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Organoidogenesis: modulating morphology and perturbation

Burak Kahveci · Ali Eren Evranos · İlayda Koçhan · Cem Batu Ören · Sinan Güven

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Abstract

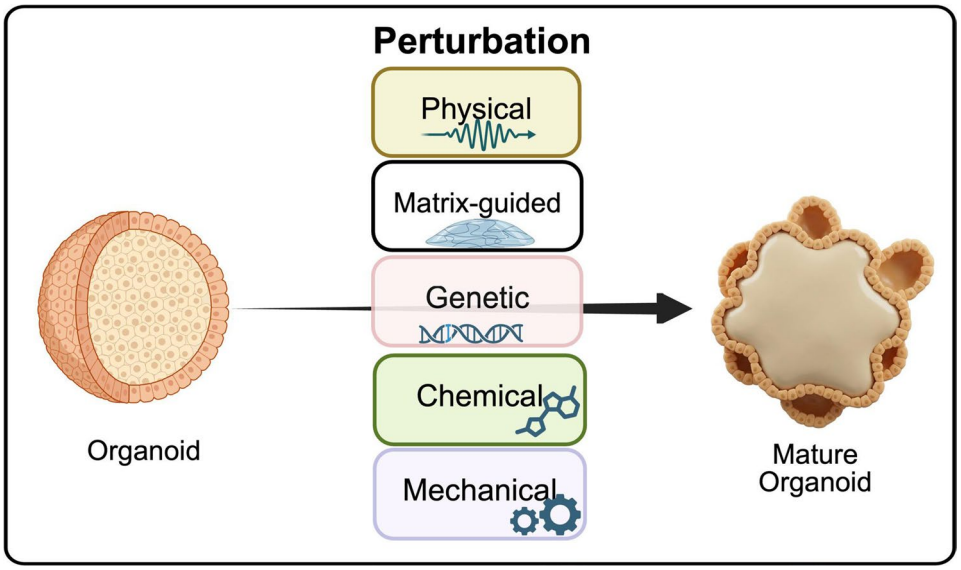
Organoids, three-dimensional (3D) structures derived from stem cells, hold immense promise for disease modeling and regenerative medicine by recapitulating native tissue architecture and function, yet their full potential is often limited by morphological heterogeneity and a lack of precise developmental control. This review provides a comprehensive overview of cutting-edge strategies to modulate and perturb organoid morphology, addressing these critical challenges. The 'top-down' bioengineering approaches, including the engineering of the extracellular matrix (ECM) and culture environment, alongside advanced fabrication techniques such as bioprinting that impose external geometric constraints were explored. Further, 'bottom-up' strategies that leverage intrinsic biological processes through biochemical and genetic perturbations, such as targeted modulation of key signaling pathways were detailed. Critically, the transformative impact of artificial intelligence (AI)-driven image analysis for robust, high-throughput quantification of morphological outcomes were highlighted. By synthesizing these complementary approaches, this review provides a roadmap for engineering next-generation organoids with enhanced control over form and function, paving the way for more predictive and reliable in vitro systems.

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Graphical Abstract



Key points

Morphology is an Indicator of Functionality An organoid's physical architecture directly reflects its functional maturity and physiological fidelity. Complex, organized structures often signify a closer recapitulation of the native organ's in vivo state.

Growth Environments Profoundly Affect Morphology Dynamic culture systems, like microfluidics, overcome the nutrient and oxygen limitations of static methods, preventing necrotic cores and promoting the development of larger, more mature organoids.

Perturbation is a Powerful Tool for Understanding Mechanisms Systematically perturbing organoid development with biochemical, genetic, or biophysical tools is a powerful strategy to dissect the fundamental mechanisms of tissue morphogenesis, organization, and disease pathogenesis.

Bioengineering Approaches Guide Morphogenesis Bioengineering techniques like scaffolding, bioprinting, and ECM engineering guide the stochastic self-assembly of organoids, enabling the formation of predictable and reproducible morphologies with desired functions.

Signaling Pathway Modulation Determines Cell Fate and Structure Precisely modulating key signaling pathways (e.g., Wnt, BMP) with small molecules is a fundamental bottom-up strategy to control cell fate decisions, differentiation, and emergent architectural organization.

AI Automates and Accelerates Morphological Analysis AI-driven deep learning models automate image analysis, enabling rapid, objective, and high-throughput quantification of morphology while minimizing human bias and allowing for longitudinal tracking of organoid development.

Keywords Organoids · Morphology · Perturbation · Morphogens · Morphogenesis · Mechanotransduction

Introduction

Stem cells are fundamental to tissue regeneration and disease modeling [1, 2]; however, traditional 2D cultures often fail to recapitulate the organizational complexity and functional maturity found in vivo [3]. To overcome these limitations, 3D organoids have emerged as a transformative technology that leverages self-organization to foster essential cell-cell and cell-matrix interactions [3, 4]. By mimicking the native physiological microenvironment,

organoids facilitate the development of specialized cell types and multicellular complexity, providing a powerful in vitro platform for investigating human physiology and pathological processes [3, 5].

The modern era of organoid research was inaugurated by Sato et al., who demonstrated that single murine Lgr5-positive stem cells could self-organize into intestinal organoids with distinct crypt-villus domains (Fig. 1) [6]. This foundational work was rapidly adapted to human pluripotent stem cells (hPSCs), facilitating the modeling of diseases such as cystic fibrosis [7]. The field subsequently expanded into the central nervous system, yielding optic cup organoids [8, 9] and cerebral organoids (CO) or cortical spheroids that recapitulate regionalized microcephaly phenotypes and brain development [10, 11]. To address the intrinsic limitations of nutrient supply and long-term maturation caused by a lack of vascularization, Takebe et al. utilized co-culture strategies to generate vascularized liver buds capable of functional integration upon transplantation (Fig. 1) [12]. Concurrently, the structural complexity of liver models was enhanced through the generation of bile-duct-forming architectures [13]. Progress in cardiac research similarly moved toward atrioventricular-separated "cardioids" that mimic heart chamber identity (Fig. 1) [14, 15]. Recent frontiers emphasize synergy between developmental biology and bioengineering to achieve greater predictability. This includes photosensitive brain organoids forming light-responsive sensory circuits [16], improved axonal guidance [17] and intestinal models with directed, reproducible morphologies [18]. The continued expansion of the field is exemplified by lacrimal gland organoids, which utilize budding and branching morphogenesis to achieve functional maturation post-transplantation [19].

The choice of starting stem cell population—either tissue-resident adult stem cells (ASCs) or pluripotent stem cells (PSCs)—is a decisive factor that dictates an organoid model's physiological fidelity and applications. ASC-derived organoids originate directly from tissue-specific pools, providing high fidelity and reproducible yields for studying homeostasis and oncology [5, 20–23]. While they offer faster generation protocols, their utility is constrained by tissue-restricted differentiation, the necessity for invasive biopsies, and technical complexities in genetic manipulation. Conversely, PSCs (ESCs and iPSCs) differentiate into all three germ layers, enabling the generation of diverse organoid types for inaccessible tissues like the brain and retina [3–5, 24–28]. The capacity

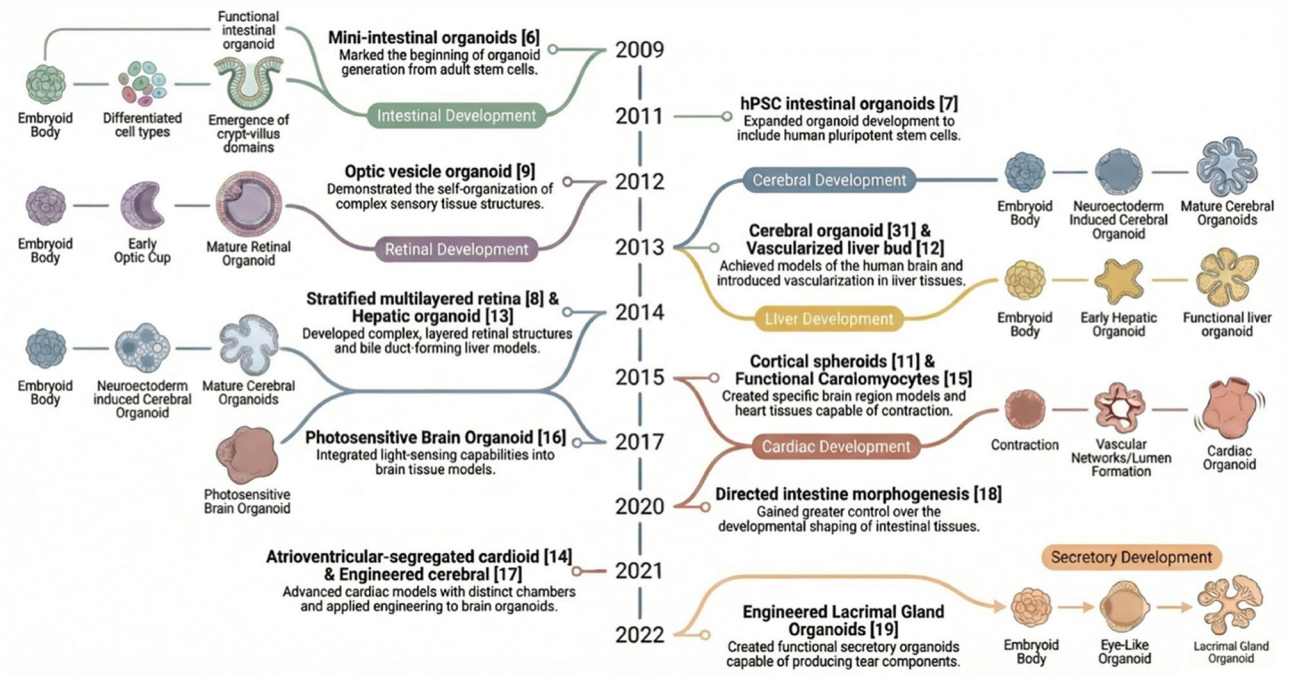


Fig. 1 Milestones in organoid development. This timeline illustrates the rapid evolution and diversification of organoid technology, highlighting seminal publications that have significantly advanced the field. Key breakthroughs are presented chronologically, demonstrating the progression from basic self-organization to increasingly complex and functional tissue models

to reprogram somatic cells into patient-specific iPSCs is particularly transformative for dissecting disease mechanisms [10, 29–31]. Crucially, PSCs provide a unique platform for modeling early embryonic development and organogenesis, processes that remain inaccessible through ASC-based systems.

The culture environment significantly dictates organoid morphology and development, typically classified into static or dynamic systems [3, 17, 32, 33]. Static cultures, characterized by stationary hydrogel droplets or microwells, are cost-effective and ideal for high-throughput screening (HTS) [32]. However, the absence of media perfusion often leads to necrotic core formation due to diffusion limits and a lack of the mechanical forces essential for in vivo tissue maturation [32]. To circumvent these limitations, dynamic systems—including spinning bioreactors, orbital shakers, and microfluidic devices—utilize active media flow to enhance nutrient exchange and metabolic waste removal [17, 32, 34–39]. By reintroducing mechanical cues fundamental to mechanobiology, dynamic cultures foster superior differentiation and long-term viability [40–42], although they necessitate specialized equipment and precise optimization to prevent excessive shear stress [43]. The successful generation of organoids requires the recapitulation of two intertwined developmental processes: histogenesis and morphogenesis [44, 45]. Histogenesis involves the differentiation of progenitor cells into specialized lineages from the primary germ layers, while morphogenesis encompasses their collective organization into complex 3D architectures. While histogenesis provides the necessary cellular building blocks, the structural fidelity and functional capacity of an organoid are ultimately determined by the precision of its morphogenesis.

An organoid's morphology serves as a critical, visually accessible proxy for its underlying functionality and physiological fidelity; for example, convoluted neuroepithelium and ventricles in cerebral models [33], or crypt-like domains and specific epithelial thicknesses in parathyroid and inner ear organoids, signify advanced maturation [30, 46]. Characterizing these features requires a multi-modal toolkit, beginning with macroscale brightfield microscopy [47] and progressing to high-resolution 3D reconstructions via confocal immunofluorescence [47, 48]. While time-lapse and light-sheet microscopy monitor dynamic processes with minimal phototoxicity [49], electron microscopy and Diffusion Weighted MRI provide essential ultrastructural and extracellular matrix organization details [50, 51]. Beyond qualitative assessment, objective quantification via platforms like ImageJ and BrAIn enables the extraction of volumetric and cellular metrics [52, 53], complemented by specialized analyses such as Sholl analysis for neuronal dendritic complexity [54]. These structural data are further refined by biomechanical assays, including micropipette aspiration to measure maturation-linked stiffness and deformability [51]. To ensure structural accuracy translates to functional activity, assays like patch-clamp electrophysiology and calcium imaging evaluate single-cell responses, while multi-electrode arrays (MEAs) record coordinated network-level electrical activity [54–56]. Ultimately, integrating these morpho-functional parameters with multi-omics and computational modeling provides a holistic understanding of developmental trajectories and responses to perturbations, though the interpretation of such vast, complex datasets remains a primary challenge in the field [27, 57–59]. Nevertheless, morphological similarity alone should not be interpreted as functional equivalence. Functional validation is essential to determine whether perturbation-induced structural changes reflect genuine maturation, disease rescue, or improved physiological fidelity. Depending on the organoid lineage, validation may include electrophysiological activity in neural and cardiac models, transepithelial electrical resistance and permeability assays in epithelial barriers, hormone or enzyme secretion in endocrine and hepatic systems, transporter localization and activity in renal and intestinal organoids, metabolic profiling, and drug-response or toxicity assays in disease models.

Analysis of organoid images obtained from microscopes with deep learning models provides advantages in terms of obtaining fast results and being able to perform analyses without human-based errors [60]. Many approaches are used for organoid analysis, including classification models, segmentation models, and object detection models [60]. Here, we define **organoidogenesis** as the in vitro process by which stem cells proliferate, differentiate, and self-organize into three-dimensional structures that recapitulate the architecture and function of a native organ. This Review provides a comprehensive synthesis of these disparate approaches. We aim to: (1) systematically categorize the physical, biochemical, and genetic methods used to modulate and perturb organoid development; (2) highlight how bioengineering principles can be leveraged to guide stochastic self-organization

towards more predictable and reproducible outcomes; and (3) emphasize the transformative role of artificial intelligence in automating the analysis and interpretation of complex morphological phenotypes.

Physical factors for perturbating of organoid morphology

Static conditions

The physical landscape of organoidogenesis is primarily governed by fundamental transport laws that dictate growth trajectories, cellular viability, and structural complexity. A universal constraint in static culture systems is the heavy reliance on passive diffusion for nutrient influx and metabolic waste efflux, which imposes a critical size threshold, typically ranging between 700 and 900 μm [61].

Beyond this biophysical limit, the escalating metabolic demands of the growing tissue outpace the rate of passive transport, leading to the accumulation of toxic byproducts and the formation of a necrotic core. This diffusion-limited stagnation severely compromises the structural integrity of the tissue, frequently resulting in significantly reduced dimensions—often up to five times smaller in static settings—across diverse lineages, including cerebral, choroid plexus, and liver organoids [61–68]. Consequently, static environments foster uncontrolled biochemical gradients that induce high morphological heterogeneity, often causing tissues to default to simplistic spheroids or underdeveloped architectures, such as immature nephron structures in kidney models [69–73]. The transition from static to dynamic systems introduces convective transport mechanisms that bypass these scaling constraints, significantly enhancing proliferation—evidenced by up to a 50-fold increase in cell number in vascular models—and steering sophisticated morphogenesis [61, 65, 67, 68]. Dynamic forces, such as fluidic shear stress and simulated peristalsis, act as essential mechanical triggers for symmetry-breaking events and functional maturation. In kidney and intestinal lineages, these environments promote **apical-basolateral polarization** and the critical alignment of transporters (e.g., OCT2), hallmarks of maturity that are typically absent in static controls [69, 70, 74]. Furthermore, dynamic cues drive advanced structural organization, fostering cortical layer separation in neural models and interconnected tubular architectures in bone and liver lineages, thereby preventing the aberrant, tumor-like lobular growth patterns observed in static 3D Matrigel cultures [67, 68, 72]. These morpho-functional refinements translate directly into superior predictive validity for pharmacological and toxicological assessments. For instance, dynamic cultures enable intestinal organoids to exhibit in vivo-like, glucose-responsive ghrelin secretion and allow for the detection of subtle neurotoxic abnormalities, such as those induced by methylmercury, which remain undetected in static systems [61, 64, 69]. However, a critical synthesis of these technologies reveals significant methodological trade-offs. While dynamic systems mitigate diffusion limits, they introduce non-physiological shear stress that can inadvertently activate mechanosensitive pathways (e.g., YAP/TAZ), potentially generating morphological artifacts. Moreover, the technical complexity and material-specific issues—such as the absorption of hydrophobic drugs by PDMS-based microfluidic devices—severely limit the scalability and inter-laboratory reproducibility of these platforms [75–77]. Ultimately, the choice between static and dynamic environments is not merely a logistical preference but a critical determinant of the model's translational relevance and scientific rigor.

Dynamic environments and microphysiological systems

Microphysiological systems (MPS) or 'organ-on-a-chip' platforms transition organoidogenesis from a stochastic self-assembly process to a spatiotemporally programmed environment. The core principle of these systems is the replacement of passive diffusion with convective transport, which allows for the precise management of the biochemical boundary layer. By maintaining continuous basolateral flow, MPS can actively remove endogenous morphogen antagonists, thereby triggering 3D structural transitions—such as villus formation or bile canaliculi maturation—that are typically suppressed in static cultures [78–82]. This dynamic control extends beyond simple

nutrient supply; fluidic shear stress acts as a primary developmental signal for apical-basolateral polarization and the reinforcement of barrier functions. The application of mechanical cues, such as peristalsis-like flow, is a fundamental rule for achieving physiologically relevant transepithelial electrical resistance (TEER) values and robust tight junction formation across intestinal, corneal, and renal lineages [81, 83–86].

Beyond mechanical induction, MPS platforms serve as high-precision tools for morphogen gradient scaling. By establishing stable, non-linear chemical gradients, these systems can recapitulate the in vivo colinearity of HOX gene expression along the rostral-caudal and dorsal-ventral axes, effectively patterning complex architectures like the neural tube [87, 88]. This principle of spatial programming is further enhanced through the integration of droplet microfluidics and surface acoustic waves, which pre-determine structural layouts and cell densities before the onset of self-organization [89–93]. Furthermore, the introduction of flow-induced shear stress is a prerequisite for vascular integration, directing endothelial alignment and maturation (e.g., HUVEC maturation) to support co-cultured tissues [38, 94, 95]. The enhanced physiological fidelity provided by MPS facilitates superior predictive validity in disease modeling and pharmacology, identifying drug-resistant phenotypes at frequencies as low as 10^{-3} [35, 96–99]. Beyond structural support, the transition of MPS from academic proof-of-concept to standardized drug development platforms is currently addressing significant regulatory challenges. As highlighted in recent evaluations of organ-on-a-chip advancements, these systems are increasingly being positioned to fulfill FDA Modernization Act 2.0 requirements by providing human-relevant alternatives to animal testing, particularly in assessing chronic toxicity and complex drug-drug interactions [100].

While dynamic systems effectively mitigate diffusion-related growth constraints, they introduce significant methodological risks and non-physiological stressors. Fluidic shear can inadvertently activate mechanosensitive pathways such as YAP/TAZ, potentially confounding results with morphogenetic artifacts or cellular damage. Furthermore, technical overhead and material-specific issues—such as small-molecule absorption in PDMS-based devices—severely limit the scalability and inter-laboratory reproducibility of dynamic platforms. Consequently, the transition to dynamic culture represents a context-dependent trade-off rather than a universal progression toward a gold standard.

Directed-morphology techniques

Directed morphology leverages extrinsic engineering cues to bypass the inherent stochasticity of self-organization, steering development toward predictable and physiologically relevant architectures. A fundamental principle in this approach is the application of geometric confinement, where physical boundary conditions dictate internal cellular tension and growth vectors [101]. By utilizing scaffolds or microwells to impose specific spatial constraints, researchers can force tissues into predefined symmetries that mirror native organ blueprints. For instance, high-resolution techniques such as melt electrospinning writing (MEW) provide the structural guidance necessary for precise spatial organization and lumenogenesis, while molded templates are used to recapitulate the crypt-villus axis by defining the physical curvature required for symmetry breaking in intestinal models (Fig. 2) [18, 102]. These engineered boundaries do not merely dictate shape; they serve as mechanical regulators of functional maturation, as evidenced by how rectangular or honeycomb templates modulate cellular aspect ratios and metabolic compactness in cardiac and hepatic lineages [103, 104].

Advanced biofabrication and material science have transitioned the field from passive confinement to deterministic 3D microenvironment programming. Hydrogel bioprinting and specialized elastomer resins enable the precise spatiotemporal modulation of cell polarity and branching morphogenesis across multiple lineages, including liver and lung tissues [105, 106]. The core principle here is the replacement of natural, diffusion-based morphogen gradients with engineered compartmentalization, as demonstrated by systems like MultiCUBE, which allow for localized perturbations within distinct hydrogel zones (Fig. 2) [107]. While the integration of these manufacturing techniques with robotic automation significantly enhances morphological homogeneity and inter-batch reproducibility, it introduces a significant methodological paradox [108].

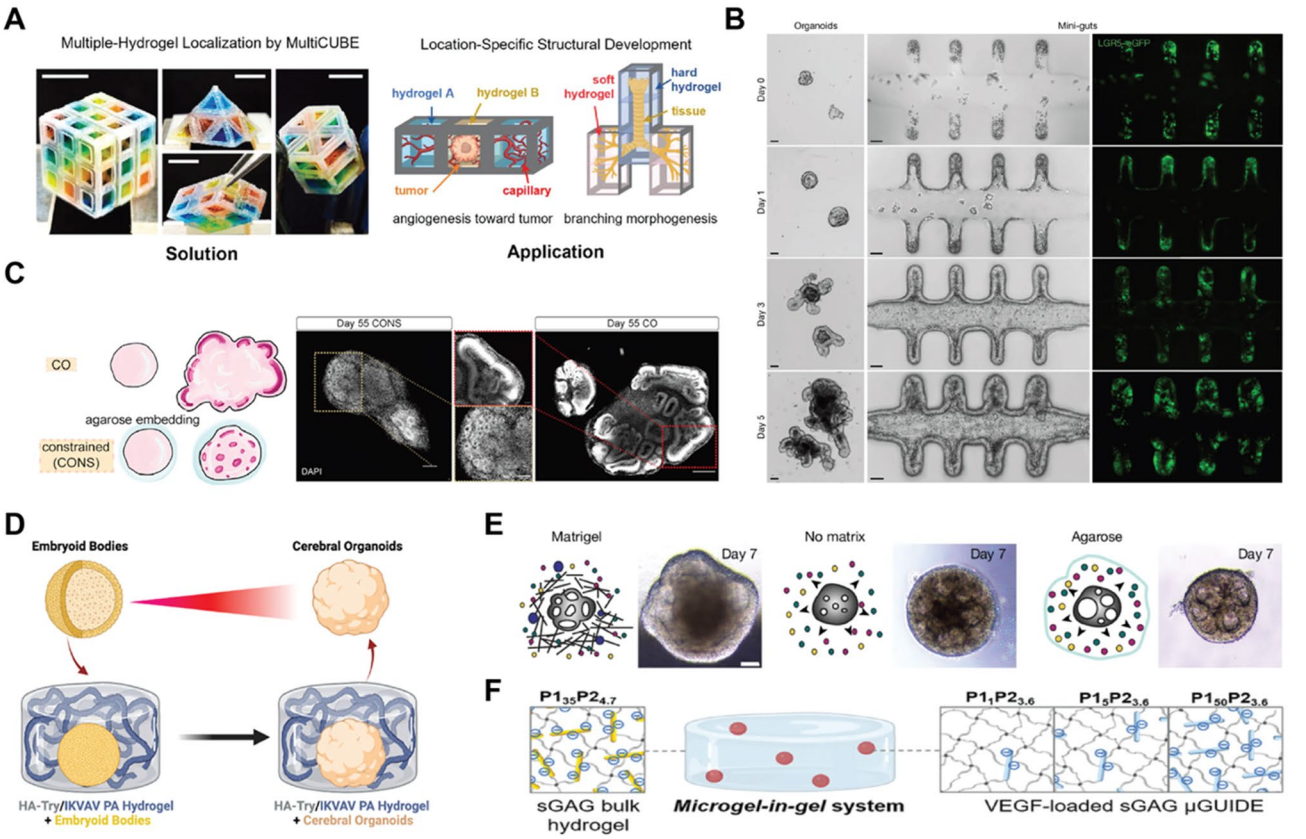


Fig. 2 Engineering organoid morphology via extrinsic cues and ECM modulation. **A)** The MultiCUBE scaffold system enables spatial control and diverse hydrogel compartmentalization to direct angiogenesis and morphogenesis. Adapted from [107]. **B)** Tube-shaped scaffold used for in vivo crypt-villus organization of intestinal stem cells [18]. **C)** Hydrogel encapsulation provides geometric specificity, which in CO resulted in smaller lumens and inhibited prominent ventricular development [33]. **D)** While abnormal ECM stiffness impairs proliferation, adjustable stiffness and non-fibrous hydrogel architectures can precisely steer CO morphology [27]. **E)** Varying culture environments—including Matrigel, low-melting-point agarose, and matrix-free conditions—produce distinct formations; Matrigel specifically facilitates luminal morphogenesis and rostral patterning [49]. **F)** Tissue development, architecture, and functionality in kidney organoids can be controlled by microgel systems that control morphogen concentration gradients [118]. Reproduced with permissions. Copyright 2025, Wiley, Nature Group, Cell Press and Elsevier

While enhancing structural homogeneity, rigid geometric constraints often impede intrinsic self-assembly, potentially limiting the stochastic diversity required for full physiological complexity. Advanced fabrication methods like MEW or MultiCUBE face significant scalability bottlenecks and high technical barriers, restricting their utility to niche mechanistic studies rather than high-throughput industrial applications. Furthermore, artificial confinement can impose non-physiological mechanical loads, risking aberrant cell fate decisions or stiffness-induced epithelial-mesenchymal transition (EMT) instead of the desired functional maturation (Table 1).

Morphology modulation and perturbation with ECM engineering

The extracellular matrix (ECM) functions as a dynamic biophysical regulator rather than a passive scaffold, dictating organoid morphogenesis through a complex interplay of biochemical and mechanical cues. A fundamental principle in ECM engineering is the trade-off between compositional complexity and experimental reproducibility. While undefined matrices like Matrigel provide a rich proteomic landscape essential for processes such

Table 1 Physical culture platforms and morphology control

Culture Strategy	Perturbation modality	Morphological outcomes	Interpretation	Reference
Static culture systems	Passive diffusion-limited culture	Necrotic core formation; reduced size; uncontrolled gradients; high heterogeneity; simple spheroids; immature architectures	Simple baseline; limited by diffusion, waste accumulation, and poor mechanical control	[32, 61–73]
Dynamic culture systems	Convective flow; shaking; spinning; perfusion	Increased growth; improved polarization; cortical layer separation; tubular architectures; reduced aberrant lobular growth	Improves transport and morphogenesis; may introduce shear stress and YAP/TAZ artifacts	[17, 32], 34– [43, 61], 64– [70, 72, 74]
Organ-on-chip / MPS	Microfluidic flow; gradient control; peristalsis-like stimulation; vascular integration	Villus formation; bile canaliculi maturation; barrier reinforcement; tight-junction organization; neural tube-like patterning; endothelial alignment	Precise spatial control; limited by technical complexity, PDMS absorption, and shear artifacts	[35, 38], 75– [100]
Directed-morphology platforms	Scaffold guidance; geometric confinement; bioprinting; compartmentalized hydrogels; robotic automation	Predefined symmetry; lumenogenesis; crypt-villus axis formation; altered aspect ratio; branching; increased homogeneity	Improves reproducibility; may restrict self-assembly and introduce non-physiological load	[18, 101–108]

as lumenogenesis and rostral patterning, their inherent batch-to-batch variability frequently perturbs structural uniformity [47, 49, 109, 110]. Conversely, the transition to defined hydrogels offers superior spatiotemporal control but often reveals a "simplicity threshold" where the absence of critical basement membrane extracts leads to truncated maturation kinetics and altered cell polarity [33, 49, 111]. The general rule emerging from diverse lineages—including pancreatic and hepatic models—is that tissue-specific ECM components (e.g., collagen vs. laminin-rich matrices) are not interchangeable; they serve as instructive templates that determine whether a tissue defaults to a simple cyst or develops complex branched architectures [112, 113].

A critical biophysical rule governing organoidogenesis is the mechanical tension threshold, primarily defined by ECM stiffness and viscoelasticity. Excessive matrix rigidity acts as a potent inducer of structural defects and aberrant cell fate transitions across multiple organoid types. For instance, high stiffness frequently triggers EMT and inhibits proliferation, a phenotype observed in both kidney models and LIS1-mutated brain organoids [27, 51, 113, 114]. In contrast, a requisite degree of stress relaxation (viscoelasticity) is essential for symmetry-breaking events and epithelial invasion. This "viscoelasticity rule" suggests that the matrix must be sufficiently plastic to allow for cellular remodeling, as evidenced by the requirement for stress-relaxing environments to promote proper ciliogenesis in renal models and prevent bronchotubule collapse in lung organoids [115–117].

Beyond structural support, the ECM serves as a mechanochemical presentation platform that modulates morphogen gradient scaling. This is achieved through electrostatic interactions between signaling molecules (e.g., SHH, BMPs, VEGF) and negatively charged glycosaminoglycans (sGAGs) within the matrix [118]. The integration of physical and biochemical cues is mediated by intracellular mechanosensors, specifically the YAP/TAZ signaling axis. A universal mechanism observed in neuroepithelial and vascular models is that the disruption of this "molecular bridge"—whether through early-stage YAP inhibition or nanoparticle-induced interference with LATS1 phase separation—precludes essential lumen formation and triggers vascular damage [119–121]. While advanced hydrogels and micropatterning techniques can recreate native-like architectures, they introduce a significant standardization bottleneck: rigid templates may constrain the intrinsic self-organization capacity of the tissue, creating a paradox where increased engineered homogeneity leads to reduced biological fidelity and limited representation of disease-induced stochasticity [121–130].

While defined hydrogels mitigate the inherent stochasticity of Matrigel, they frequently fail to replicate the synergistic proteomic landscape required for full physiological maturation, often forcing a trade-off between experimental reproducibility and biological fidelity. Moreover, mechanical interventions used to rescue aberrant morphologies—such as stiffness modulation in LIS1-mutated models—may inadvertently mask underlying genetic pathologies rather than restoring authentic cellular circuitry. Furthermore, the high technical overhead and cost associated with engineering tissue-specific viscoelasticity or microgel-based gradients (e.g., GUIDEs)

remain significant barriers to scalability, restricting these advanced scaffolds to low-throughput mechanistic studies rather than standardized industrial drug discovery pipelines (Table 2).

Table 2 Perturbations of ECMs on organoids

ECM Type	Perturbation Effects	Organoid Type	Reference
Agarose	Smooth surface Small-lumen rosettes Thin proliferative layers	Cerebral Organoid	[33]
Matrigel	Diameter and Morphology Change	Brain Organoid	[51]
	Supporting lumen morphogenesis and rostral patterning	Brain Organoid	[49]
	Different Matrigel types affect the average size of the neuroepithelium	Cerebral Organoid	[109]
	Excessive use of Matrigel can prevent the formation of uniform organoids	Cerebral Organoid	[110]
	Negative effect on dynamic morphogenesis	Intestinal Organoid	[131]
	Size heterogeneity	Intestinal Organoid	[126]
	Damaged morphology and dark granule formation in cases where there is no Matrigel regeneration	Hepatic Organoid	[111]
	Only sphere-like spheroids or cysts are formed, no branched tubules with luminal and terminal budding	Pancreatic Ductal Adenocarcinoma Organoid	[112]
	Size heterogeneity	Fallopian Tube Organoid	[128]
Geltrex	Concentration change affects neuroepithelial cyst and organoid organization	Neuroepithelial Organoid	[119]
Defined ECMs	Abnormal stiffness causes a decrease in proliferative capacity.	Cerebral Organoid	[27]
	Viscoelastic properties affect direct tissue growth and structural symmetry	Intestinal Organoid	[115]
	Increased local endothelial cell development	Kidney Organoid	[118]
	No effect on morphology	Kidney Organoid	[114]
	Soft and stress-relaxing hydrogels cause polarization of large kidney segments and apical proximal tubule	Kidney Organoid	[113]
	Uniform Morphology	Pancreatic Cancer Organoid	[125]
	Vascular Remodeling	Blood Vessel Organoids	[120]
	Supporting controllable branching and development of bronchotubules	Lung Organoids	[117]
	More uniformly sized and more compact organoids compared to those grown with Matrigel	Lung Organoids	[127]
Collagen	Larger and higher viability organoids	Tumoroid	[122]
	Branched tubules are formed by luminal and terminal budding and reach sizes up to 2000 μm	Pancreatic Ductal Adenocarcinoma Organoid	[112]
Gelatin	More rounded morphology	Pancreatic Ductal Adenocarcinoma Organoid	[123]

Biochemical clues

Modulations of signaling pathways during organoidogenesis

Organoidogenesis is fundamentally governed by the precise titration of internal signaling pathways, where morphogens act not as simple binary switches but as concentration-dependent regulators of cell fate. A universal principle across lineages is dose-dependent fate specification, primarily controlled via the Wnt/β-catenin axis. In neural models, the frequency and dimensions of fluid-filled ventricles are tightly regulated by a cocktail of EGF, FGF2, and the Wnt activator CHIR99021 (Fig. 3) [33, 110]. This sensitivity to titration is a recurring theme; while precise Wnt activation is essential for maintaining homeostasis, excessive activation via CHIR99021 can aberrantly shift cell fate from telencephalic to more caudal progenitor identities or induce abnormal gastric morphologies [49, 126, 128, 130, 132]. Conversely, the complete inhibition of Wnt signaling entirely precludes organoid formation in models like the fallopian tube, highlighting its role as an indispensable survival and patterning cue [128].

A second governing rule is morphogen gradient scaling, which drives the establishment of dorsoventral (D-V) and anteroposterior (A-P) axes through competitive signaling loops. Across neural, renal, and choroid plexus models, SHH acts as a ventralizing factor while BMP (e.g., BMP4, BMP7) serves as a dorsalizing agent, defining the spatial boundaries of tissue domains (Fig. 3) [55, 57, 65, 70, 129]. These gradients are complemented by Wnt, FGF, and Retinoic Acid (RA) signaling, which facilitate A-P patterning and induce specific lineages, such as FOXA2-expressing cells or polarized bile canaliculi [55, 64]. The spatial efficiency of these gradients is often contingent on initial parameters, such as cell seeding density, which dictates the embryoid body size and subsequent morphogenetic potential [110, 133]. Furthermore, TGF-β signaling acts as a global regulator of epithelial integrity, promotes neuroepithelium expansion, and modulates organoid polarity during structural invagination and closure (Table 3) [30, 49, 70, 130].

Beyond early development, conserved signaling modules are leveraged to drive functional maturation and branching morphogenesis. In glandular and ductal tissues, the initiation of branching follows a shared regulatory circuit triggered by FGF2 and EGF (Fig. 3), a process critically dependent on Sox9 in bile duct models [19, 45, 134]. Similarly, in renal models, the complex stages of nephrogenesis and vascularization are directed by the coordinated action of VEGF and FGF9 [69, 135]. These same pathways are often hijacked in oncological models; for instance, tumoroids within the tumor microenvironment rely on Wnt3a, EGF, R-spondin-1, and TGF-β1 to drive survival and invasive phenotypes, with genetic perturbations like SMAD4 mutations shifting invasive patterns from mesenchymal to collective modes (Table 3) [136–139].

The emerging concept of mechanochemical feedback suggests that these biochemical axes are inextricably linked to physical cues. Intracellular mechanosensors, specifically the RhoA/ROCK and YAP/TAZ pathways, recalibrate cellular sensitivity to morphogens based on extrinsic geometric constraints [103, 140, 141]. For example, mechanical disruption can autonomously activate Wnt signaling in colorectal models, increasing stemness and therapeutic resistance [140]. However, achieving reproducible results remains a challenge due to the inherent instability of ligands like Wnt3a within undefined matrices, which often necessitates microphysiological systems to maintain precise signaling thresholds [121, 126, 141, 142]. By integrating these biochemical perturbations with imaging and multi-omics analysis, we move toward a holistic understanding of how diverse signaling axes dictate organoid structural and functional fidelity [132, 136, 141].

Despite the perceived precision of biochemical titration, the heavy reliance on high-cost recombinant protein cocktails (e.g., R-spondin-1, Noggin) introduces significant batch-to-batch bioactivity fluctuations, severely undermining inter-laboratory reproducibility and industrial scalability. Furthermore, the common narrative often overlooks the mechanochemical crosstalk, where extrinsic physical cues—such as ECM stiffness—can fundamentally recalibrate cellular sensitivity to morphogen gradients like Wnt or BMP, explaining frequent discrepancies in phenotypic outcomes across static and dynamic models. Consequently, these signaling 'blueprints' remain

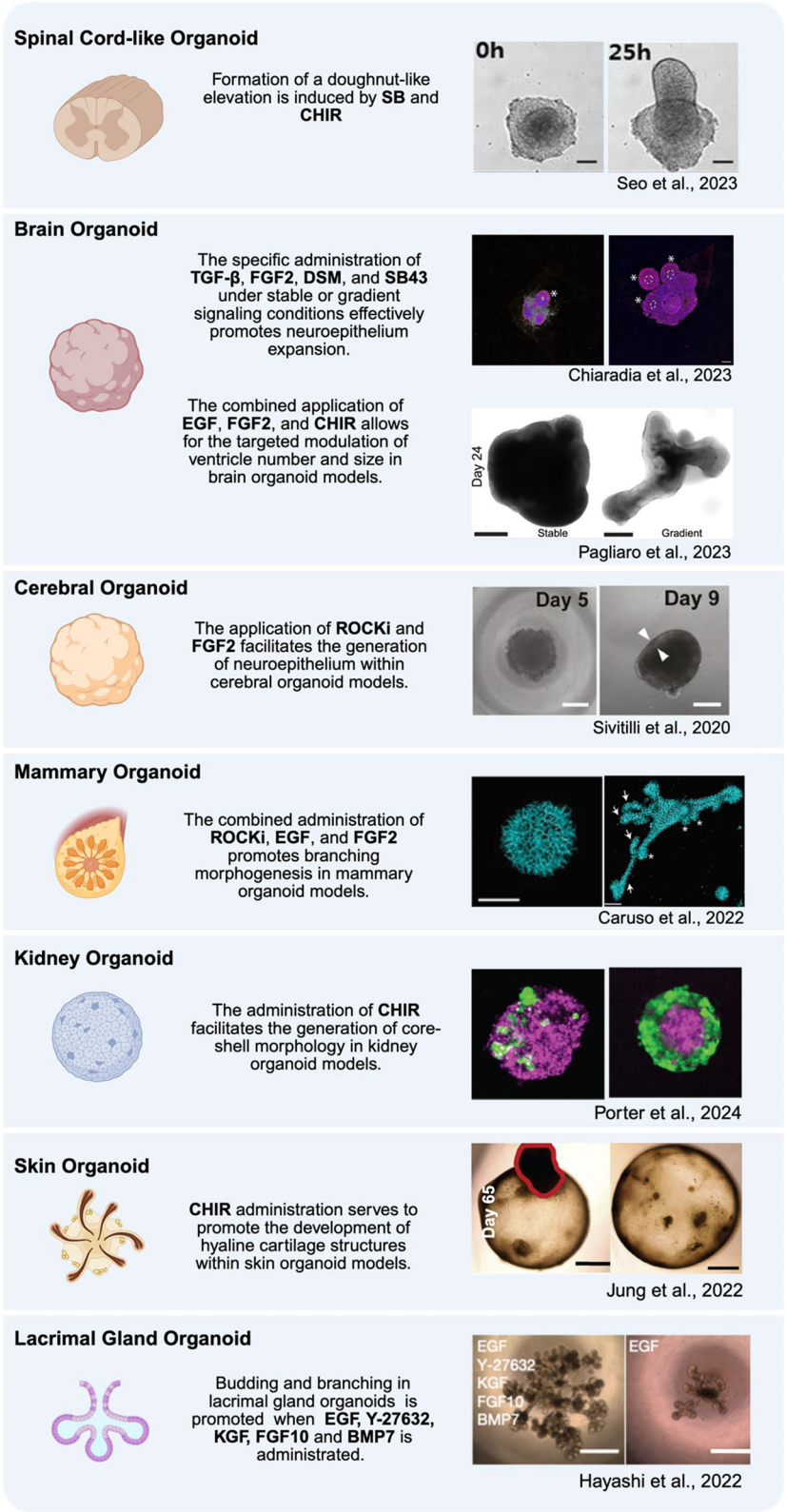


Fig. 3 Small molecules used in organoid morphology modulation and their effects. SB & CHIR treatment performed on Day 3 provides doughnut-like elevation [129]. EGF, FGF, and CHIR affect ventricle numbers and sizes in COs [33]. ROCK inhibitor was used to support the viability and pluripotency of human embryonic stem cells, while FGF2 was used to induce neuroepithelium formation after embryoid body formation [110]. To examine the effect of TGF-β on neuroectoderm induction enhancement, different signal modulations were created, from high to low inhibition, and it was determined that decreasing TGF-β administration over time was important for expanded neuroectoderm [130]. FGF2 induction on days 3–4 of mammary organoid culture causes branching initiation in the organoid. Subsequent addition of FGF2/EGF produces extended primary branches and gives rise to secondary branches [45]. Budding and branching in lacrimal gland organoid is promoted when EGF, Y-27,632, KGF, FGF10 and BMP7 is administrated [19]. Reproduced with permissions. Copyright 2025, Wiley, Nature Group, Cell Press, Life Science Alliance and Frontiers Group

Table 3 Signaling pathways and their morphological modulations

Potential Effect	Core Signaling Axes	Morphogenetic Outcomes	Organoid Models	References
Dose-Dependent Fate Specification	Wnt/β – catenin, TGF − β	Dictates regional identity (caudalization vs. telencephalic); regulates ventricle size and epithelial expansion.	Neural, Gastric, Fallopian tube, Parathyroid, Lung.	[30, 33, 49, 110], 126–[130, 132]
Morphogen Gradient Scaling	SHH (V), BMP (D), Wnt/FGF/RA (A-P)	Competitive signaling loops establish D-V and A-P axes; induction of specialized lineages.	Neural, Renal, Choroid Plexus, Hepatic.	[55, 57, 64, 65, 70, 110, 129, 133]
Conserved Branching Circuitry	FGF2, EGF, Sox9, VEGF/FGF9	Initiation of ductal/glandular architecture; promotes vascularization and progenitor differentiation.	Mammary, Bile duct, Lung, Renal, Pancreatic.	[45, 69, 134, 135, 141]
Mechanochemical Feedback	RhoA/ROCK, YAP/TAZ	Recalibration of morphogen sensitivity based on ECM geometry and stiffness, thereby regulating lumen formation.	Cardiac, Colorectal, Neuroepithelial, Blood vessel.	[102, 103, 118], 140– [142]
Pathological Hijacking (Tumor microenvironment Induction)	TGF-β1, IL-6, CXCL12, SMAD4	Transition from mesenchymal to collective invasion; increases stemness and therapeutic resistance.	Pancreatic (PDAC), Colorectal, Lung tumoroids.	[136–140]

highly context-dependent; without accounting for the reciprocal feedback between biochemical inputs and the physical microenvironment, achieving a truly standardized 'organoidogenesis' protocol remains an elusive goal.

Genetic and biological perturbation

Organoid-based disease models provide invaluable insights into how genetic defects or external biological agents perturb tissue morphology and function (Fig. 4). These platforms allow researchers to recapitulate disease phenotypes in vitro and dissect the underlying mechanisms driving abnormal morphogenesis. Organoids serve as robust platforms for investigating how biological agents and genetic mutations compromise tissue integrity. In skin models of Atopic Dermatitis, *Staphylococcus aureus* infection induces significant epidermal-dermal damage and epithelial barrier disruption—characterized by stem cell depletion and increased inflammatory cytokines—though *Cutibacterium acnes* pretreatment can confer protection [141]. Genetically, *Sox9* silencing impairs branching morphogenesis through elevated TGF-β signaling and transcriptomic immaturity, a morphological defect that is partially reversible via Activin A inhibition [134]. Similarly, while heterozygous *RB1* mutants develop normally, the complete loss of this tumor suppressor induces the disorganized photoreceptor differentiation characteristic of retinoblastoma [143]. Finally, neurodevelopmental models of Pretzel Syndrome demonstrate that mTOR hyperactivation triggers a cascade of structural abnormalities, including abnormal organoid expansion, excessive budding, and the loss of neural progenitor apical-basal polarity [144].

While CRISPR-based and viral-mediated edits provide high mechanistic precision, the inevitable cellular mosaicism and variable genomic penetrance in complex 3D architectures often generate significant phenotypic noise, undermining inter-batch reproducibility. Furthermore, the reliance on clonal selection introduces a sampling bias that masks the natural stochasticity of human development, posing a major bottleneck for the large-scale standardization required in clinical-grade disease modeling.

Pharmaceutical and chemical treatment

Organoids serve as potent platforms in pharmacology and toxicology for assessing the impact of small molecules, environmental toxins, and therapeutics on tissue viability, morphology, and molecular signaling [122, 125, 136, 138, 145–148]. These models facilitate quantitative profiling of drug efficacy and toxicity by monitoring

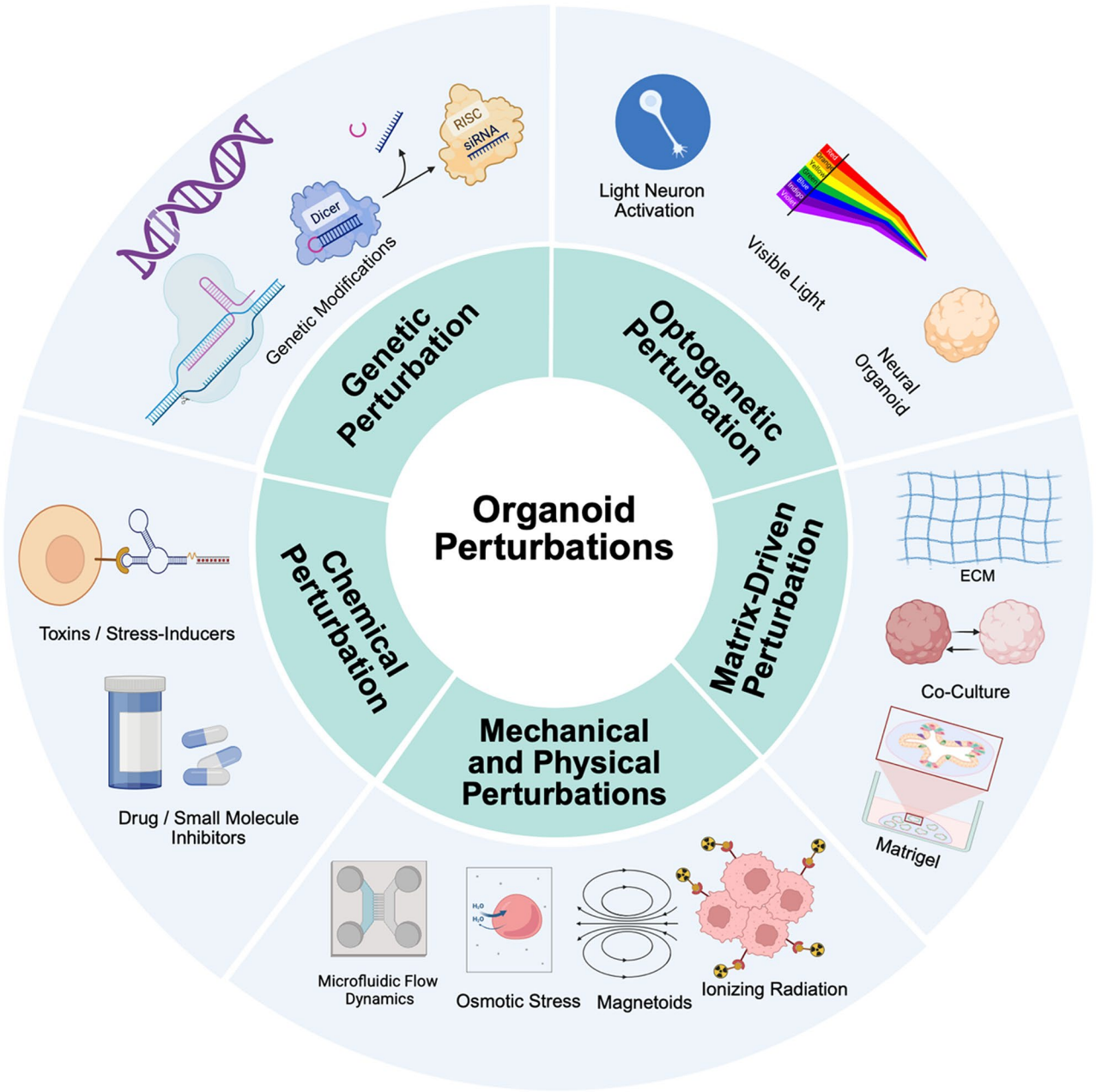


Fig. 4 Schematic representation of organoid perturbation strategies. Various modalities enable precise probing and maturation of organoid models: Genetic (e.g., siRNA, CRISPR/Cas9) for targeted gene modulation; Optogenetic for light-mediated control of signaling; Matrix-Driven (e.g., scaffolds) to refine tissue architecture; Mechanical (e.g., acoustic, X-ray) to influence stress responses; Physical (e.g., microfluidics, shakers) to optimize nutrient/oxygen exchange; and Chemical (e.g., small molecules) to guide differentiation pathways. Together, these strategies highlight the versatility of organoids as dynamic mechanistic and translational platforms

dose-dependent shifts in organoid size, viability, and cellular composition [136, 147]. Nervous system organoids are pivotal for modeling neurodegeneration; specifically, midbrain models treated with neurotoxins (6-OHD, rotenone) recapitulate Parkinson's disease through dose-dependent dopaminergic neuron loss [50, 149]. In forebrain models, stress-induced dendritic defects can be reversed by Trazodone, highlighting their utility in therapeutic screening [54]. Chemical toolkits also deconstruct branching morphogenesis in pancreatic organoids:

Table 4 The morphological-functional dictionary: quantitative descriptors of organoid states

To transition from qualitative observation to predictive modeling, organoid morphology must be decoded into standardized quantitative metrics. These descriptors serve as high-dimensional functional proxies, enabling the objective assessment of maturation, cellular stress, and therapeutic rescue:

Area & Perimeter: Quantitative measurement of the 2D projected surface and boundary length: Growth Kinetics & Biomass Accumulation. These are the primary indicators of proliferative capacity. Rapid changes in area-to-perimeter ratios often precede macroscopic structural shifts, serving as a baseline for tracking expansion rates and volumetric scaling across all organoid lineages.

Circularity & Sphericity: Degree of deviation from a perfect circle ($4\ A/P^2$): Developmental Stability. High circularity indicates uniform radial growth; significant deviations suggest localized mechanical stress or stochastic symmetry-breaking events. This is a "gold-standard" quality control metric for early-stage cerebral organoids and hepatic spheroids.

Branching Index (Sholl Analysis): Quantification of secondary and tertiary protrusions from a central core: Morphogenetic Complexity. A direct proxy for ductal maturation, glandular development, or dendritic arborization in neural models. In lung and mammary organoids, branching fractals correlate with functional capacity, such as surfactant production or secretory efficiency.

Lumen-to-Tissue Ratio (L/T): The volumetric or area ratio of internal fluid-filled spaces to total tissue mass: Epithelial Maturation. Reflects the formation of functional tight junctions and secretory/transport capacity. It is a critical determinant for neuroepithelial folding (ventricle size) and assessing the functional activity of ion channels and transporters.

Surface Roughness (S): Vertical deviation of the surface profile and the fractal dimension of the tissue edge: Invasive Potential and Budding Efficiency. High roughness values correlate with Epithelial-Mesenchymal Transition (EMT) or high-frequency budding in intestinal crypts. In oncological models, it quantifies the transition from a dormant to an invasive tumoroid phenotype.

Polarity Alignment Score: Vectorial alignment and spatial distribution of markers (e.g., ZO-1, E-cadherin) relative to the lumen: Barrier Integrity. A numerical measure of structural polarization required for directional nutrient transport and pharmacological sensitivity (drug permeability). This score is a prerequisite for high-fidelity kidney, gut, and blood vessel models.

Pixel Density & N/C Ratio: Local intensity variations and the ratio of nuclear area to cytoplasmic area: Metabolic Health & Viability. High pixel heterogeneity or a shifted N/C ratio predicts cellular stress, apoptosis, or drug-induced necrotic core formation significantly earlier than traditional biochemical assays (predictive toxicity).

Major/Minor Axis Length (Aspect Ratio): The ratio between the longest and shortest diameters of the organoid: Structural Symmetry & Elongation. This metric distinguishes between isotropic (spherical) growth and anisotropic (polarized/elongated) morphogenesis. It is a critical determinant for monitoring symmetry-breaking events in neural tube, vascular, or gastruloid models

while aphidicolin and MMP inhibitors (batimastat) impair structural complexity, ROCK inhibition (Y-27632) prevents tubulogenesis, whereas forskolin promotes lumenogenesis [112]. Furthermore, chemical perturbations mimic diverse pathologies: Poly(I: C) treatment induces a biliary atresia-like morphological decline in liver models [150], high calcium disrupts the kidney's glomerular-to-tubular ratio [116], and Bisphenol A promotes pro-tumorigenic branching in mammary organoids [151]. Accordingly, pharmacological perturbation studies should pair morphological endpoints with functional assays such as viability, lineage-specific activity, secretion, barrier integrity, electrophysiology, metabolic profiling, and dose-response sensitivity. This orthogonal validation is particularly important for distinguishing true therapeutic rescue or toxic injury from culture-condition-dependent changes in organoid size, shape, or viability.

Although organoids provide high-resolution phenotypic readouts for drug screening, their lack of integrated vasculature and systemic clearance mechanisms often results in an incomplete representation of pharmacokinetic (PK) profiles and chronic toxicity. Moreover, the propensity of basement membrane extracts (e.g., Matrigel) to sequester hydrophobic compounds can lead to skewed IC_{50} values, compromising the reproducibility of pharmacological benchmarks across different scaffolds. Ultimately, relying on static end-point morphological metrics fails to capture the dynamic physio-chemical fluctuations of a living system, highlighting a critical gap between high-throughput 'screening' and authentic clinical 'prediction'.

Engineering morphogenesis with targeted physical cues

Beyond static cues, dynamic physical forces—ranging from mechanical stimulation to microgravity—enable precise steering of organoid architecture. For instance, mechanical perturbations can modulate radioresistance in colorectal cancer models [140], while simulated microgravity in rotating bioreactors induces longitudinal resorption in bone organoids, distinct from the reticulate patterns of static cultures [72]. Retinal organoids demonstrate

frequency-dependent responses to oscillatory stretching, with 0.5 Hz stimulation promoting rounder phenotypes [152].

Furthermore, fabrication techniques are decisive; low-shear volumetric bioprinting preserves liver organoid structural integrity better than high-shear extrusion methods [76]. Finally, micropatterning effectively standardizes initial aggregation, yielding liver organoids with significantly lower variability in size and distribution compared to traditional Matrigel dome cultures [153]. 4D photostimulable materials enable unparalleled spatiotemporal control over guided assembly by responding to light-induced stimuli [131]. These smart platforms facilitate on-demand modulation through photodegradation for niche creation, photoinduced viscoelasticity for real-time epithelial shaping, and photostiffening to direct tissue development via regional rigidity [131].

Targeted physical cues often impose non-physiological mechanical loads that risk deviating from authentic developmental trajectories. High-precision modalities like 4D photostimulation face significant phototoxicity hurdles, while the reliance on specialized hardware creates 'technical silos' that severely limit scalability and inter-laboratory reproducibility.

Image based monitoring organoidogenesis

High-throughput classification

Microscopy enables the real-time, longitudinal tracking of organoidogenesis, yet the shift from subjective human assessment to objective computational analysis is essential for identifying sub-visual phenotypic signatures. A fundamental principle in AI-driven monitoring is the use of high-throughput classification architectures—specifically YOLO-based models to automate artifact exclusion and developmental stage identification. By leveraging architectures such as Deep-Orga (YOLOX) and Orga-Dete (YOLOv11), researchers can objectively quantify size dynamics and fine morphological features in densely packed cultures, such as lung or intestinal organoids [154, 155]. This approach effectively mitigates manual assessment errors and facilitates the categorization of organoids into distinct maturation phases—including cystic, early budding, late-stage, and spherical morphologies—as demonstrated by the Deliod (YOLOv8) framework [156].

Precise pixel-level segmentation

Beyond simple detection, the extraction of high-dimensional metrics requires advanced Convolutional Neural Networks (CNNs) and U-Net architectures to perform precise pixel-level segmentation. A core governing rule for evaluating culture stability is the calculation of geometric parameters such as area, perimeter, and axis length, (BOX 1) facilitated by tools like OrganoID and OrgaExtractor [157, 158]. To address the challenges of high-density cultures, architectures leveraging StarDist (e.g., OrganoIDNet) are employed to classify health status via pixel density and size-based treatment groups (Fig. 5) [159].

Specifically targeting the inherent complexity of neural lineages, the BrAIn application utilizes deep learning to automate the morphological analysis of brain organoids, providing a specialized framework for quantifying structural health and developmental progression [52]. Similarly, MOrgAna integrates machine learning models, such as Multi-Layer Perceptron, with the Watershed algorithm to quantify large, heterogeneous brain organoids and gastruloids (Fig. 5) [160]. These specialized tools address the high stochasticity of neural maturation, bridging the gap between raw pixel data and biologically relevant neuroanatomical readouts.

Advanced architectures for complex morphologies and multimodal integration

Recent advancements focus on solving the segmentation of overlapping and nonhomogeneous structures, a common bottleneck in tumoroid analysis. Principles such as the Convexity-Preserving Level-Set Method or Mask

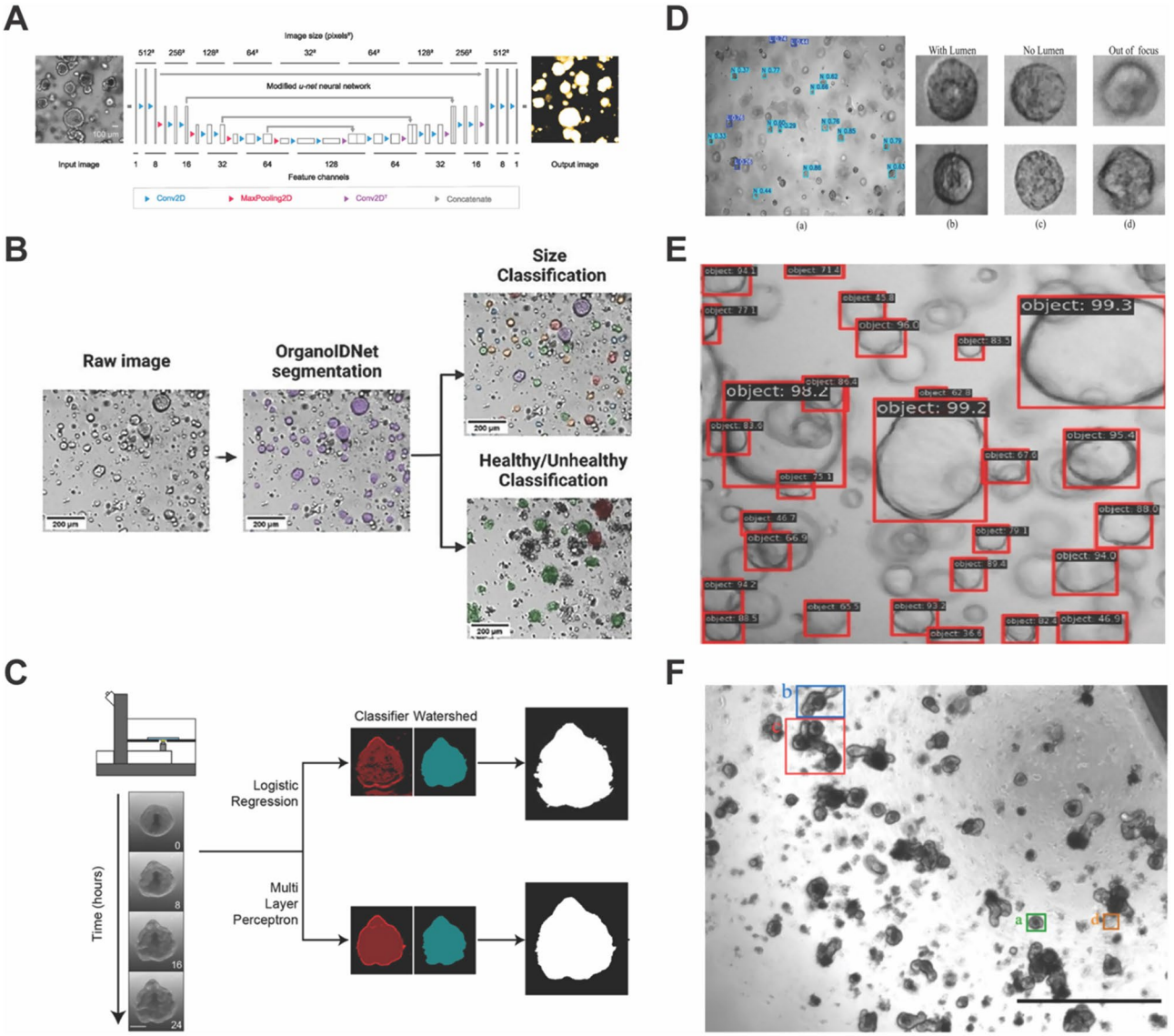


Fig. 5 AI platforms for high-throughput organoid morphology analysis. **(A)** OrganoID facilitates automated identification and longitudinal tracking of organoid count, size, and dynamic morphology. **(B)** OrganoIDNet pipeline integrates segmentation with parallel classification of size and health status (via pixel density). **(C)** MOrgAna leverages a hybrid ML-Watershed approach for robust analysis of large, evolving organoid structures. **(D)** Orga-Dete identifies specific differentiation markers, such as lumen presence in lung organoids. **(E)** Deep-Orga utilizes YOLO-based object detection for rapid counting and size quantification in drug screening applications. **(F)** Deliod enables automated subtype classification within heterogeneous populations, identifying budding (**a**), cystic (**b**), and spherical (**c**) morphologies. Reproduced with permissions. Copyright 2025, Nature Group, Springer Science, The Company of Biologists, MDPI and Elsevier

R-CNN (OrgaSegment) ensure geometric fidelity by maintaining convexity during segmentation, allowing for the separation of irregularly shaped or clustered organoids directly from brightfield images [161, 162]. Furthermore, the integration of deep learning with multimodal imaging, such as Optical Coherence Tomography (OCT), enables the 3D functional characterization of hollow architectures in retinal and cardiac lineages [163]. Finally, foundation models like the Segment Anything Model (SAM) allow for zero-shot classification of diverse growth patterns (e.g., dense vs. grape-like morphologies), offering a universal tool for morphological fingerprinting [164]. The true potential of AI-driven morphological monitoring lies in its convergence with microphysiological systems, creating a synergistic 'game-changer' for the drug discovery pipeline. This integration allows for the

real-time, autonomous adjustment of fluidic and biochemical parameters based on AI-derived phenotypic feedback, effectively streamlining the transition from lead identification to clinical validation [165].

Methodological bottlenecks: from the standardization paradox to biological validation

Despite the rapid proliferation of AI architectures, the field faces a significant standardization paradox: while AI models provide unparalleled speed and throughput, their clinical and industrial utility is frequently hampered by dataset bias and a lack of cross-platform generalizability. A model trained on a specific high-numerical aperture imaging setup often fails to maintain accuracy when applied to different hardware configurations, creating hardware-software silos that prevent the universal adoption of standardized metrics. This lack of robustness is compounded by the human-labeler biasinherent in training sets, where models inherit the subjective "ground truths" of individual researchers, often failing to account for the natural stochasticity of organoid development.

Furthermore, the 2D-projection fallacy remains a critical technical barrier in 3D modeling. Most current YOLO-based or CNN systems operate on maximum intensity projections (MIP), which inherently ignore the loss of signal at depth caused by light scattering in complex assembloids. This leads to a systematic underestimation of internal volumetric fidelity and lumen dynamics. Beyond these physical constraints, the "black-box" nature of deep learning complicates biological interpretation; statistical correlations in pixel density or surface texture do not always mirror specific molecular pathways or transcriptomic states.

For AI-derived morphology scores to become biologically interpretable, they must be benchmarked against independent functional ground truths rather than treated as stand-alone labels. Such ground truths may include electrophysiological recordings, TEER and permeability measurements, secretory outputs, transporter activity, metabolic flux, single-cell transcriptomics, or validated drug-response phenotypes. This validation step is especially important when perturbations alter shape indirectly through stress, necrosis, matrix remodeling, or imaging artifacts rather than through authentic maturation. Ultimately, achieving clinical-grade reliability requires a paradigm shift toward Explainable AI (XAI). In this framework, morphological shifts are not merely calculated as abstract scores but are directly mapped to transcriptomic or functional ground truths (e.g., scRNA-seq or metabolic flux). By establishing this "Morpho-Molecular" alignment, researchers can ensure that AI-derived metrics represent authentic biological states rather than computational artifacts, transforming AI from a descriptive tool into a predictive methodology for high-stakes drug discovery [158, 162, 165, 166].

Towards a unified standard: the future of organoid data harmonization

The lack of interoperability between disparate AI architectures and multi-omics pipelines remains the "Achilles' heel" of the field. To overcome this, we propose a shift towards Standardized Benchmarking Ecosystems, where AI models are validated against open-source, multi-center "Gold Standard" datasets—analogous to the ImageNet paradigm in computer vision. Furthermore, the integration of BioCompute Objects and Organoid Ontologies is essential to harmonize metadata across laboratories, ensuring that a "morphological health score" or "transcriptomic maturation index" generated in one facility is semantically and statistically equivalent in another. Beyond hardware standardization, the adoption of Federated Learning could allow for the training of robust AI models on decentralized datasets, bypassing data privacy issues and technical silos while maintaining high cross-platform reproducibility. Ultimately, bridging the gap between raw data and clinical translation requires a unified "Organoid-Data Roadmap" that prioritizes the FAIR principles, transforming organoid engineering from a fragmented artisanal craft into a standardized, high-throughput diagnostic methodology.

Conclusion & future direction

The future of the organoid field will focus on improving the physiological relevance and reproducibility of existing models. Instead of undefined matrices like Matrigel, which vary from batch to batch, the development of synthetic and bioactive hydrogel systems with precisely tunable biochemical and mechanical properties is critical for standardization. Multi-organ-on-chip systems, where different organoid types are combined on microfluidic platforms, will open new avenues for modeling systemic diseases and studying interorgan interactions. The use of dynamic materials that can change shape or function over time, such as 4D biofabrication, will enable more realistic simulations of tissue morphogenesis. Sensor technologies and advanced imaging methods integrated into culture systems to monitor organoid development non-invasively and in real time will become widespread. Artificial intelligence systems that automate morphological analysis are expected to evolve into closed-loop systems that actively guide organoid development by instantly adjusting culture conditions. Future perturbation studies should therefore report morphology together with lineage-appropriate functional endpoints to strengthen reproducibility and translational interpretation. All these advances will enable the creation of more complex and functional tissue analogs that model human development and diseases with unprecedented precision.

Review criteria

This narrative review was based on a targeted literature search rather than a formal systematic review. Articles were identified primarily through the Science Citation Index Expanded (SCIE) within the Web of Science Core Collection using combinations of the following keywords: organoids, morphology, morphogens, morphogenesis, perturbation, mechanotransduction, static culture, shaker-based culture, dynamic culture, microfluidic culture, and organ-on-chip. The main search window covered January 2020 to April 2026 and was restricted to English-language original research articles involving human cells or human-relevant organoid platforms.

Studies were prioritized when they directly examined physical, biochemical, genetic, pharmacological, or computational perturbations that altered organoid morphology, morphogenesis, maturation, reproducibility, or morpho-functional interpretation. Foundational studies published before 2020 were also included when needed to introduce key concepts, historical milestones, or widely used organoid platforms.

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Author contributions B.K and S.G. conceptualized the idea and drafted the manuscript. B.K., A.E.E., I.K., and C.B.O. conducted the literature searches and reviews. The manuscript was reviewed and approved by S.G. All authors contributed to writing and editing the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Artificial intelligence (AI) Use During manuscript preparation, Gemini was used only to improve text flow and presentation. All scientific content, interpretations, and references were reviewed and verified by the authors, who take full responsibility for the final manuscript.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Burak Kahveci[1,2] · Ali Eren Evranos[1,2] · İlayda Koçhan[1,2] · Cem Batu Ören[2] · Sinan Güven[1,2,3]

✉ Sinan Güven
sinan.guven@deu.edu.tr

[1] İzmir International Biomedicine and Genome Institute, Dokuz Eylül University, İzmir 35340, Türkiye

[2] İzmir Biomedicine and Genome Center, İzmir 35340, Türkiye

[3] Department of Medical Biology and Genetics, Faculty of Medicine, Dokuz Eylül University, İzmir 35340, Türkiye