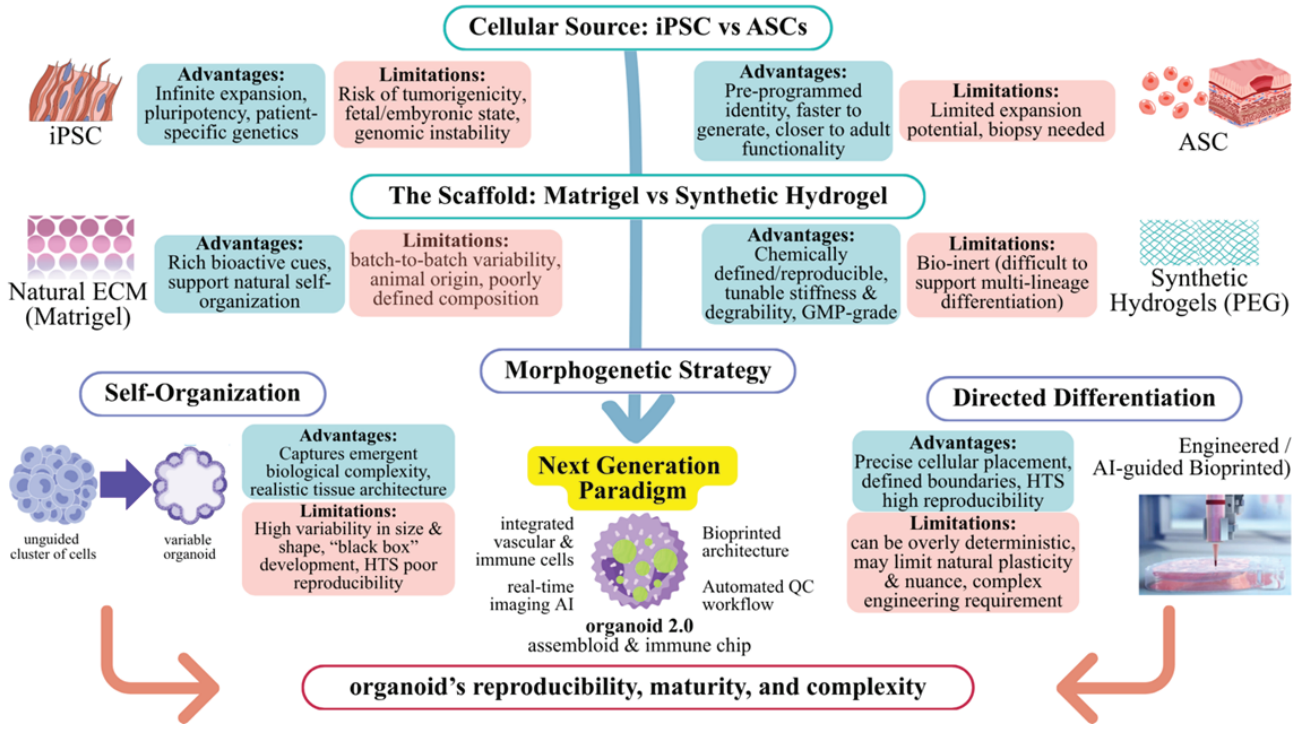

Figure 1. The organoid engineering landscape: navigating foundational trade-offs for the next generation. This figure shows the key organoid model clinical translatability parameters. Researchers can switch from stochastic self-assembly to deterministic bioprinted designs by choosing cellular sources and adjustable synthetic scaffolds. The "Next Generation Paradigm" connects laboratory prototypes to clinical "avatars" using real-time AI and integrated vascular/immune components.

from fundamental research to a clinical decision-making tool, particularly for individuals who are excluded from trials due to unusual mutations.

The problem of hepatocyte expansion in the hepatic field was resolved in 2025 by Keio University. Researchers were able to obtain a million-fold multiplication of human liver organoids that sustain near-human levels of albumin and metabolic function for six months by using a hormone cocktail and Oncostatin M.(67–69) The "maturation bottleneck" that previously restricted liver organoids to fetal-like states is resolved by this.

Neurological Organoids

While intestinal models focus on metabolic function, neurological and pulmonary models prioritize structural complexity. Brain organoids derived from human pluripotent stem cells have become powerful models for studying human brain including zika virus (ZIKV). (56,70–72) Compared to traditional 2D cultures, brain organoids offer a more physiologically relevant platform to study virus-specific cellular responses and to test antiviral strategies, including RNA interference enhancers that can prevent ZIKV-induced microcephalic phenotypes.(73) Their lack of adult-level maturity, however, continues to be their fundamental drawback. This has been addressed by the "Assembloid" Era (especially the Paşca Lab), which has created "mini-systems", like the human ascending sensory pathway, by combining region-specific organoids. These are now employed to investigate how signals move from the periphery to the brain, a task not achievable in conventional monoculture models, and have been verified using synchronized electrical activity.(74)

Cardiovascular & Pulmonary Organoids

Organoid and organ-on-chip technologies have enabled the development of physiologically relevant models for organoids and "lung-on-a-chip" systems which replicate the alveolar–capillary barrier, they are particularly well suited for researching infectious diseases such as SARS-CoV-2. (11) These chips precisely replicate COVID-19-associated "endothelialitis" and hypercoagulopathy, which 2D methods are unable to capture, according to benchmarking studies. (75) The discovery that SARS-CoV-2 inhibits host lipid metabolism pathways is a significant recent discovery. This host response was confirmed in lung organoids and offers a novel target for metabolic-based antivirals.(56)

Renal and Pancreatic Models

In contrast to the systems above, renal and pancreatic organoids remain primarily in the basic research stage and become the maturation bottleneck in organoid technology. While kidney organoids successfully express injury markers like kidney injury molecule (KIM)-1 in response to drugs like cisplatin (64,76), they still lack the complete vascularization and flow required to model chronic filtration diseases. Similarly, pancreatic organoids can produce insulin-secreting β-cells, but their use in type 1 diabetes remains primarily in basic research as scientists work to integrate the autologous immune compartment to model autoimmune destruction.(69,77)

Table 2 systematically compares organoid cell sources, key cell types, gold-standard assays, primary limitations, optimal use cases, and clinical readiness levels, illustrating the progression from basic cell sources to validated assays and their present clinical applicability.

The "Assembloid" Era

The rise of assembloids, which integrate multiple organoids to stimulate complex tissue interactions, has significantly advanced the study of neural circuit connectivity. In contrast to conventional co-culture techniques, which often require the combination of several cell types within a singular, frequently chaotic environment, assembloids are characterized by an intentional combination of pre-patterned, distinct three-dimensional units. Co-cultures provide a "soup" of cellular diversity, whereas assembloids function as a "circuit" of distinct modules. By fusing region-specific brain organoids, such as cortical and striatal organoids, assembloids recreate inter-regional projections critical for brain function, enabling the formation of synaptic connections and electrophysiological maturation of neurons, as demonstrated in cortico-striatal assembloids where medium spiny neurons exhibit calcium activity following cortical stimulation.(78) More than just reflecting a “mini organ”, we can think of assembloids as a “mini system”. Figure 2 highlights this hierarchy, where the spheroids represent the most basic structures composed of homogeneous cell populations; organoids replicate a singular organ or specific regional cytoarchitecture; and assembloids connect these elements into a higher-order system, creating functional interfaces that are mostly unachievable in dissociated or two-dimensional co-culture systems.

Beyond brain-brain connections, assembloids integrating cerebral organoids with motor neuron spheroids mimic brain-spinal cord circuitry, allowing monitoring of neural signal transmission and providing platforms to study neurochemical modulation of motor pathways.(79) These advances underscore the potential of assembloids to model hierarchical and modular neural networks with enhanced input-output functionality, supporting investigations into development, disease mechanisms, and therapeutic screening by recapitulating complex circuit connectivity *in vitro*. The maturation of organ-specific organoid models has extended their use beyond developmental biology, allowing their application across diverse area of biomedical research. These advances have established organoids as functional platforms for disease modeling, drug discovery, and emerging clinical applications.(80)

Table 2. Evaluation of organoid models: Source, validation, and clinical maturity.

Organ System	Cell Source	Unique Advantage (*vs.* 2D/Animal)	Gold-Standard Assay / Benchmark	Key Milestone (2024–2025)	Clinical Readiness (TRL*)
Gastrointestinal	ASC / iPSC	Captures patient-specific 3D crypt architecture	FIS Assay: r > 0.9 *vs.* patient drug response (74)	"Clinical Avatar" status for rare CF mutations	9 (Standard of Care)
Hepatic	iPSC / Primary	Recreates metabolic flow and matrix stiffening	Albumin/Cyp3A4: Maintenance of adult-like metabolism	Million-fold expansion via Oncostatin M (77)	5 (Pre-clinical)
Neurological	iPSC / hESC	Models human-specific neural progenitor tropism	MEA/Patch-clamp: Synchronized circuit firing	Multi-tissue assembloids for pain pathways (75)	4 (Translational)
Pulmonary	iPSC / Primary	Recreates alveolar-capillary barrier under stretch	Viral Entry: Recapitulates COVID-19 endothelialitis (81)	Discovery of viral lipid metabolism suppression (80)	4 (Pre-clinical)
Renal	iPSC	Recapitulates nephron-like segmented tubules	KIM-1/NGAL: Upregulation in response to toxins	Scaling of high-throughput nephrotoxicity screens	3 (Basic Research)
Pancreatic	ASC / iPSC	Enables study of human-specific insulin kinetics	GSIS: Glucose-stimulated insulin secretion	Integration of autologous immune cells for T1D (86)	3 (Basic Research)

*TRL: Technology Readiness Level Scale; 1–3: Basic research; 4–6: Pre-clinical/translational; 7–9: Clinical application/standard of care. (TRL levels adapted from the NIH/FDA Technology Readiness Level framework for regenerative medicine.)

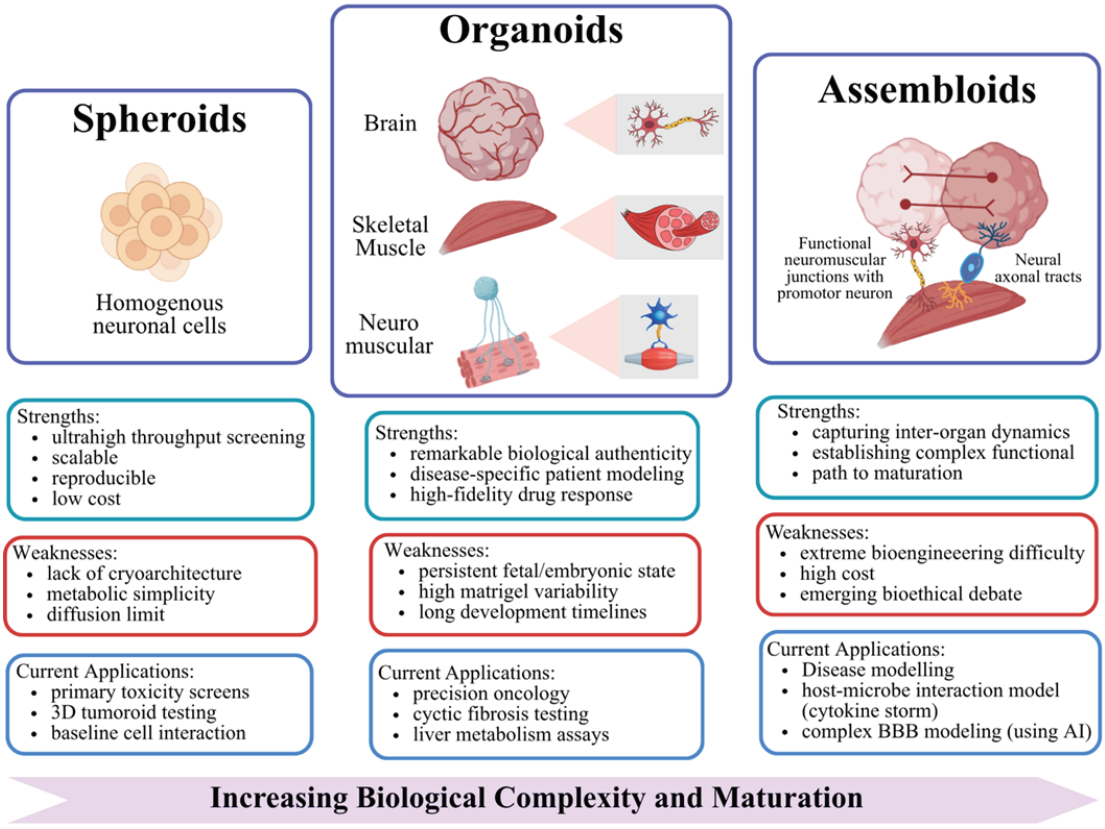

Figure 2. Hierarchy of 3D modeling systems in biomedical research. A comparison of spheroids, organoids, and assembloids by structure and clinical use. The continuum shows the trade-offs between repeatability (left) and physiological integrity (right) as biological complexity increases. Key applications for each model include primary toxicity tests, host-microbe interaction modeling, and systemic inter-organ research.

Applications of Organoids in Toxicity Testing: Current Status and Level of Validation

Organoid and assembloid technologies provide high-fidelity *in vitro* models that surpass traditional 2D cultures and animal models in mimicking human physiology.(33) By retaining patient-specific genetic profiles, these platforms have transformed drug discovery and personalized medicine, enabling accurate high-throughput screening for efficacy and toxicity.(81) This transformative approach is particularly vital for refining clinical decision-making in oncology and rare genetic diseases.(37,82)

Disease Modeling: Patient-specific Organoids as "Avatars" for Rare Genetic Diseases

Patient-specific organoids, derived from iPSCs, provide a controlled 3D microenvironment to study these disorders, allowing for genotype-phenotype correlation, interrogation of pathogenic pathways, and evaluation of candidate drugs, which is often challenging with scarce patient samples or less accurate animal models.(83) However, the clinical maturity of these systems exhibits considerable variation within fields. The most substantial clinical validation now exists in oncology and cystic fibrosis, where patient-derived organoids (PDOs) serve as functional "avatars" to forecast specific medication reactions and inform tailored treatment strategies. In many instances, PDOs have transcended laboratory settings, exhibiting a strong association between *in vitro* drug sensitivity and *in vivo* therapeutic results. Conversely, applications in neurology and cardiology predominantly reside in the experimental proof-of-concept phase. Patient-specific organoids produced from iPSCs offer a regulated 3D microenvironment for investigating genotype-phenotype correlations and pathogenic pathways in rare genetic diseases and complex multi-organ disorders. Although models like neuromuscular organoids for amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), or cardiac organoids for Duchenne muscular dystrophy (DMD) have effectively replicated disease phenotypes and identified potential therapeutic targets *in*

vitro, they have not been extensively utilized as primary instruments for the real-time clinical decision-making.(84)

The combination of organoids with organ-on-chip systems and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene editing signifies the forthcoming advancement in closing this divide. The utilization of DMD patient-derived cardiac organoids to evaluate personalized antisense oligonucleotides has revealed the possibility of phenotypic reversal, underscoring a scalable approach to individualized therapy.(85) Nonetheless, until extensive prospective clinical trials validate that organoid-guided interventions reliably enhance patient survival or quality of life relative to standard care, these applications are classified as high-potential translational research rather than recognized clinical practice.(86)

Drug Discovery and Toxicology: High-throughput Screening (HTS) and Reducing Late-stage Clinical Trial Failures

Organoids improving the prediction of efficacy and toxicity and helping to reduce late-stage clinical trial failures. The pharmaceutical industry has long struggled with high development costs and low success rates, as many compounds fail in clinical trials because of unpredicted toxicity or inadequate efficacy. Organoids help bridge gap by offering models that more closely reflect human physiology than conventional 2D cell cultures or animal models, which often poorly predict human responses.(34,87) However, the extensive implementation of organoids in HTS depends on stringent standardization and quality control (QC) to address intrinsic biological variability.

Organoid-specific response metrics, particularly in kidney and neural organoids, have been established by recent developments in lab-automation-driven HTS techniques to guarantee reproducibility across huge batches. Strict QC requirements, such as consistent organoid size distribution, cellular composition ratios, and metabolic activity thresholds prior to drug exposure, are necessary to achieve "assay readiness" to support large-scale phenotypic screening, quantifying structural or functional changes and distinguishing efficacy from adverse effects in complex disease models.(88) This includes applications in conditions such as acute kidney injury and Huntington's disease, where organoids enable detailed assessment of drug responses at the level of individual organoid.(76,89)

Furthermore, multi-organoid "body-on-a-chip" platforms that integrate liver, heart, lung, brain, and other tissues, can uncover both organ-specific and systemic toxicities. Bioprinting and microfluidic assembly was applied to lessen the "batch-to-batch" fluctuation that has historically hampered organoid research in order to preserve the dependability of these preclinical experiments. Lab-automation-driven HTS protocols, with organoid-specific response metrics, further enhance scalability and analytical accuracy, supporting biomarker discovery and more reliable prioritization of candidate therapies.(90,91) The 2026 Food and Drug Administration (FDA) draft guidance on New Approach Methodologies (NAMs) and the 2025 ISO/AWI 25430-2 standards, which require strict quality control, such as size uniformity, cellular composition ratios, and automated AI-driven morphological validation, as prerequisites for high-throughput clinical translation, have further cleared the way for regulatory acceptance.(92)

Applications in Precision Medicine (Preclinical *vs.* Clinical Implementation)

Regenerative Approaches for Precision Medicine

Patient-derived organoids (PDOs) retain the unique genetic makeup and pathological characteristics of a patient's organ, including tumor.(35) These 3D *in vitro* models provide a strong foundation for functional precision medicine, enabling direct evaluation of diverse therapies, including chemotherapeutic drugs, on a patient's own cancer cell. This approach aims to move beyond genomic-centric precision medicine by providing a functional assessment of drug response, which is crucial for tailoring treatment strategies for individual cancer patients and predicting the efficacy of therapies that may lack established genetic biomarkers.(82) A key application of PDOs lies in predicting chemotherapy sensitivity and guiding treatment decisions in oncology. Research has shown a close alignment between drug responses measured in PDOs and the real-world clinical outcomes of patients receiving the same chemotherapy treatments. For example, research in colorectal cancer has shown that PDO-based drug tests can predict patient response with high sensitivity and specificity, outperforming empirically guided treatment selection. This capability allows oncologists to select effective treatments and avoid those unlikely to work, thereby minimizing unnecessary exposure to toxic side effects and reducing treatment costs.(93,94)

The application of PDOs in personalized medicine provides major advantages over conventional preclinical models. They accurately preserve the architectural features, genetic profiles, and drug sensitivities of the original tumors,

making them powerful tools for drug screening and the discovery of new therapeutic targets. Additionally, PDOs enable direct comparison of treatment effects by culturing both tumor and healthy organoids from the same patient, allowing for the selection of therapies that selectively target cancer cells while minimizing harm to normal tissues. This advancement is crucial for optimizing therapeutic strategies and advancing functional precision oncology.(35)

Despite of PDO's potentials, numerous significant bottlenecks prevent their widespread use in clinical decision-making. Logistically, tissue collection, expansion, and high-throughput screening take 4–8 weeks, which often surpasses the urgent clinical window needed to treat advanced or aggressive cancers.(95) Furthermore, biopsy quality and cellularity are crucial to these models' success. Small or necrotic samples sometimes lack biomass for robust testing, resulting in a "take rate" or success rate that varies by cancer type (commonly 60%–90%). Due to this diversity, the method may only be useful to a subgroup of patients with more culturable malignancies.(96)

Most common PDO platforms struggle with structural and clonal representation. Most models have epithelial cells instead of the complex stromal, vascular, and immunological components of the tumor microenvironment. This restriction greatly restricts PDOs' potential to predict immunotherapy or tumor stroma-targeted medication responses.(97) In addition, *in vitro* expansion may cause clonal evolution, where culture circumstances favor certain cellular clones. This can cause a model to misrepresent the patient's tumor's heterogenic landscape, skewing drug sensitivity assay results.(98)

Regenerative Medicine: Potential for Organoid-based Transplantation and Tissue Repair

Organoid technology offers substantial promise for regenerative medicine by providing potential alternatives to conventional cell replacement therapies and organ transplantation.(38,99) As self-organizing three-dimensional cultures that replicate key aspects of native organ structure and function, organoids can be generated from patient-specific cells, thereby lowering the risk of immune rejection associated with allogeneic transplants.(65,100) This capability positions them as valuable tools for generating functional tissues to treat degenerative diseases and organ failure, directly addressing the critical shortage of donor organs.(101,102)

Preclinical success and engineering innovations numerous studies have highlighted the potential of organoids in tissue repair and transplantation in animal models. Numerous studies have highlighted the potential of organoids in tissue repair and transplantation in animal models. For example, kidney organoids derived from patient-specific pluripotent stem cells are being investigated as alternatives to kidney transplantation.(37) Similarly, intestinal organoids transplanted into mice have shown the ability to regenerate colonic mucosa injuries and repair the colon after damage.(56) Liver organoids have also proven effective, with orthotopic transplantation of human fetal hepatocyte organoids exhibiting high engraftment efficiency in acute liver failure mouse models. In addition, 3D bioprinting is being explored as a means to generate more complex tissue structure and transplantable mini-organs from organoids.(38,101)

Despite this potential, several challenges remain before organoid-based therapies can be broadly adopted in clinical settings. These challenges include achieving full functional maturation, ensuring sufficient vascularization of transplanted tissues, and addressing scalability and standardization requirements for producing clinical-grade cultures.(103) The need for non-animal-based alternatives to traditional Matrigel for scaffolding and efficient delivery procedures also remains critical for their broader clinical adoption.(37) Ultimately, collaborative efforts across biology, bioengineering, computational science, and clinical research, are crucial to fully harness the potential of organoids for improving human health.(65)

The shift from benchtop methods to clinical-grade manufacture of PSCs encounters considerable technical and biological challenges. To obtain the substantial cell numbers needed for therapeutic applications, which often surpassing 109 cells per patient, a transition from manual 2D cultures to automated 3D bioreactor systems is essential. But this transition introduces mechanical shear stress and nutritional gradients that may jeopardize cell viability or induce undesired, spontaneous differentiation. Preserving genomic stability throughout extended expansion is also a critical safety issue, since pluripotent stem cells are susceptible to recurring genetic aberrations that may elevate tumorigenic risk or modify functional efficacy in the ultimate therapeutic product.(104) The tumorigenicity risk, particularly the possibility of remnant undifferentiated cells leading to teratoma formation after transplantation, necessitates stringent purification and validation techniques. Ensuring long-term functional stability and enough vascularization to avert graft necrosis is crucial for clinical viability. Collaborative endeavors across biology, bioengineering, and regulatory science are essential for the safe utilization of organoids in promoting human health.(105)

In addition to biological limitations, the "maturation gap" and elevated cost of goods (COGS) continue to pose significant obstacles to extensive clinical use. PSC-derived tissues frequently maintain fetal-like functional attributes, lacking the complete physiological maturity necessary to adequately substitute adult organ function. The dependence on costly, medical-grade growth hormones and specialized synthetic matrices renders large-scale production economically difficult. Resolving these challenges necessitates the adoption of standardized QC frameworks, such as the 2025 ISO/AWI 25430-2 standards, and the establishment of closed-loop, automated systems to guarantee batch-to-batch reproducibility and adherence to regulatory requirements for "assay-ready" clinical goods. (92)

Figure 3 summarizes the diverse biomedical applications of organoid technology recently in four key areas: disease modeling, where iPSCs and CRISPR/Cas9 are used to recapitulate organ-specific pathologies; drug discovery and toxicology, which utilizes organoids for high-throughput screening of chemical and biological agents; personalized medicine, involving the creation of PDO from biopsies for tailored therapeutic testing; and regenerative medicine, which integrates organoid expansion with 3D-printing and scaffolds for tissue engineering. Together, these pillars underscore the role of organoids in connecting conventional cell culture systems with real-world clinical applications.

Current Challenges and Limitations

Despite their potential, organoids cannot yet fully recapitulate the maturity and systemic complexity of native human tissues. A major conceptual challenge lies in balancing natural biological self-organization with the engineered control needed to minimize variability and direct morphogenesis. Many current models still lack key physiological features such as vascularization, mechanical forces, and fluid flow, resulting in anatomical limitations.(64,103,106,107) Addressing these limitations through interdisciplinary integration of bioengineering

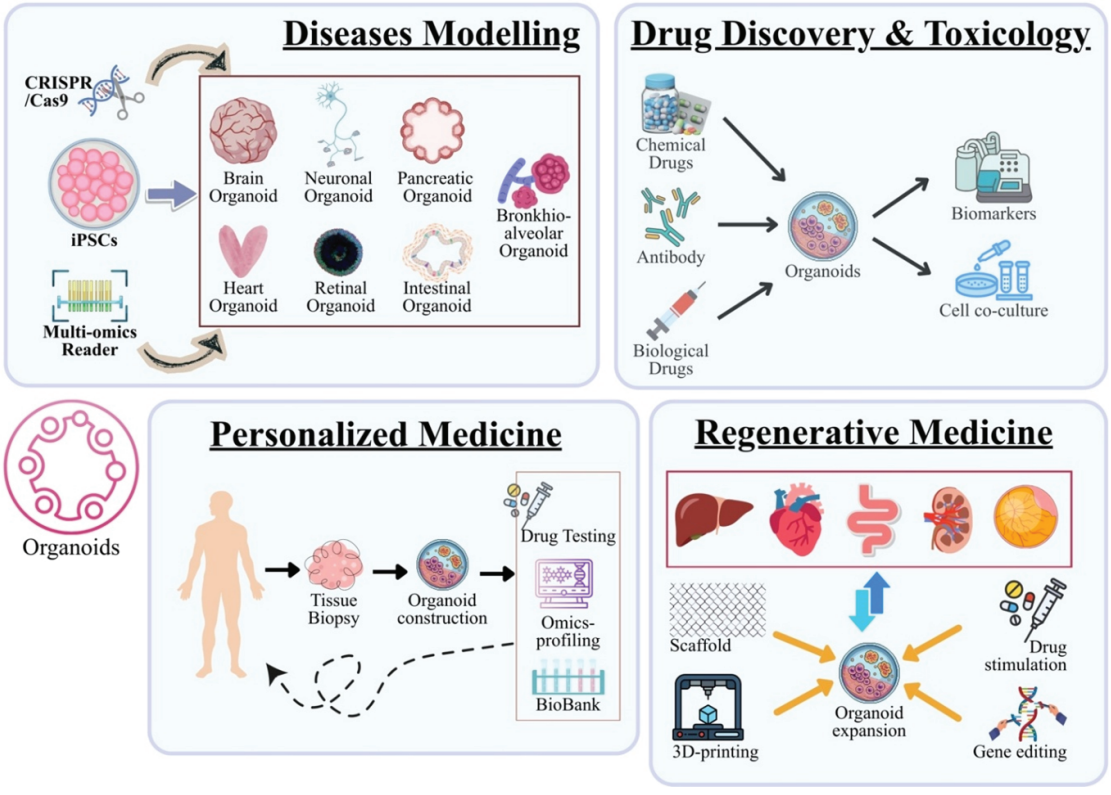

Figure 3. The multidisciplinaryapplications of organoid technology in translational medicine. This schematic shows the four main pillars of organoid utility: (A) Disease Modeling, using iPSCs, CRISPR/Cas9, and multi-omics to replicate organ-specific pathologies; (B) Drug Discovery & Toxicology, a high-fidelity platform for testing chemical, antibody, and biological therapeutics; and (C) Personalized Medicine, the "bench-to-bedside" pipeline where patient biopsies are used to create diagnostic "avatars" for drug testing and (D) Regenerative Medicine, using 3D bioprinting and gene editing to grow functioning tissues for transplantation. These examples show how organoids advance biological research and clinical diagnosis and treatment.

and computational science is essential to prevent the overinterpretation of data and to develop next-generation platforms that faithfully approximate human physiology.

Vascularization: The Oxygen Diffusion Limit and the Emergence of Necrotic Cores

One of the most enduring challenges in organoid technology is the lack of a functional vascular system. Although organoids can reproduce key features of tissue structure and cellular diversity, their expansion is limited by diffusion constraints on oxygen, nutrients, and metabolic waste, resulting in hypoxia, metabolic stress, and necrotic cores in larger culture.(7) In conventional culture, organoids depend on passive diffusion, limiting their size to roughly 200–300 μm before a necrotic core forms due to oxygen and nutrition deficiency.(108,109)

Current strategies to address the vascularization deficit face significant trade-offs. Although co-culture with endothelial cells can spontaneously network formation, these structures frequently remain immature and non-functional. Bioengineering tools like organ-on-a-chip improve mass transport via fluid flow but introduce complexity that limits scalability and can disrupt natural cell behavior. Conversely, *in vivo* transplantation provides functional perfusion but reintroduces animal dependency and inter-species data conflicts.(7,107) Researchers are also progressively shifting from stochastic self-organization to AI-optimized microfluidics and 3D bioprinting to address this issue. Recent studies conducted in 2025 and 2026 have shown that machine learning algorithms can dynamically modify "system-level parameters", including fluid flow rates, shear stress, and oxygen tension, to generate perfusable, hierarchical vascular networks that replicate the neurovascular unit or blood-brain barrier. Somehow, attaining active physiological remodeling remains a challenge. Although we can fabricate "pipes," we have yet to emulate the dynamic processes of angiogenesis or self-pruning in natural vessels in response to local tissue requirements without manual intervention.(110)

Developmental Maturation

Despite their ability to reproduce complex tissue structures, most organoids consistently resemble fetal or embryonic stages rather than fully mature adult organs. This developmental immaturity is a persistent challenge across various organoid types and significantly limits their applicability for modeling adult-onset diseases, chronic pathologies, and drug metabolism, where mature physiological functions are crucial.(8)

This developmental stagnation arises from both their cellular origins and the absence of appropriate physiological cues. iPSCs are "reset" to an embryonic state, necessitating a long developmental timeline, whereas ASCs retain epigenetic memory that facilitates more mature, tissue-specific functionality.(111) However, both types are often constrained by simplified, static culture environments that favor rapid proliferation over specialization. Without incorporating systemic maturation signals, such as blood flow, mechanical forces, and hormonal changes, organoids lack the critical biological cues needed to advance beyond early developmental stages.(8,112)

To address the "embryonic" characteristics of iPSC-derived cells, researchers are employing electromechanical conditioning and specific epigenetic "aging" techniques. In cardiac organoids, administering electrical shocks to the tissue simulates the workload of an adult heart, whereas in brain models, co-culturing with microglia has demonstrated an enhancement in synaptic pruning and electrophysiological development. However, most organoids predominantly exhibit characteristics of a second-trimester fetal condition. A universal "aging cocktail" that can effectively transition these models into an adult phenotype without causing cellular senescence or mortality is currently unavailable. (113,114) Therefore, the goals of recent studies is to identify the "extrinsic cues", including mechanical tension, hormonal fluctuations, or electrical stimulation, that can induce an organoid to mature into an adult state.

Reproducibility: The Challenge of Batch-to-Batch Variability

Even with the standardized protocols, reproducibility continues to be a major obstacle to the broad use of organoids in both academic research and industrial settings. Variability in size, cellular composition, morphology, and functional performance complicates data interpretation, weakens statistical power, and undermines confidence in cross-study comparisons, ultimately limiting their clinical applicability.(9)

Organoid variability arises from three main factors: biological heterogeneity, matrix variability, and manual handling. Even within identical genetic backgrounds, pluripotent stem cells display differences in epigenetic states and differentiation tendencies, which together with variations in cell density and timing will lead to divergent developmental outcomes.(115) Matrigel, while effective, introduces batch-specific differences in stiffness and growth factor content that hinder reproducibility and regulatory approval, driving the shift toward defined synthetic or tissue-

specific hydrogels. Finally, reliance on manual handling introduces operator-dependent variables that accumulate across laboratories.(116)

The approval of ISO/AWI 25430-2 (2025) set up the initial global quality control standards for organoid production. Lab automation, particularly through robotic liquid-handling systems and microwell arrays like AggreWell HT, has diminished operator-dependent variability by 80%, facilitating high-throughput screening that satisfies pharmaceutical industry standards. The remain challenge is the “matrix mystery”. Regardless the emergence of synthetic hydrogels, numerous intricate organoids continue to necessitate Matrigel, an animal-derived substance. The variability across batches poses a considerable obstacle for rigorous regulatory approval.(92,117)

Biological Complexity: Missing Immune and Microbiome Interactions

Most organoid systems are typically derived from epithelial or parenchymal stem cells and maintained under “sterile” conditions that exclude immune cells and microbiome influences.(10) This lack constitutes a major limitation, especially for investigating inflammatory diseases, infections, cancer immunology, and host-microbe interaction.(36) Organoids are often conceptualized as isolated tissue replicas, yet *in vivo* organs function as integral nodes within complex biological and ecological systems, which organoid models currently fail to fully capture.(118)

Researchers at Erasmus MC and additional institutions have effectively incorporated yolk-sac-derived macrophages into liver and intestine organoids created an “immunized organoids” (119), where these models may now replicate a "cytokine storm" and tissue damage in reaction to viral infections (such as SARS-CoV-2 or Hepatitis E) without the necessity of animal models. To better reflect the systemic complexity of human physiology, the field is shifting toward modular and integrative strategies, such as assembloids and multi-organ platforms. By integrating diverse cell populations and facilitating inter-organ communication, these "interoperable biological units" greatly improve the translational relevance of organoid models.(120) Although we can incorporate a single immune cell type, such as macrophages, the comprehensive reconstruction of the adaptive immune system—encompassing T-cell trafficking and microbiome-mediated "education" of the immune system—continues to pose an unresolved "interoperability" challenge.(97) The advancement of organoids from experimental models to therapeutic 'avatars' needs a methodical resolution of current biological and technical impediments. Table 3 delineates these problems alongside the associated bioengineering innovations, such as AI-driven perfusion and automated standardization, that characterize the forthcoming generation of high-fidelity tissue modeling.

Future Directions: Bioengineering The Next Generation

The transition from current experimental models to clinically predictive platforms depends on overcoming the structural and maturation bottlenecks identified above. The future of vascularization and systemic modeling lies in organ-on-a-chip integration, which moves beyond static wells to introduce microfluidic perfusion. By mimicking blood flow and dynamic nutrient exchange, organ-on-a-chip platforms alleviate hypoxia-induced necrosis and provide essential biomechanical cues, such as shear stress, to drive tissue specialization. Future iterations of these platforms are expected to emphasize modular, fluid-linked systems that

Table 3. Current challenges, limitations, and future bioengineering directions.

Category	Current Challenges and Limitations	Biological & Translational Impact	Future Directions & SOTA Solutions (2025–2026)
Vascularization	Lack of functional perfusion; reliance on passive diffusion.	Oxygen Diffusion Limit: Hypoxia-induced necrotic cores in organoids >300 μm.	AI-Driven Microfluidics: Real-time, machine-learning-optimized flow and 3D-bioprinted hierarchical vascular trees.
Maturation	Developmental stagnation in fetal/neonatal stages.	The "Fetal Gap": Inability to accurately model adult-onset diseases (e.g., Alzheimer’s, T2D).	Electromechanical Conditioning: Use of shear stress, electrical pacing, and targeted epigenetic "aging" cocktails.
Reproducibility	High batch-to-batch variability; manual handling errors.	The "Black Box" Problem: Weak statistical power in HTS; hurdles for regulatory (FDA) approval.	Automated Workflows: Robotic liquid handling, ISO/AWI 25430-2 standards, and bioactive synthetic scaffolds.
Complexity	Missing immune and microbiome interactions.	"Immune-Blind" Models: Failure to capture neuroinflammation or immunotherapy responses.	Immunized Assembloids: Integration of yolk-sac-derived macrophages and multi-organ "body-on-a-chip" systems.

enable inter-organ communication, offering a more realistic representation of systemic drug metabolism and multi-organ physiology.(11,121)

To solve the challenge of batch-to-batch variability, 3D bioprinting is progressing from a simple patterning tool into a framework for "guided self-organization." Rather than attempting to print every individual cell, future directions involve the precise placement of "bio-inks" and bioactive scaffolds that establish an architectural foundation. This allows researchers to engineer complex features, like layered luminal compartments and vascular channels, while still allowing the biology to self-assemble within those defined geometries. The integration of engineering determinism with emergent biological complexity seeks to attain the reproducibility necessary for industrial and regulatory acceptance.(12,61,62)

Ultimately, the shift toward AI-assisted live imaging and real-time analytics addresses the current "black box" nature of organoid development. By interpreting developmental trajectories in real time, machine learning algorithms can enable continuous quality control and detect early signs of disease or drug response. As these datasets expand, integrating AI with multi-omics will allow researchers to forcast and refine differentiation protocols, effectively creating a digital twin of the biological model. Together, these convergent technologies (perfusion, architectural guidance, and real-time analytics) will systematically bridge the gap between lab-grown "avatars" and clinical human physiology.(13,122)

Conclusion

Organoid technology has fundamentally transformed our capacity to model human physiology, surpassing the static limitations of 2D cultures and the interspecies differences inherent to animal models. The evolution from early stochastic self-assembly to the sophisticated assembloid era reflects a shift toward modeling the human body as an interconnected ecosystem. Bioengineering and computational AI are also integrated as the crucial link for clinical translation, amidst prevalent discussions on the successful reproduction of specific tissue types. There are shift from "bespoke" laboratory models to "regulatory-grade" platforms by correlating existing biological limitations, such as vascularization and fetal-like immaturity, with innovative engineering solutions, including AI-optimized microfluidics and standardized manufacturing frameworks. By guiding biological self-organization within carefully engineered environments, we can build a more ethical, accurate, and personalized foundation for next-generation personalized medicine.

Authors Contribution

AM, AC, RA, BW, and AW were involved in the conception and design of the research. AM, AC, RA, and BW performed data acquisition, conducted the data analysis, interpreted the results, prepared the manuscript, and designed the figures and/or tables. IL contributed through critical revision of the manuscript. AW provided overall supervision of the study.

Ethical Statement

Ethical approval and informed consent were not required for this work.

Conflict of Interest

The authors declare no conflicts of interest.

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