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Synthetic Morphogen Gradient Engineering in Human Organoids: Toward Programmable Tissue Patterning for Next-Generation Regenerative Medicine

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ABSTRACT

Despite impressive self-organisation, conventional human organoids (cell aggregates) fail to reproduce intricate, reproducible, and scalable in vivo-like structures and organ formations because they rely on uniform (constant) pro-differentiation morphogen gradients. To address this developmental impasse, the field has evolved toward synthetic gradient engineering with morphogen signals, which combines developmental signalling cues with cutting-edge synthetic biology and bioengineering platforms. Here, we present a comprehensive overview of cutting-edge advances in four main areas: novel gradient engineering systems (ranging from microfluidic chips to sustained-release hydrogels, optogenetics, and DNA-barcoded microbeads); the types of organoids that respond to these spatial cues; the engineered "Morphogenic Programming" circuits that allow cells to automatically interpret morphogen thresholds and record positional history; and the profound translational implications of these advances. The growing body of evidence across these platforms suggests an interesting convergence: tissue patterning is largely achieved through hybrid approaches that combine external spatial cues with intrinsic self-organisation. Synthetic gradient engineering provides the means to transform cell aggregates into customised structures, enabling spatially resolved drug screening, reliable modelling of monogenic diseases, and novel therapeutic strategies for tissue regeneration.

Keywords: Morphogen gradients, Organoids, Synthetic biology, Gene circuits, Tissue engineering, Regenerative medicine.

1. Introduction

1. 1. A Developmental Paradox and a Technological Gap

A long-standing paradox in developmental biology is how a seemingly uniform blastula yields hundreds of distinct cell types arranged into reproducible anatomical structures, largely guided by noisy, diffusing morphogen signals. Turing's reaction-diffusion theory proposed that patterns could arise de novo from instabilities between short-range activators and long-range inhibitors, whereas Wolpert's positional information framework emphasised graded morphogen concentrations that cells interpret as coordinates (Turing, 1952; Wolpert, 1969). Contemporary studies of neural tube, limb, and gut development have confirmed the positional information framework originally proposed by Wolpert (1969). We now know that this process is driven by specific families of morphogens, especially Wnt, Bone Morphogenetic Protein (BMP), Sonic hedgehog (Shh), Fibroblast Growth Factor (FGF), and retinoic acid (RA), which form spatial and temporal gradients to assign positional identity (Green & Sharpe, 2015; O'Grady et al., 2019).

Organoid technology offers three-dimensional human stem cell models that recreate developmental processes. These systems can develop structures resembling the brain, intestines, kidneys, lungs, liver, and heart. They often grow from unpatterned stem cell aggregates exposed to simple, time-based sequences of morphogen addition and removal, mirroring natural embryo formation. However, while their ability to self-organise is impressive, standard organoids usually fail to develop the precise, repeatable patterns and large-scale architecture found in the human body. For example, cortical layers may be scattered, gut structures may be incomplete, and heart chambers may be inconsistent across different batches. To address this, researchers have designed environments that provide multiple signalling gradients, mechanical cues, and long-range interactions, replacing the uniform liquid media traditionally used in standard cultures.

1. 2. The Problem of Controlled Tissue Patterning in Organoids

It is becoming apparent that human organoids can produce complex cell fate diversity in response to modified regimens of morphogens or their antagonists. However, the proportion and configuration of cell fates vary between cell lines and even between aliquots of the same cell line (Anand et al., 2023; Sanchís-Calleja et al., 2026; Scuderi et al., 2025). For instance, single-cell RNA-seq-based morphogen screening in neural organoids has shown that gradient exposure to Shh, Wnt, FGF8, RA, or BMP modulators dramatically alters the proportions of dorsal-ventral and anterior-posterior neurones but also shows a significant inter-line variability in morphogen responses and patterning (Sanchís-Calleja et al., 2026). Likewise, microfluidic tools that deliver opposing gradients of Wnt agonists and antagonists, or gradient exposure to orthogonal Wnt and Shh agonists, to endodermal or neural spheroids can achieve spectacular anterior-posterior and dorsal-ventral patterning of endodermal or neuronal tissues. However, patterning outcomes vary between genetic models and even within cultures (O'Grady et al., 2019; Scuderi et al., 2025; Yaman & Ramanathan, 2023). These findings suggest two related issues. First, external morphogen presentation must be much better controlled in space and time than simple bath application (especially when multiple pathways need to be altered), which can be achieved by simple bath application (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Repina et al., 2023). Second, the gene regulatory networks that respond to these gradients are not static; without engineered mechanisms to bring the proposed gradients to bear on network thresholds, memory, and noise, even clear-cut external gradients may not translate into robust tissue-scale patterns (Dupin et al., 2022; Prochazka et al., 2022; Trentesaux et al., 2023). Therefore, a major challenge remains in turning organoids, which only roughly self-organise under gross morphogen manipulations, into engineered platforms for programmable tissue-scale organisation at reproducible scales across donors and batches.

1. 3. Modern Gradient Biology: From Reaction Diffusion to Positional Information

Contemporary gradient biology merges the ideas of Turing and Wolpert to provide a more sophisticated view in which multiple contact-dependent and diffusible information sources cooperate to expand and deform tissues (**Figure 2**) (Turing, 1952; Wolpert, 1969). Reaction-diffusion can produce primary axes or spotted and striped patterns, whereas positional information often sharpens such founder asymmetries into sharp boundaries through threshold gene expression and cross-repressive transcription factor wiring (Green & Sharpe, 2015; Wolpert, 1969). In experiments on the vertebrate neural tube, for instance, the neural tube interprets opposing gradients of Shh (floor plate) and BMP/Wnt (roof plate) via cross-regulatory TFs to produce distinct progenitor regions (Green & Sharpe, 2015; Wolpert, 1969). Similarly, in the gut, Wnt activity is high at the base of the crypts, while BMP/Notch gradients regulate stem cell activity and progenitors along the crypt-villus axis (Xiang et al., 2024; Xu et al., 2025). The interpretation of gradients is more than a mere concentration reading. While historical theories have emphasised the importance of positional information and reaction-diffusion (Turing, 1952; Wolpert, 1969), contemporary experimental systems have highlighted the importance of mechanical restraints (O'Grady et al., 2019) and tissue-scale feedback and growth (Green & Sharpe, 2015) in shaping effective morphogen landscapes.

This shift in the understanding of development has two lessons for organoid engineering. First, the simple mimicry of a single snapshot of the concentration profile of a particular morphogen is unlikely to recapitulate in vivo developmental outcomes; instead, dynamic landscapes with complex morphogen interactions need to be designed, often in conjunction with mechanical and matrix interactions (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Zhang et al., 2026). Second, to achieve embryo-like quality control, programmed gradients may need to be complemented by engineered intracellular networks that implement robust state switching mechanisms, including bistability, oscillations, and epigenetic memory (Barkai & Shilo, 2009; Gierer & Meinhardt, 1972; Prochazka et al., 2022). Why Current Organoid Protocols Fail to Recapitulate Authentic Tissue Architecture

Several converging lines of evidence suggest that standard organoid protocols struggle to reproduce authentic tissue architectures. The uniform application of high concentrations of morphogens or their inhibitors often drives organ-wide adoption of a particular cell fate, abolishing regional diversity and collapsing gradients that should exist at the scale of hundreds of micrometres (Sanchís-Calleja et al., 2026; Xiang et al., 2024; Xu et al., 2025). Even when morphogens are pulsed or globally titrated, differences in diffusion across Matrigel (a 3D cell-culture matrix), local cell density, and endogenous ligand production create uncontrolled microgradients that vary between wells and over time, contributing to stochastic organization (Ahammed & Kalangi, 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). In addition, important structures in vivo, such as signaller zones and organisers, result from spatiotemporal, localised production of morphogens, which are difficult to achieve using bulk medium manipulations (Green & Sharpe, 2015; Marti-Figueroa & Ashton, 2017).

Another limitation is the biophysical environment. Many organoids develop in spherical geometries that lack clear boundaries or axes; therefore, even when external gradients are imposed, they may be misaligned with intrinsic symmetry-breaking events (Yaman & Ramanathan, 2023). Limited vascularisation leads to hypoxia and nutrient gradients that can either confound or override the intended morphogen patterns, particularly in larger organoids (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Finally, the absence of stromal, immune, and vascular compartments removes important sources and sinks of morphogens and antagonists that dynamically sculpt gradients in vivo (Ahammed & Kalangi, 2024; Yao et al., 2024). Collectively, these factors mean that current protocols often succeed in producing the right "parts list" of cell types but fail to achieve the correct spatial arrangement or organ-scale geometry.

1. 4. The Synthetic Biology Opportunity: Engineering Programmable Gradient Systems

As an alternative to modifying the external culture environment, synthetic biology provides powerful tools for genetically programming cells. Scientists can now design cells that respond predictably to specific morphogen thresholds, introduce entirely synthetic signalling pathways that function alongside natural ones without interference, and equip cells with genetic memory to

track and respond to their spatial history (Prochazka et al., 2022; Pulecio et al., 2017; Shi et al., 2025; Trentesaux et al., 2023). Synthetic morphogen systems in microbial and cell-free droplets have demonstrated that relatively simple circuits, such as the mutual inhibition of transcription factors or two-node feedback modules, can extract positional information from gradients and form stable expression domains, even in the presence of noise (Dupin et al., 2022; Lam et al., 2022; Schaerli et al., 2014). Recent work on human pluripotent stem cells has used optogenetic activation of Wnt signalling in spatially restricted subpopulations to drive self-organisation and epithelial mesenchymal transition dependent patterning, illustrating that engineered signalling asymmetries can trigger tissue-scale morphogenesis (Repina et al., 2023).

Concurrently, microfluidic, hydrogel, and nanoengineered DNA bead platforms now allow externally imposed gradients with single-organ and even intra-organoid resolution (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Zhang et al., 2026). Organoid development is a revolutionary process. Beyond leveraging global cues to orchestrate simple self-organisation, organoids are now being viewed as engineerable materials. Both spatiotemporal environments and genetic networks that read them can be tailored. Critically, we observe that tuning the external environment and the internal network together, not in isolation, significantly improves tissue patterning fidelity and reliability (Dupin et al., 2022; Prochazka et al., 2022; Repina et al., 2023; Trentesaux et al., 2023).

1. 5. Aim and Outline of this Review

This review draws on research in the fields of developmental gradient biology, organoid engineering, and synthetic biology to develop a new paradigm for synthetic morphogen gradient engineering in human organoids (**Figure 1**). It describes a conceptual framework and reviews four key areas: (i) gradient engineering platforms, (ii) gradient-patterned organoid systems, (iii) molecular circuits for gradient computation, and (iv) translational considerations in regenerative medicine and drug discovery. It further provides a critical integration of consensus and controversy; identifies less-studied dimensions such as interactions between gradient signaling and the immune system and stroma; and explores research, clinical, and conceptual advances. The focus is on human organoid systems and programmable patterning versus a comprehensive review of all organoid types or animal systems.

To synthesise the current state of synthetic morphogen gradient engineering, a comprehensive literature search was conducted across major electronic databases, including PubMed, Web of Science, Scopus and Google Scholar, with an emphasis on peer-reviewed articles published through late 2025. The search strategy utilized Boolean combinations of core concepts, including "organoid", "morphogen gradient", "synthetic biology", "optogenetics", and "microfluidics". Article selection prioritised foundational theoretical frameworks alongside modern primary research, and reviews focused specifically on programmable patterning in human pluripotent or adult stem cell-derived organoid systems. To maintain a rigorous focus on validated, human-relevant applications, studies investigating invertebrate or plant models were intentionally excluded, along with non-peer-reviewed preprints and conference proceedings. Ultimately, rather than providing an exhaustive catalogue of all synthetic biology research, the retrieved literature was critically appraised and synthesized into four thematic domains: gradient engineering platforms, gradient-patterned organoid systems, molecular circuits for gradient computation, and translational implications for regenerative medicine.

2. Conceptual Framework

2. 1. Core Morphogen-Mediated Signaling in Patterning

During development, core morphogens, such as Wnt/β-catenin, BMP, Shh, FGF, Notch, and RA, are expressed in complex, overlapping concentration gradients, which are translated into patterning and boundary restriction cues by cells to propagate tissue axes and borders. These evolutionarily conserved signaling pathways are gaining momentum as control levers of choice to direct spatial patterning and tissue regionalization in engineered organoids. **Table 1** provides an overview of these key morphogens, their roles in development, and their use in engineered organoid systems.

2. 2. Organoid Self-Organisation: Successes and Randomness

During the last decade, organoids have convincingly shown that human stem cells are endowed with strong self-organising powers. Brain organoids produce primitive regions of cortical layering, ventricular zones, and subtype-specific neurones; gut organoids produce crypt-villus-like structures; kidney organoids produce glomerulus- and tubular-like structures; and liver, lung, and heart organoids recapitulate elements of organ-specific physiology (Ahammed & Kalangi, 2024; Huang et al., 2021; Wang et al., 2025; Xiang et al., 2024; Xu et al., 2025; Yao et al., 2024). These structures arise from initially random aggregates through relatively straightforward protocols of morphogen exposure and removal, a process reminiscent of natural development (Ahammed & Kalangi, 2024; Wang et al., 2025). However, organoid self-organisation is unpredictable. Experimental studies comparing the outcomes of different human-induced pluripotent stem cell (iPSC) lines have shown considerable variability in sensitivity to morphogens and the resulting tissue composition, even when using the same protocol (Anand et al., 2023; Sanchís-Calleja et al., 2026; Scuderi et al., 2025).

The timing and location at which these cell aggregates initiate the formation of structure, such as polarisation, lumen formation, or bud production, occur in a stochastic manner. As a result, organoid structures assume variable shapes and irregular internal structures (**Figure 3**) (Anand et al., 2023; Yaman & Ramanathan, 2023). Furthermore, the inherent production of Wnt, BMP, and other ligands by differentiating cells provides dynamic sources of poorly understood gradients that vary among organoids (Ahammed & Kalangi, 2024; Marti-Figueroa & Ashton, 2017; Sanchís-Calleja et al., 2026). These are crucial for studying the properties of developmental robustness but are less desirable for organoids used in clinical applications.

2. 3. Principles of Synthetic Biology Design for Gradient Engineering

Synthetic biology enables a design-driven engineering approach to organoid systems based on the concepts of modularity and orthogonality. First, it is possible to create new independent ('orthogonal') signaling networks based on engineered receptor-ligand pairs or novel transcriptional regulatory systems. This modular approach enables researchers to establish synthetic gradients of morphogens. Surrogate signals, such as secreted fluorescent proteins or synthetic peptides, are used because they do not cross-talk with native signaling pathways (Prochazka et al., 2022; Trentesaux et al., 2023). Second, nonlinear input-output: bistable switches, ultrasensitive promoters, and mutual inhibition can sharpen external, smooth gradients, sharpening domain transitions to improve sharpness and boundary detection (Dupin et al., 2022; Lam et al., 2022; Schaerli et al., 2014). Third, feedback and feedforward: positive feedback can help commit cell fates once a threshold is passed, whereas negative feedback and incoherent feedforward can act as noise minimization or filtering to modulate dynamic responses (Dupin et al., 2022; Lam et al., 2022). Fourth, memory and recording: CRISPR-based epigenetic editing and recording systems can maintain a memory of past morphogen exposure, allowing cells to respond to a time-averaged history (Pulecio et al., 2017; Shi et al., 2025).

For organoids, these ideas point to a design strategy in which control over external gradients (engineered via microfluidics, hydrogels, and optogenetics) is paired with control over the transfer function inside cells, which together dictate tissue patterns. Remarkably, we found that genetic circuits designed in microbes or two-dimensional (2D) cultures often preserve their qualitative behaviour in three-dimensional (3D) organoid contexts but need to be "retuned" in terms of thresholds, response times, and integration with endogenous processes (Lam et al., 2022; Prochazka et al., 2022; Trentesaux et al., 2023).

2. 4. Gradient Systems in Organoids

For theoretical purposes, it is helpful to conceptualise three types of gradient systems in organoid patterning (**Figure 2**). Endogenous gradients self-organise via intrinsic morphogen secretion, diffusion, and decay, such as the intrinsic Wnt and BMP gradients in growing intestinal organoids or polarity-driven axial elongation in human trunk-like organoids (Anand et al., 2023; Xiang et al., 2024; Xu et al., 2025). Externally imposed gradients are provided by engineered devices and materials, such as stable linear gradient devices using simple diffusion, hydrogels with microfluidic channels that deliver opposing morphogens to the organoid, or DNA-containing microbeads that are microinjected into the organoid (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). Hybrid systems exploit engineered gradients to initiate or bias intrinsic self-organization,

which then develops and stabilizes through feedback processes. For example, optogenetic induction of Wnt in a subpopulation of cells, which in turn secretes more morphogens for nearby wild-type cells, can be used (Repina et al., 2023).

In reality, the most promising organoid applications are of this hybrid nature, in which engineered gradients provide initial biases or induce symmetry breaking, allowing intrinsic patterning processes to complete the pattern development. The trade-off between control and beneficial self-organization has been the subject of many contemporary debates and can also be found in the following sections on limitations and prospects.

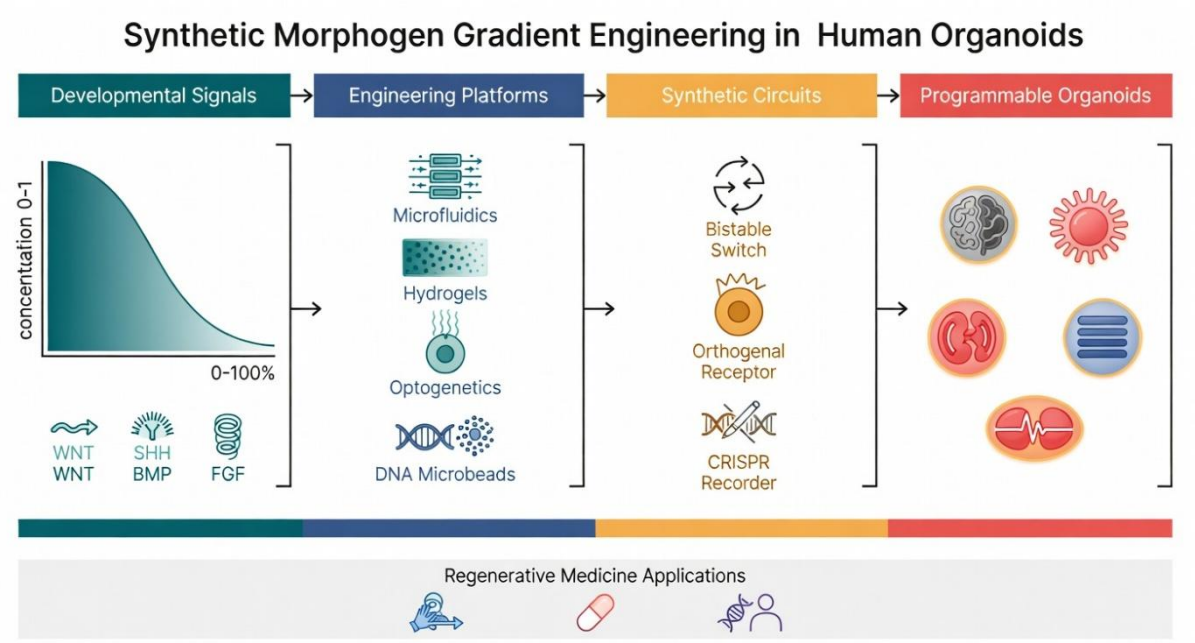

Figure 1. Synthetic engineering framework for human organoids. The spatiotemporal control of morphogen gradients using physical platforms and synthetic circuits enables the generation of programmable organoids with targeted applications in regenerative medicine.

Table 1. Key Morphogen Signalling Pathways and Their Roles in Tissue Patterning Relevant to Organoid Engineering.

Pathway	Representative ligands/molecules	Canonical downstream effects	Developmental/tissue context	Relevance to organoid patterning	Key references
Wnt/β-catenin	Wnt3a, Wnt7a, CHIR99021 (Glycogen Synthase Kinase 3-GSK3 inhibitor)	It stabilizes β-catenin, activates T-cell factor/lymphoid enhancer factor (TCF/LEF) target genes, and promotes proliferation and posterior/ventral fates.	Neural tube anterior-posterior/dorsal-ventral (AP/DV) patterning, intestinal crypt stem cell niche, and early cardiac and kidney specification.	Controls cortical arealization in brain organoids and the crypt-villus axis in intestinal organoids; used in many protocols as a stemness and posteriorizing factor.	(Green & Sharpe, 2015; Repina et al., 2023)
BMP/Transforming Growth Factor Beta (TGFβ)	BMP2/4/7, TGFβ1, Activin A, Noggin (inhibitor).	It phosphorylates Small Mothers Against Decapentaplegic (SMAD)1/5/8 (BMP) or SMAD2/3 (TGFβ) and regulates germ layer specification and DV patterning.	Gastrulation and germ layer allocation, dorsal-ventral neural and mesodermal patterning, and intestinal differentiation gradients.	It is widely used to direct ectoderm vs. mesendoderm fates and to modulate villus vs. crypt identity; spatial BMP gradients affect intestinal and lung organoid architecture.	(O'Grady et al., 2019; Xiang et al., 2024)
Sonic hedgehog (Shh)	Shh ligand, Smoothened Agonist (SAG) (agonist), cyclopamine (antagonist).	It Activates Glioma-Associated Oncogene-GLI transcription factors, ventralizes neural tissues, and patterns limb buds.	Ventral neural tube, forebrain, spinal cord, and patterning of motor neuron and interneuron domains.	Local Shh sources or gradients in forebrain organoids specify ventral fates; dual Shh–Wnt gradients generate complex brain regionalization.	(Scuderi et al., 2025; Yao et al., 2024)
FGF	FGF2, FGF4, FGF8, and FGFR agonists were used.	Activates Rat Sarcoma – Mitogen-Activated Protein Kinase (RAS–MAPK) and Phosphoinositide 3-Kinase – Protein Kinase B(PI3K–AKT), promotes proliferation, survival, and patterning at organizer boundaries.	Midbrain–hindbrain organizer; limb outgrowth; kidney and lung branching morphogenesis.	Supports the expansion of progenitors in brain, kidney, and lung organoids; FGF8 gradients help specify neural regional identities when combined with Wnt and RA.	(Green & Sharpe, 2015; Yaman & Ramanathan, 2023)
Notch	Delta-like Canonical Notch Ligand (DLL)1/4, Jagged ligands, and γ-secretase modulator.	Lateral inhibition maintains progenitors and sharpens boundaries by repressing neighbouring cell fates.	Somite segmentation; intestinal crypt stem cell vs secretory fate decisions; vascular sprouting.	Modulates differentiation patterns in intestinal, liver, and vascular organoids; it often acts downstream of primary morphogen gradients to refine architectures.	(Xiang et al., 2024; Xu et al., 2025)

Retinoic acid (RA)	RA, retinaldehyde dehydrogenase (RALDH) enzymes.	Nuclear receptor signalling specifying posterior neural and endodermal identities.	Anterior–posterior patterning of spinal cord, hindbrain, and gut; limb proximodistal patterning.	RA gradients in microfluidic or hydrogel systems drive spinal motor neuron differentiation and axial patterning in neural and endodermal organoids.	(Anand et al., 2023; O'Grady et al., 2019)
EGF family	EGF, heregulin, EGF-like ligands.	Epidermal Growth Factor / Receptor (EGFR)–RAS–MAPK activation promotes proliferation and survival of epithelial lineages.	Intestinal and gastric epithelium; skin and other barrier tissues.	Maintains stem/progenitor compartments in intestinal and gastric organoids; contributes to crypt domain expansion and villus patterning.	(Wang et al., 2025; Xiang et al., 2024)
VEGF	VEGF-A, VEGF-C, VEGF-D.	Drives endothelial proliferation, migration, and tube formation via Vascular Endothelial Growth Factor / Receptor (VEGFR2/3).	Vascular network formation in many organs; tip/stalk cell patterning.	Used in vascularized liver and cardiac organoids, VEGF gradients are potential tools for engineering perfusable vascular domains within organoids.	(Yao et al., 2024; Zhang et al., 2026)

Note. Data synthesised from Green & Sharpe (2015), Xiang et al. (2024), Yao et al. (2024), and O'Grady et al. (2019). AP/DV: Anterior-Posterior / Dorsal-Ventral; BMP: Bone Morphogenetic Protein; CHIR99021: Glycogen Synthase Kinase 3 (GSK3) inhibitor; FGF: Fibroblast Growth Factor; RA: Retinoic Acid; SAG: Smoothened Agonist; Shh: Sonic hedgehog; TGFβ: Transforming Growth Factor Beta, VEGF: Vascular Endothelial Growth Facto.

3. Engineering Synthetic Morphogen Gradients, Platforms, and Technologies

3. 1. Microfluidic Gradient Generators

Microfluidic devices remain the most mature platform for engineering defined morphogen gradients in stem cell cultures. While foundational studies in the early 2000s relied on active-flow branched channels for 2D cultures, modern systems utilise passive dual-reservoir diffusion devices to generate stable, pumpless linear gradients across trapped 3D organoids (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). Mathematical models confirm that these devices successfully establish reproducible gradients of key morphogens (e.g., Wnt, Shh, and BMPs) at spatial scales appropriate for human development (Yaman & Ramanathan, 2023). These gradient dynamics are fundamentally governed by the reaction-diffusion equation 1:

$$\frac{\partial C}{\partial t} = D\nabla^2 C - R(C) \quad (1)$$

Here, C represents the morphogen concentration, D is the diffusion coefficient, and $R(C)$ accounts for the reaction or cellular consumption rate. A comprehensive comparison of these microfluidic gradient configurations and their capabilities is detailed in **Table 2**.

Applied to human neural and endodermal systems, microfluidic gradients have generated countervailing Wnt/inhibitor fields that pattern anterior–posterior and dorsal–ventral axes within organoids and spheroids (Sanchís-Calleja et al., 2026; Scuderi et al., 2025; Yaman & Ramanathan, 2023). Orthogonal-gradient platforms, such as the Dual Microgradient Array Patterning System (Duo-MAPS), expose iPSC aggregates to intersecting gradients of Wnt and Shh agonists, producing organoids containing forebrain, midbrain, and hindbrain-like regions whose transcriptomes map onto foetal brain atlases (Scuderi et al., 2025). Passive diffusion-based gradient generators have also been used to apply combined Wnt, BMP, and RA gradients that drive forebrain-hindbrain and dorsal-ventral patterning in Matrigel-free neural organoids, highlighting the feasibility of scalable, matrix-independent devices (Yaman & Ramanathan, 2023).

Microfluidic systems are powerful in precision and flexibility but have limitations. Chip fabrication and use, while improving, remain technically challenging, and scaling up the number of organoids while ensuring reproducible and consistent flow and gradient profiles between devices remains difficult (Huang et al., 2021; Marti-Figueroa & Ashton, 2017). Further, organoid culture in microfluidic chips can be affected by shear stress and challenging for downstream imaging or analysis, although organoid-on-chip systems increasingly include gentle perfusion and modular retrieval capabilities (Ahammed & Kalangi, 2024; Huang et al., 2021; Wang et al., 2025). Critically, what we learn from microfluidics literature is that exogenous gradients can lead to robust biases in organoid patterning, but how to balance them with intrinsic signalling and morphogen production remains challenging and needs to be studied for each system (O'Grady et al., 2019; Sanchís-Calleja et al., 2026; Scuderi et al., 2025).

3. 2. Slow-Release and Channel Gradients in Hydrogels

Hydrogels offer an alternative approach in which gradients are not imposed upon the gel but rather encoded within by sources and sinks. A notable variant employs channels lithographically inscribed within a thick hydrogel containing stem cells; fluid channels are perfused with different solutions containing morphogens to generate steady gradients by diffusion through the matrix (O'Grady et al., 2019). O'Grady and colleagues showed fluidic-channel hydrogels can establish opposing gradients of RA and Transforming Growth Factor Beta (TGFβ) /BMP to mesenchymal stem cells, resulting in spatially varying differentiation from osteoblasts to chondrocytes in a single patch (O'Grady et al., 2019). Simulations of diffusion constants and population-level consumption rates allow the design of gradient profiles and time windows of exposure; these are tested via reporter cell lines (O'Grady et al., 2019).

More generally, gels containing microparticles or microspheres are able to slowly release morphogens such as BMP2, TGFβ1, or synthetically active Wnt proteins, whose release profile can be influenced by particle type and loading method (Marti-Figueroa & Ashton, 2017; Zhang et al., 2026). Co-delivery of two particles with different degradation times enables step- or spatial patterns of combined exposure to two factors, more closely mimicking natural development (Marti-Figueroa & Ashton, 2017). Although many of these examples have involved studies of mesenchymal or skeletal tissue-like structures, the general principles can be applied to epithelial organoids in extracellular matrix (ECM)-like hydrogels. A recent study of organoids embedded in controlled synthetic materials, including dynamic hydrogels with user-defined stiffness and ligand availability, highlighted the role of multi-scale mechanical and biochemical gradients in organogenesis (Zhang et al., 2026).

Hydrogels are appealing to many researchers because they are macroscopically simple and can be used with standard furnaces and microscopes, making them easier to adapt. But it can be difficult to control gradients at the scale of individual organoids when many aggregates are embedded in a shared gel, and diffusion through complex organoid-matrix geometries can result in unexpected distributions of morphogens (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). In addition, temporal control is generally more difficult than in microfluidic systems, for example, to produce sharp on-off pulses unless combined with an active component such as photolabile linkers and release-on-demand.

3. 3. Optogenetic and Chemogenetic Approaches

A powerful way to achieve precise temporal control over signaling proteins is through optogenetics. In this approach, the effective "morphogen" is not the physical concentration of a molecule but rather the activity level of a light-sensitive signalling pathway. For instance, Repina et al. developed optoWnt in human embryonic stem cells (hESCs). This system uses the clustering of a Wnt pathway component, triggered by blue-light illumination, to produce rapid kinetic control and activation of β -catenin-dependent canonical Wnt signalling. By selectively illuminating different cell populations, researchers spatially patterned Wnt activation in hESC co-cultures. This induced epithelial-mesenchymal transitions and mesendoderm differentiation, ultimately resulting in beautifully self-organised epithelial and mesenchymal domains. Similarly, optogenetic activation has been used in cardiac differentiation to control mesoderm induction and patterning.

In theory, illuminating subregions of a 3D organoid could be used to create arbitrary virtual gradients of pathway activity without the need to expose the tissue to a gradient of a soluble ligand. While most existing examples are from 2D or shallow 3D cultures, which are restricted by the ~100–300 μm penetration limit of blue light, the adoption of redshifted optogenetic tools takes advantage of the biological optical window. By utilizing red and far-red wavelengths, these tools enable significantly deeper tissue penetration (up to ~1 mm depending on tissue opacity), facilitating intricate spatial and temporal patterning even within larger, maturing organoids (Hellwarth et al., 2021; Repina et al., 2023). Chemogenetic control formats like synthetic receptors triggered by non-native small molecules similarly allow appealing capabilities, particularly when combined with microfluidic or hydrogel-based control of ligand spatial patterning (Prochazka et al., 2022; Trentesaux et al., 2023).

A major advantage of opto- and chemogenetic systems is that they decouple activation of receptors from native ligand kinetics, enabling clear dissection of pathway roles and interactions. However, such approaches require genetic manipulation of stem cells, complex illumination or drug-delivery systems, and careful consideration of potential off-target effects and toxicity (Hellwarth et al., 2021; Repina et al., 2023).To translate such systems, the integration of optogenetic patterning into good manufacturing practice (GMP)-compatible processes will be challenging, but cell pre-patterning early in the process, followed by device-free maturation, remains a possibility.

3. 4. DNA-Based Programmable Morphogen Scaffolds

The emerging "nano-engineered" DNA-based hydrogels and microbeads represent a new type of gradient engineering platform in which the morphogen is linked to a DNA network that can be precisely positioned and activated (Afting et al., 2024). Afting et al. delivered microbeads made of DNA with variable stiffness and surface chemistry into medaka retinal organoids via microinjection to provide localised, light-cleavable sources of a mimic for the Wnt morphogen (Afting et al., 2024). The attachment of the morphogen to the DNA through photocleavable linkers resulted in spatiotemporal release of the morphogen from within the retina, generating inside-out gradients more akin to some in vivo situations where the signalling centre is in the interior of the tissue (Afting et al., 2024). These gradients led to the preferential formation of pigmented epithelium (PigE) at the expense of neuroretinal ganglion cells (NG) in the organoid, thereby enhancing its realism (Afting et al., 2024).

Theoretically, DNA scaffolds could serve as 'smart' scaffolds. By incorporating molecular logic gates, these scaffolds will be able to respond to tissue-specific cues and selectively deliver morphogens, providing dynamic feedback to the state of the tissue (Lam et al., 2022; Schaerli et al., 2014). While such integrated systems remain (mostly) theoretical, the field of DNA nanotechnology has reached a stage where it is possible to program such "smart" scaffolds today. Lastly, translating these technologies into human clinical use may be accompanied by concerns about immunogenicity and degradation; however, pathways may be cleared by existing clinical use of DNA-based therapeutics.

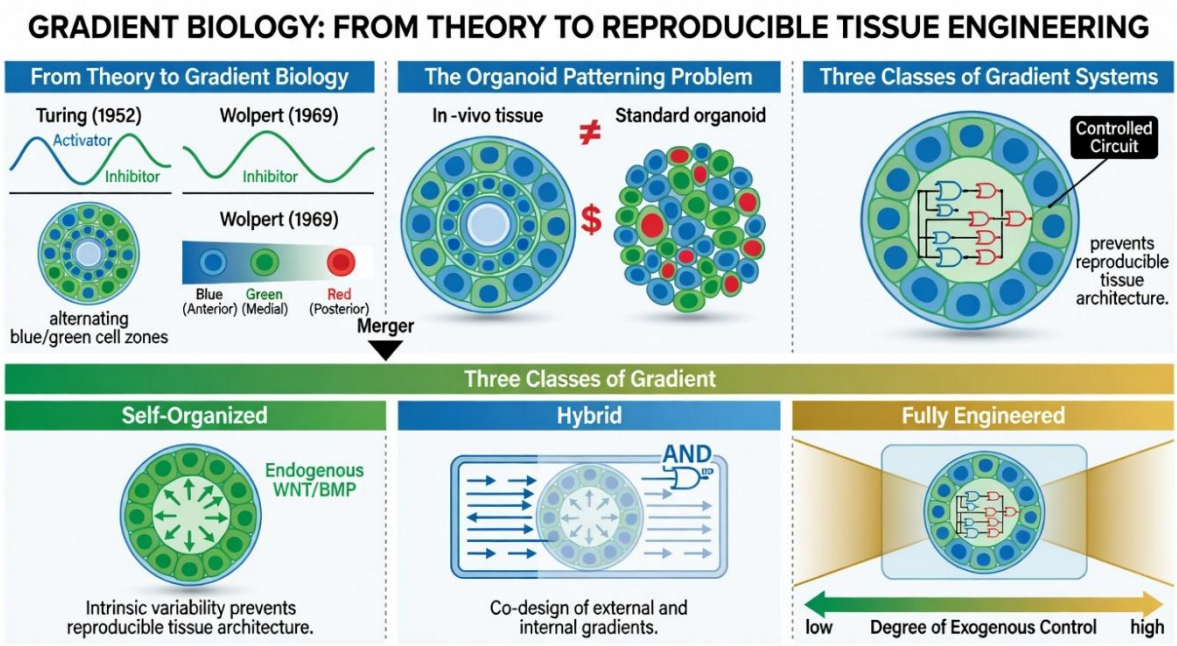

Figure 2. A graphical abstract illustrating the evolution of gradient biology from foundational theories (Turing, 1952; Wolpert, 1969) to modern tissue engineering, categorising approaches into self-organised, hybrid, and fully engineered systems to overcome the organoid patterning problem.

Table 2. Comparison of Synthetic Gradient Engineering Platforms for Organoid Patterning Applications.

Platform type	Mechanism of gradient formation	Spatial resolution	Temporal control	Organoid compatibility	Key limitations	Representative studies
Passive-diffusion microfluidic chips	Morphogens diffuse from side reservoirs into the central organoid channel, establishing steady linear gradients.	Tens to hundreds of micrometres across the channel; shape tunable by geometry and concentrations.	Stable over 24-48 h; gradient can be reconfigured by changing reservoir contents.	Neural and endodermal organoids; embryoid bodies; Matrigel-free systems.	Device fabrication/operation complexity; shear stress; limited throughput if each device holds few organoids.	(Sanchís-Calleja et al., 2026; Yaman & Ramanathan, 2023)
Orthogonal-gradient microfluidics (Duo-MAPS)	Two perpendicular diffusion axes with distinct morphogens forming orthogonal gradients around spheroids.	Approximate quadrant-level patterning with regional domains on a 100-500 µm scale.	Independent control of each gradient over days via reservoir changes.	Brain organoids and neural spheroids are adaptable to other organoids with size constraints.	Alignment of organoids with axes; variable responses across iPSC lines.	(Scuderi et al., 2025; Yao et al., 2024)
Hydrogel-embedded fluidic channels	Channels patterned inside hydrogels perfused with distinct morphogen-containing media generate diffusion-based gradients.	Length scales from hundreds of micrometres to millimetres; multi-gradient configurations are possible.	Good temporal control via perfusion pumps; reversible by switching channel contents.	Mesenchymal constructs, organoids embedded in gels, multi-tissue assemblies.	Complex fabrication; diffusion through tissue yields non-trivial profiles; limited single-organoid targeting.	(Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019)
Microparticle-based slow-release hydrogels	Morphogens loaded into degradable or mineral-coated microparticles embedded in ECM-like gels create long-lived gradients.	Coarse to moderate; depends on particle size and distribution, typically 100–1000 µm.	Long-term, days to weeks; temporal profiles set by degradation and diffusion.	Bone, cartilage, and potentially epithelial organoids with appropriate ECM.	Less precise spatial boundaries; difficult to rapidly modulate or pulse signals.	(Marti-Figueroa & Ashton, 2017; Zhang et al., 2026)
	Light-controlled clustering or	Potentially single-cell	Millisecond-to-hour timescales;	hESC-derived monolayers	Requires genetic modification and optical hardware; deep-tissue	

Optogenetic activation (e.g., optoWnt)	conformational change activates the signalling pathway in illuminated cells.	resolution in 2D. In 3D organoids, depth depends on the wavelength: standard blue light is limited to ~100–300 µm, while redshifted/far-red light can penetrate up to ~1 mm	arbitrary patterns and duty cycles are programmable.	and embryoid bodies; early-stage organoids; thin or translucent constructs.	patterning in large organoids requires redshifted tools to overcome penetration limits.	(Hellwarth et al., 2021; Repina et al., 2023)
Chemogenetic synthetic receptors	Engineered receptors respond to synthetic ligands absent from the endogenous milieu.	Defined by ligand distribution method (microfluidic, hydrogel, or diffusion); in principle, high.	Tunable through ligand dosing schedules.	Broad, including organoids where genetic modification is feasible.	Off-target pharmacology; need for careful Pharmacokinetics/pharmacodynamics (PK/PD) modelling in 3D tissues.	(Prochazka et al., 2022; Trentesaux et al., 2023)
DNA microbeads and hydrogels	Nanoengineered DNA beads injected into organoids release tethered morphogens upon light or chemical triggers.	Intrinsic localisation within organoid; gradients emanate from the bead on 50–200 µm scales.	High control via photocleavable linkers and bead design; repeated triggering possible.	Retinal organoids; potentially generalizable to other organoid types.	Microinjection labour; potential immunogenicity; regulatory novelty.	(Afting et al., 2024)

Note. Data synthesised from O'Grady et al. (2019), Yaman & Ramanathan (2023), Repina et al. (2023), Trentesaux et al. (2023), and Afting et al. (2024).

When critically comparing these platforms, distinct trade-offs between precision, reproducibility, and scalability become evident. Microfluidic devices offer unmatched spatiotemporal precision and robust gradient maintenance, yet they often suffer from low throughput, complex fabrication, and shear stress-related issues that complicate large-scale manufacturing. Conversely, hydrogel-based systems excel in scalability and 3D organoid compatibility, but they lack the sharp boundary control and rapid reversibility seen in microfluidics. Optogenetics provides unparalleled non-invasive, dynamic control with high resolution, but it is fundamentally limited by the depth of light penetration in dense tissues and requires extensive genetic modification. Finally, DNA microbeads present a highly localised, programmable solution from within the tissue itself, though their reliance on manual microinjection currently restricts reproducibility and high-throughput applications.

4. Organoid Systems Amenable to Gradient Engineering

4. 1. Cerebral and Brain Organoids

Brain organoids are ideal platforms for synthetic gradient engineering, as endogenous neural differentiation is strongly dependent on multiple axes of morphogens. Through orthogonal Wnt and Shh gradients, researchers have been able to successfully pattern forebrain, midbrain and hindbrain regions that match human foetal atlases. Moreover, the "fine-tuning" of critical morphogen concentrations can selectively regulate the regional cell fate composition of brain organoids. **Table 3** provides a summary of current patterning challenges and opportunities to engineer gradients specific to brain and other organoid models. Yet, these studies also highlight important limitations, such as individual variability and line-to-line differences in morphogen responses, that produce patterning differences.

Moreover, strong and/or global application of high levels of morphogens often obliterates fine-scale organisation, yielding so-called "monoclonal" fates (Sanchís-Calleja et al., 2026; Scuderi et al., 2025). Finally, gyri, fascicles, and laminar structures are poorly mimicked even in organoids with correctly specified regions, suggesting that by itself, morphogen gradients cannot produce all of the neural cytoarchitecture and that mechanical and activity-dependent processes play an essential role (Ahammed & Kalangi, 2024; Yao et al., 2024). Thus, gradient engineering in brain organoids provides a robust means to specify regional identities but only a partial means to establish higher-order cytoarchitecture.

4. 2. Intestinal Organoids

Intestinal organoids are well-positioned to benefit from engineered gradients because, in vivo, they exhibit distinct crypt and villus domains with opposing underlying Wnt/BMP gradients (Xiang et al., 2024; Xu et al., 2025). Conventionally, intestinal organoids develop budding crypt-like structures and central lumens; however, they are rarely observed across long-range directions or with villus-like morphologies like those found in vivo (Huang et al., 2021; Xiang et al., 2024). Theoretically, microfluidic- or hydrogel-based gradients of Wnt agonists, BMPs, and Notch modulators could promote the development of long-range crypt-villus axes, but few studies have been reported (compared to neurones) (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Xiang et al., 2024).

Recent reviews are right to stress that the adoption of microfluidics, genome editing, and innovative hydrogels is rapidly expanding the utility of intestinal organoids for disease modelling, drug screening and therapies (Xiang et al., 2024; Xu et al., 2025; Yao et al., 2024).

4. 3. Kidney Organoids

Organoids derived from human pluripotent stem cells with nephron-like units comprising podocytes, proximal and distal tubules, and collecting ducts typically have patterning and segmentation along the corticomedullary axis that are scrambled in comparison to a native kidney (Ahammed & Kalangi, 2024; Huang et al., 2021). Wnt, FGF, and BMP gradients orchestrate segmentation of nephrons in vivo, so engineered gradients could help optimise segmentation and alignment of tubuli in vitro (Meng et al., 2025; Yao et al., 2024). However, so far, kidney organoid studies have typically modulated these pathways temporally and homogeneously.

Microfluidics and gradient hydrogels can be used to impose, for example, proximal versus distalising cues along the x-axis of a kidney organoid, and synthetic circuits that sense combinations of RA, Wnt and FGF may coordinate segment-specific gene expression (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). The sparse gradient-engineered studies in kidney organoids can be attributed to technical difficulties, including organoid fragility and 3D structure and the lack of effective readouts of segment identity in 3D. The combination of gradient engineering and better readouts of segment identity, such as spatial transcriptomics and multiplexed imaging, will likely see increased development of gradient-engineered progenitors into nephrons in the future.

4. 4. Cardiac and Vascular Organoids

Heart organoids and engineered heart tissues have made rapid progress, exhibiting chamber-like structures, beating capabilities, and simple vascular networks (Huang et al., 2021; Yao et al., 2024). Wnt and BMP processes play vital roles in early cardiac mesoderm differentiation and later atria versus ventricle differentiation, while VEGF and Notch processes shape blood vessels (Hellwarth et al., 2021; Meng et al., 2025; Yao et al., 2024). Optogenetic tools, such as optoWnt, have been used early in differentiation to induce cardiac mesoderm patches that subsequently form patterned heart muscle (Hellwarth et al., 2021; Repina et al., 2023).

However, strong chamber specification and orientation, like in the four-chambered human heart, have been elusive. This challenge is partly due to the complex interplay between mechanical factors and gradients in heart development; growth and form, lumen (hole) formation, and blood flow interfere with the reading of gradients (Hellwarth et al., 2021; Yao et al., 2024). In this case, engineered gradient reading may have to be complemented with mechanical cues, such as stretch and flow, as well as bioelectricity, to achieve functionally realistic architectures. Vascular organoids that form microvessel networks are a more accessible platform for VEGF gradient engineering, and they have been used to build pre-vascularised tissue grafts (Huang et al., 2021; Yao et al., 2024)

4. 5. Lung and Hepatic Organoids

Lung organoids model proximal–distal patterning, branching morphogenesis, and alveolar differentiation, which are processes known to depend on gradients of FGF, Wnt, BMP, and RA (Meng et al., 2025). However, most lung organoid protocols use uniform factor exposure, resulting in mixtures of proximal and distal cell types without clear spatial segregation (Meng et al., 2025). Synthetic gradients, implemented either through microfluidic chips mimicking airway–alveolar transitions or through hydrogel-embedded growth-factor sources, could help impose more physiological proximal–distal organisation and branching geometries.

Microfluidic liver-on-chip systems have begun to implement oxygen and nutrient gradients, but explicit morphogen gradient programming in hepatic organoids remains rare (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Interestingly, CRISPR-based editing and patient-derived hepatic organoids are already being deployed for disease modelling and drug screening, suggesting that integrating synthetic gradient engineering into these genetically edited, patient-specific models could yield zonation-aware pharmacology platforms (Pulecio et al., 2017; Yao et al., 2024)

Table 3. Organoid Systems and Their Current Patterning Limitations Addressable by Synthetic Gradient Engineering.

Organoid type	Target tissue architecture	Key patterning signals required	Current patterning limitation	Synthetic engineering opportunity	Representative references
Cerebral/brain organoids	Regionalised forebrain, midbrain, and hindbrain with proper cortical layering and DV domains.	Wnt, Shh, BMP, FGF8, RA gradients along AP and DV axes.	Stochastic regionalisation; incomplete laminar structure; poor long-range connectivity.	Orthogonal microfluidic gradients (Duo-MAPS), optogenetic Wnt, and synthetic circuits that sharpen regional boundaries.	(Sanchís-Calleja et al., 2026; Scuderi et al., 2025)
Intestinal organoids	Organised crypt–villus units with aligned axes and appropriate stem cell and differentiated cell zoning.	Wnt/BMP countergradient; Notch modulation; EGF support.	Budding crypt-like structures without consistent villus alignment; batch variability.	Microfluidic or hydrogel-based Wnt/BMP gradients along a single axis; Wnt/BMP-sensitive synthetic gene circuits to reinforce crypt vs villus fates.	(Xiang et al., 2024; Xu et al., 2025)
Kidney organoids	Repeated nephron units with correct segment order (glomerulus, proximal, distal, collecting duct) and cortico-medullary organisation.	Wnt, BMP, FGF, RA gradients; Notch for segmentation.	Disordered segment arrangement; absence of a clear cortico-medullary axis.	Gradient hydrogels delivering proximalizing vs distalizing cues; CRISPR-based reporters and circuits to enforce segment identities.	(Yao et al., 2024; Zhang et al., 2026)
Cardiac organoids	Chambered structures with aligned atrial and ventricular regions and integrated vasculature.	Wnt, BMP, RA, VEGF, Notch gradients; mechanical cues.	Incomplete chamber specification; limited spatial separation of cardiomyocyte subtypes.	Optogenetic Wnt gradients during early mesoderm induction; VEGF gradients to guide vascular network topology.	(Hellwarth et al., 2021; Yao et al., 2024)
Vascular organoids	Hierarchical vascular trees with perfusable lumens and appropriate tip/stalk cell patterning	VEGF, Notch, Angiopoietin gradients.	Isotropic microvessel networks lack hierarchical organisation.	Microfluidic VEGF gradients; Notch-modulating circuits in endothelial cells to stabilise tip/stalk decisions.	(Zhang et al., 2026)
Lung organoids	Proximal–distal airways with branching bronchioles and alveolar regions.	FGF, Wnt, BMP, RA gradients; mechanical stretch.	Mixed proximal and distal cell types without clear spatial segregation.	Organ-on-chip devices imposing FGF/Wnt gradients along the channel; hydrogel scaffolds with patterned morphogen sources.	(Meng et al., 2025)
Liver organoids	Zonation along periportal–pericentral axis with metabolic and signalling gradients; organised bile duct networks.	Wnt, oxygen, nutrient, and hormone gradients; Notch for ductal patterning.	Limited zonation; homogeneous hepatocyte-like populations.	Microfluidic oxygen and Wnt gradients; synthetic circuits reading oxygen and Wnt to enforce zonal gene expression.	(Yao et al., 2024)
Retinal organoids	Stratified retinal layers with correct distribution of photoreceptors, ganglion, and pigment epithelium.	Wnt, Shh, RA gradients; intrinsic Notch signalling.	Incomplete layering; limited pigment epithelium formation.	DNA microbead-based internal Wnt gradients to drive localised pigment epithelium while preserving neural retina.	(Afting et al., 2024)

Note. Data synthesised from Xiang et al. (2024), Yao et al. (2024), Sanchís-Calleja et al. (2025), Afting et al. (2024), and Xu et al. (2025).

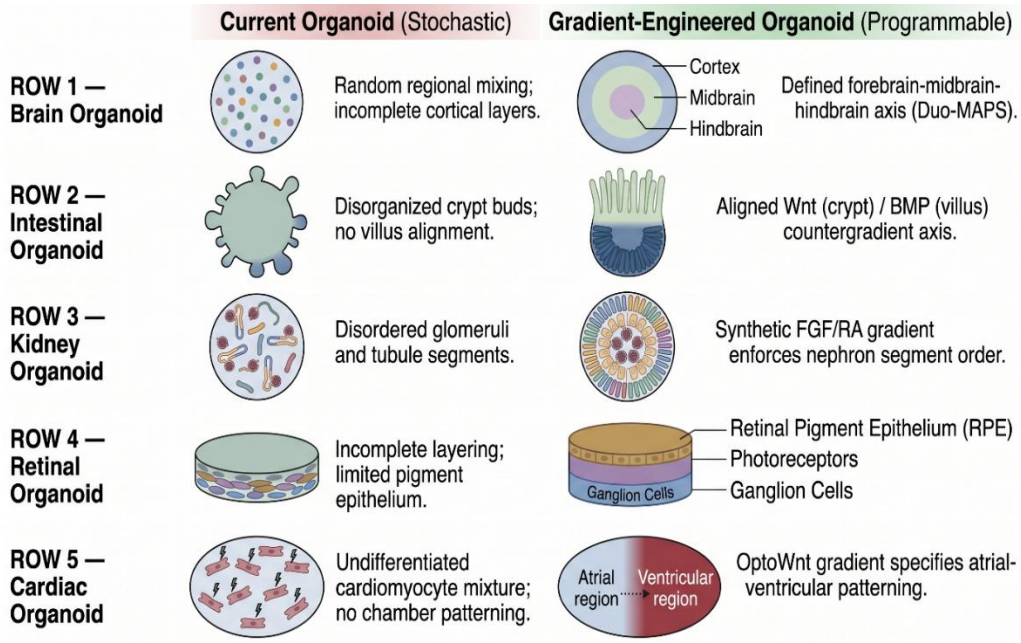

Figure 3. Structural advantages of programmable organoids. A comparison demonstrating how gradient engineering (right) enforces organised cellular patterning and defined spatial axes across various tissue types, overcoming the disorganised, random mixing typical of standard stochastic organoids (left).

5. Molecular Circuits for Gradient Computation in Living Cells

5. 1. Synthetic Gene Circuits Sensing Morphogen Thresholds

Microfluidic liver-on-chip has started to apply oxygen and nutrient gradients, and explicit morphogen gradient programming in hepatic organoids is uncommon (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Remarkably, CRISPR-based editing and patient hepatic organoid models are already being implemented to model diseases and screen drugs, implying that integration of systematic gradient engineering into such genetically engineered, patient-specific models could result in zonation-aware pharmacology scaffolds (Pulecio et al., 2017; Yao et al., 2024). To fully maximise the potential of these organoids, scientists can engineer their cells with synthetic circuits that automatically detect and respond to local chemical signals. As an example, artificial gene networks that can respond to changes in local oxygen and Wnt concentrations have the potential to impose strict zonal gene expression, thereby autonomously stabilising the tissue architecture to environmental noise. Table 3 will give a comprehensive description of how these molecular circuits and external gradient tools can ameliorate existing constraints in patterning hepatic and other organoid systems.

Prochazka and others modified circuits in human pluripotent stem cells that commit to readings of combinations of endogenous microRNAs (as cell state proxies) and enable optimisation of output protein levels, including developmental morphogens such as BMP4 (Prochazka et al., 2022). Combined with exogenous BMP addition, these circuits enabled greater control over the composition of the germ layers of the organism, implying a modular approach where morphogen exposure and intrinsic state are combined to inform fate choices (Prochazka et al., 2022). Based upon these designs, programmable circuits may be extended into a secondary morphogen or adhesion molecule only after a cell has undergone a specific extent and duration of an exogenous gradient, which helps to sharpen boundaries within tissues.

5. 2. Bidirectional Feedback Loop Design: Bistable Switches and Oscillations

Feedback is often needed to stabilise fates and form dynamic patterns, like segmentation clocks, through developmental systems (Green & Sharpe, 2015; Wolpert, 1969). Sharp on/off domains can be recapitulated by synthetic bistable switches that consist of mutually repressive transcription factor pairs, amplifying positional information by extending the range of

concentrations in which cells that self-transcribe distinct fates are stable (Dupin et al., 2022; Lam et al., 2022). Experimental and modelling of synthetic multicellular assemblies of drops containing two-node feedback circuits have measured the influence of gradient shape, noise and circuit parameters on positional information, and they found trade-offs between precision and robustness (Dupin et al., 2022).

Gradient phase-locking oscillatory circuits, such as synthetic Notch-based loops or repressilator-like loops, could theoretically be phase-locked to external gradients to form stripe or segment patterns akin to somitogenesis. Although direct testing of such circuits in human organoids is relatively scarce, recent organoid experiments of the effects of segmentation clock-like oscillations in response to relevant morphogen environments have indicated that synthetically amplifying such endogenous oscillators is accessible (Anand et al., 2023; Yaman & Ramanathan, 2023). Importantly, designs with multiple feedback loops need tuning to avoid undesired dynamics like spurious oscillations or multi-stability that are unmatched to the desired patterns.

5. 3. Orthogonal Receptor Engineering

One of the key issues in tissue engineering is how to control particular cell pathways which cannot cause intracellular cross-talk in the sophisticated native signalling context. To overcome it, scientists use orthogonal receptor-expressed proteins that bind only synthetic ligands or non-native epitopes to overlay artificial gradient systems and clean overlaying normal networks (Trentesaux et al., 2023). An example would be the methods described by Trentesaux and colleagues, in which an arbitrary molecule, such as secreted Green Fluorescent Protein (GFP), is turned into a functional morphogen by expressing a chimeric receptor containing a GFP-binding nanobody in conjunction with an intracellular signalling (Trentesaux et al., 2023). With designed controls of receptor affinity, expression levels, downstream effectors, and their combination, scientists are striving to create highly accurate, gradient-responsive behaviours, which are completely independent of natural ligand distributions (Trentesaux et al., 2023).

Such orthogonal systems might be used in organoids to incorporate microfluidic or DNA-bead-based sources of synthetic ligands to realise clean, quantifiable gradients without the confounding influences of endogenous ligand production and consumption. Further, orthogonal morphogens decrease pleiotropic side effects, which is critical for translational safety. A practical problem is how to integrate these receptors without overloading the cellular signalling machinery or putting them into unwanted cross-talk with the scaffold proteins and the subsequent networks.

5. 4. Positional Information CRISPR-Based Epigenetic Recordings

CRISPR-based epigenome editing is not only confined to non-chromatin or chromatin states of static transcriptional repression or activation, but it could also serve to engrave environmental histories in DNA (Shi et al., 2025). Morphogen-responsive promoters can be coupled with CRISPR base editors or dCas9-linked epigenetic enzymes, record cumulative exposure to a gradient as a stable mark, which is subsequently read by sequencing or imaging (Shi et al., 2025).. For example, a Shh- or Wnt-responding promoter-driven base-editing guide RNA could encode a positional barcode of historic gradient levels, which, even when the gradient decays, would allow recapitulation of past gradient dynamics.

Although the majority of currently used CRISPR-based epigenetic tools have been used to regulate gene expression in vivo or investigate disease pathophysiology, their use to develop organoid-like structures is conceptually uncomplicated and technically implementable considering existing infrastructures (Pulecio et al., 2017; Shi et al., 2025). Notably, these recording circuits need not per se affect fate but can be implemented as passive sensors, maintaining native developmental dynamics, but with strong readouts to gradient-engineering experiments. This strategy may, in the future, allow the generation of closed-loop applications where the stored positional data proactively influences the future cell fate choices via programmable changes in epigenetics.

5. 5. Juxtacrine and Paracrine Signalling

The synthetic juxtacrine and paracrine signalling circuit consists of cell-cell communication pathways. Lateral inhibition and community effects correlate with gradient reading; tissue patterns are heavily influenced by them (Green & Sharpe, 2015; Wolpert, 1969). Positional information can be enhanced, and boundaries refined by synthetic cell-cell communication circuits driven by engineered juxtacrine signalling, such as synthetic Notch (synNotch) or paracrine circuits. The user-defined transcriptional program can be activated by SynNotch receptors, in which a cell responds when it interacts with a neighbour presenting a particular surface ligand. Gradients or domains of synNotch–ligand complexes can create patterns in which cells change fate based on the local composition of their neighbourhood (Morsut et al., 2016; Trentesaux et al., 2023).

In patterned organoids at gradients, these circuits may help transduce a continuous morphogen field into discrete domains by imposing self-exclusive cell fates that not only depend on their own morphogen exposure but also that of their neighbours. An example is by having cells surpassing a Wnt threshold to express a synNotch ligand that, when contacted, causes adjacent subthreshold cells to switch their fate to a complementary one, sharpening interfaces. Initial experiments in mammalian cell aggregates indicate that synthetic cell-cell communication can induce self-organised patterning and can be translated to human organoids (Morsut et al., 2016; Trentesaux et al., 2023).

6. Bench-to-Bedside Therapeutic and Translational Implications

6. 1. Ready-to-Be Transplanted Organoids and Patterning Fidelity

To be effective in clinical transplantation, organoid-derived grafts need to achieve specific tissue-relevant structures to achieve appropriate host integration, perfusion, and functioning (Ahammed & Kalangi, 2024; Huang et al., 2021; Yao et al., 2024). Providing an alternative design strategy, synthetic gradient engineering is an incredibly potent strategy to pre-pattern these key geometries, including aligned intestinal crypts or layered retinal tissue, potentially overcoming the structural constraints of stochastic self-assembly **(Figure 3)** (Afting et al., 2024; Xiang et al., 2024). Nevertheless, even with this promise, there is an urgent need to conduct systematic preclinical studies that would satisfactorily compare the long-term engraftment results of the grafts grown by the light of gradient engineering and the traditional growth. An overall summary of these translational applications and barriers to development is outlined in **Table 4**.

6. 2. Monogenic Disease Modelling and Patient-Derived Organoids

Patient-derived organoids have been positioned at the core of the organoid disease modelling of monogenic diseases, such as cystic fibrosis, liver metabolic diseases, and inherited kidney diseases (Alnasser, 2025; Xiang et al., 2024; Yao et al., 2024). In these conditions, like cystic fibrosis, liver metabolic diseases, and inherited kidney diseases, structural defects typically occur not only at the cellular scale but also within the broader tissue architecture, leading to altered crypt-villus ratios, disorganised bile duct networks, or maladaptively formed nephron segments. (Ahammed & Kalangi, 2024; Huang et al., 2021; Yao et al., 2024). Synthetic morphogen gradients provide a means of standardising space, on which such mutations are examined, avoiding confounding variability in individuals caused by disorganised patterning.

Also, gradient engineering could enable patterning correction strategies in which patient-derived organoids with a repaired genotype, using CRISPR-Cas9 editing, i.e., exposed to standardised morphogen programmes, would determine whether restored signalling is converted into normal tissue architecture (Pulecio et al., 2017; Yao et al., 2024). This method, in particular, is useful in investigating diseases that are the result of mutations that impair the capacity of a cell to respond to morphogens. By putting the mutant cells on board with the genetically corrected cells, researchers are in a position to expose the mutant cells to the same gradients as the genetically corrected cells. This direct and side-by-side comparison will enable the scientists to chart out precisely the development of tissues in relation to the presence of a particular genetic mutation.

6. 3. Pharmacodynamic Testbeds and Drug Screening

Organoids are also available in an increasing variety of drug screening and toxicity tests, but they are considered uniform tissues (Alnasser, 2025; Huang et al., 2021; Wang et al., 2025; Yao et al., 2024). Nonetheless, in vivo distribution and response of drugs to various tissue regions delimited by morphogen and metabolic gradients, such as periportal versus pericentral hepatocytes or crypt stem cells versus villus enterocytes, are challenging (Huang et al., 2021; Yao et al., 2024). It can also be more realistic and spatially resolved, so gradient-patterned organoids can give a drug response.

Microfluidic organoid-on-chip systems can subject different parts of an organoid or organoid array to a discussable amount of various drugs, but internal morphogen gradients mean that they can read out region-specific toxicity and efficacy (Ahammed & Kalangi, 2024; Alnasser, 2025). One practical application is the use of intestinal organoids patterned by synthetic Wnt/BMP gradients to model gastrointestinal toxicity. Researchers propose that this system can elucidate the preferential damage chemotherapeutics inflict on crypt stem cells compared to villus cells, thereby guiding strategies to mitigate mucositis (Xiang et al., 2024; Xu et al., 2025). On the same note, liver organoids reconstituted to be zonated are suggested to become a way to narrow down the predictions of drug-induced liver injury by probing the zone-specific susceptibility (Yao et al., 2024).

6. 4. Organ-on-Chip Integration

Microfluidics, synthetic biology, and tissue engineering: an obvious overlap of gradient-engineered organoids with organ-on-chip platforms is a logical progression (Ahammed & Kalangi, 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). These systems enable sustained perfusion, mechanical feedback (e.g., stretch or fluid shear), and intricate biochemical gradients and allow providing high-content imaging capabilities and sampling (Ahammed & Kalangi, 2024; Alnasser, 2025; Wang et al., 2025). Organoid-on-chip devices have already enhanced oxygenation, maturation, and vascularisation in various models, and more frequently include morphogen gradients in diverse models to recapitulate developmental microenvironments (Ahammed & Kalangi, 2024; Yao et al., 2024).

In the context of regenerative medicine, organoid-on-chip platforms may play the role of pre-conditioning bioreactors in which grafts are conditioned via gradient programmes that are defined to shape architecture and behaviour before transplant. Applying multiplexed chips with organoid arrays of gradient patterns of individual patients to precision medicine pipelines that capture realistic tissue heterogeneity would support drug discovery. The difficulty is to harmonise during the connectivity between chips and organoids, control the batch-to-batch variation, and meet the regulatory demands of the device-tissue assemblies.

6. 5. Regulatory, Manufacturing, and Clinical Limitations

From a regulatory and manufacturing perspective, scaling up gradient-engineered organoids introduces distinct challenges. Although programmable gene circuits and CRISPR-based positional recordings present revolutionary theoretical possibilities for custom tissue design, their current application remains strictly experimental. Fully programmed tissue structures have yet to be realised in human clinical trials, and researchers must carefully distinguish between these conceptually supported models and experimentally validated applications.

Translating these technologies into the clinic will require overcoming significant manufacturing bottlenecks, particularly regarding scalability and reproducibility across massive batches. Furthermore, long-term clinical application is complicated by biological risks like insertional mutagenesis, off-target genetic effects, and immunogenicity against engineered or foreign proteins. Ethical considerations also remain a significant hurdle, particularly concerning the development of advanced, regionally specified brain organoids. Finally, DNA-based materials and microfluidic chips require rigorous biocompatibility and sterility validations before transitioning from the lab bench to human patients. Because of these limitations, practical clinical pathways will likely adopt a progressive approach, beginning with *ex vivo* diagnostics and drug screening, before eventually advancing to direct patient transplantation.

Table 4. Translational Applications of Gradient-Patterned Organoids Across Disease and Therapeutic Contexts.

Application area	Disease/condition	Organoid system	Gradient engineering approach	Stage of development	Major translational barrier	Representative references
Regenerative transplantation	Intestinal failure, short bowel syndrome	Intestinal organoids	Wnt/BMP countergradients to align crypt–villus architecture before engraftment.	Preclinical concepts; limited *in vivo* engraftment studies.	Achieving scale, vascularisation, and functional integration with host mucosa.	(Marti-Figueroa & Ashton, 2017; Xiang et al., 2024)
Regenerative transplantation	Retinal degeneration	Retinal organoids	DNA microbead–mediated internal Wnt gradients to generate layered retina and pigment epithelium.	Preclinical in animal models; *in vitro* optimisation ongoing.	Long-term survival, synaptic integration, and immune compatibility.	(Afting et al., 2024)
Disease modelling and drug testing	Cystic fibrosis, Inflammatory Bowel Disease (IBD), Colorectal Cancer (CRC)	Intestinal and colonic organoids	Microfluidic and hydrogel gradients of inflammatory cytokines and drugs along crypt–villus axes.	Widely used organoids; gradient engineering is emerging.	Standardisation and high-throughput compatibility; patient variability.	(Xiang et al., 2024; Xu et al., 2025)
Metabolic and liver disease	Non-Alcoholic Fatty Liver Disease (NAFLD), Non-Alcoholic Steatohepatitis (NASH), hepatotoxicity	Liver organoids and liver-on-chip	Oxygen/nutrient and Wnt gradients to model zonation; drug concentration gradients.	Organ-on-chip devices in advanced preclinical use.	Complex culture systems; regulatory acceptance of microfluidic platforms.	(Yao et al., 2024)
Neurodevelopmental disorders	Microcephaly, autism spectrum, epilepsy	Brain organoids	Orthogonal Wnt/Shh gradients to pattern regions; optogenetic circuits to model activity-dependent development.	Brain organoids are already used extensively in gradient systems in early-stage studies.	Ethical issues, model variability, and limited maturation.	(Sanchís-Calleja et al., 2026; Scuderi et al., 2025)
Cardiovascular disease	Cardiomyopathies, arrhythmias	Cardiac organoids and engineered heart tissues	Wnt/BMP gradients during mesoderm induction; VEGF gradients for vascularisation; optogenetic patterning of conduction tissue.	Preclinical models for drug testing and disease modelling.	Reproducing mature conduction systems and chamber architecture.	(Hellwarth et al., 2021; Yao et al., 2024)
Precision oncology	Tumour heterogeneity and microenvironment	Tumour organoids	Gradients of growth factors, oxygen, and	Patient-derived tumour organoids	Modelling immune and stromal	

		and co-culture systems	drugs to mimic tumour cores vs margins.	in early clinical decision-making.	components; intratumoral heterogeneity.	(Alnasser, 2025)
Gene and cell therapy development	Vector targeting and off-target toxicity	Multi-organoid arrays	Controlled gradients of viral vectors or genome editors across organoid systems on chips.	Conceptual and early experimental work.	Biosafety of gene editing; integration with manufacturing workflows.	(Pulecio et al., 2017; Shi et al., 2025)

Note. Data synthesised from Xiang et al. (2024), Yao et al. (2024), Afting et al. (2024), Marti-Figueroa & Ashton (2017), and Ahammed & Kalangi (2024).

7. Future Perspectives: The Era of Programmable Organoids

Looking forward, the transition from stochastic self-organisation to fully programmable tissue engineering represents a paradigm shift in regenerative medicine. We envision a future where patient-derived stem cells are combined with "smart" microfluidic scaffolds and synthetic genetic circuits that automatically guide their own development. In this framework, transient external gradients will reliably initiate early symmetry breaking, while internal, CRISPR-recorded genetic switches will permanently lock in mature tissue architectures. Ultimately, integrating these programmable organoids into scalable, closed-loop bioreactors will bridge the gap between basic developmental biology and the reliable mass manufacturing of personalised, transplantable organs.

8. Critical Synthesis and Analysis

8. 1. Emerging Patterns of Convergence

There are a few trends that emerge from papers that use the various platforms and organoid models described above. First, gradient engineering of any sort (e.g., microfluidics, hydrogels, optogenetics or DNA microbeads) always increases control of fate speciation in organoids versus simple feeding with medium (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Repina et al., 2023; Trentesaux et al., 2023). This impact is variable depending on the tissue type and culture conditions, but the trend is very consistent. Second, platforms that engineer gradients and also provide at least some basic synthetic circuitry or stateful reporters mediate more intuitive and reproducible outcomes because they directly couple environmental profiles to genetic decision-making and readouts (Dupin et al., 2022; Prochazka et al., 2022; Shi et al., 2025; Trentesaux et al., 2023). Third, combined strategies that employ engineered gradients to bias early symmetry breaking and leave the hard work of development to intrinsic programmes seem to be more effective than attempts to impose pre-patterning on pre-existing developmental programmes in a completely deterministic fashion (Anand et al., 2023; Repina et al., 2023; Sanchís-Calleja et al., 2026; Yaman & Ramanathan, 2023).

In all, these three emerging trends suggest that the field is coming to a consensus that synthetic gradients do not replace development; rather, they steer it. They constrain self-organisation and leave fine-scale architecture to the cells themselves. Engineered gradients are shifting from controlling development to guiding it.

8. 2. Paradoxes and Controversies

As the field comes together on these underlying principles, there are considerable debates and challenges. A common question is the level of engineered control desirable for self-organisation. Microfluidic experiments demonstrate that there are strong, persistent exogenous gradients that can override endogenous patterning to produce tissues with natural-looking but somewhat forced domain boundaries or unnatural lineage distribution (O'Grady et al., 2019; Sanchís-Calleja et al., 2026; Scuderi et al., 2025). Others report that, despite well-calibrated gradients, organoids show variable results, with evidence of stochastic fluctuations and line variability, suggesting that this variation may be unavoidable or even beneficial (Anand et al., 2023; Sanchís-Calleja et al., 2026; Yaman & Ramanathan, 2023).

There is also no consensus about the complexity of gradient programmes. Some argue that rather simple, monotonic gradients of one or two morphogens are sufficient, as endogenous regulatory networks will be capable of filling in the gaps. Others envision more and more complex gradients with multiple morphogens and time-dependent profiles, hoping to mimic the dynamics of the embryo (O'Grady et al., 2019; Sanchís-Calleja et al., 2026; Trentesaux et al., 2023). Synthetic biology introduces yet another dimension, with some designs opting for simple circuits that minimally interfere with endogenous networks, whereas others aim to build very sophisticated engineered cell lines with extensive orthogonal recording and signalling capacities (Prochazka et al., 2022; Shi et al., 2025; Trentesaux et al., 2023). The right balance between simplicity, robustness and faithfulness is unclear.

8. 3. Technological Challenges: Reproducibility, Scalability, and Vascularisation

Technically, many gradient-engineered organoid studies are proof-of-concept studies involving small numbers of organoids, implementing gradient techniques that are technically difficult to replicate elsewhere, such as custom-designed setups (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Repina et al., 2023). Sources of variability include changes in the stem cell differentiation and culture matrix (for instance, lots of Matrigel) (Ahammed & Kalangi, 2024; Huang et al., 2021; Wang et al., 2025). Very few studies evaluate the influence on variability of gradient parameters like slope, amplitude, and time, or include robust statistical designs and quality of pattern evaluations by blinded third parties.

Challenges in reproducing these systems aside, a key challenge is how to translate these technologies into manufacturing at scale. Microfluidic chips and microinjection-based DNA beads are amazing for exploring biology but are hard to scale up to screen or manufacture hundreds or thousands of organoids (Afting et al., 2024; O'Grady et al., 2019; Trentesaux et al., 2023). Hydrogels containing channels or beads can be scaled up, but they lose resolution (Marti-Figueroa & Ashton, 2017; Zhang et al., 2026). Another common problem is a lack of vascularisation; without perfusable networks, large organoids will develop necrotic cores and unnatural oxygen and nutrient gradients that hinder patterning by morphogen gradients (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Even gradient-engineered vascular organoids have yet to be routinely integrated into other organoids to alleviate this issue at scale.

8. 4. Closing the Gap: Immune Stromal and Gradient Microenvironments

Although the first series of organoid models, created by Sato and co-workers in 2009, has laid the foundation for organoid models made purely of epithelium, the overwhelming majority of studies in the past 15 years continue in this vein, still primarily focused on parenchymal tissues (Sato et al., 2009; Yao et al., 2024). As a result, there remains a paucity of immune and stromal cell types critical for the correct functioning of mature tissues. The inclusion of these cells remains a major challenge because cell lineages frequently require different (and, at times, competing) morphogen conditions to survive and/or self-organise (Green & Sharpe, 2015; Huang et al., 2021). However, in vivo, fibroblasts, endothelial cells, pericytes, and immune cells respond to and modify the diffusion and transport of morphogens by releasing ligands, antagonists, and extracellular matrix (ECM) components (Ahammed & Kalangi, 2024; Alnasser, 2025; Meng et al., 2025). In the absence of these factors in most gradient-engineered organoids, it is likely that effective gradients are modified and may blunt the maturation and stability of the patterned tissue.

New types of co-culture organoids with integrated immune and stromal cells, and even microbiota, have started to shed light on how these interactions influence organoid function, but gradient engineering in these more complex organoid systems is just beginning (Alnasser, 2025; Xiang et al., 2024; Xu et al., 2025). An important avenue for future research is to engineer gradients of both traditional morphogens and chemokines, cytokines, and ECM gradients to recapitulate immune-stromal-parenchymal communication. This would be especially important for tissue regeneration strategies, where grafts will have to respond to host gradients of immune and stromal cues.

8. 5. Can Existing Gradient Tools Pattern Tissues?

The question arises whether synthetic gradient tools have now matured to a level of refinement at which they can be used for tissue patterning, much as they occur in development, or whether they are merely "toy" systems. These tools appear to have varying degrees of success. For example, systems like Duo-MAPS and DNA microbeads are able to provide multi-axis and internal gradients, yielding readily identifiable, patterned tissues (Afting et al., 2024; Scuderi et al., 2025). Also, optogenetic and chemogenetic tools show that selective activation for subsets of cells is sufficient to achieve global self-organisation and boundary formation (Repina et al., 2023; Trentesaux et al., 2023).

By contrast, very few, if any, studies present organoids with global shape, multilayered architecture and functions, such as electrochemical pathways, peristaltic waves, or bile transport, that match in vivo organs as a result of engineered gradients. They typically revolve around regional differentiation and domain formation, rather than whole organ morphogenesis (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). We must find out whether improvements to our existing platforms, better

microfluidics, more versatile hydrogels, and more reliable optogenetics are enough or whether new ideas, such as integrated closed-loop artificial intelligence-driven cultivation strategies or co-designed mechanical and morphogen fields, will be needed to reach this milestone.

9. Implications

9. 1. Research Implications

There are several evident research challenges arising from this marriage of developmental cues, microfluidic devices and synthetic biology. First, there is a need for systematic gradient design experiments, in which characteristics such as gradient amplitude, slope, and temporal features are systematically adjusted and tested in high-throughput conditions. Ideally, these experiments will involve automated fluorescence imaging and single-cell transcriptomics to reveal how different combinations of multiple morphogens lead to different cell fates (Huang et al., 2021; Sanchís-Calleja et al., 2026; Shi et al., 2025). This will help us progress from anecdotal experimentation toward a quantitative understanding of how gradient features affect pattern formation. Second, when combined with synthetic reporter/recording gene circuits, these experiments will allow novel measurements of how cells sample, integrate and interpret gradients over time to identify information bottlenecks (Dupin et al., 2022; Prochazka et al., 2022; Shi et al., 2025).

Third, certain organoid systems deserve particular attention because of their specific developmental challenges and clinical promise. Best among these are brain organoids, for which the pairing of orthogonal gradients with CRISPR-based molecular time stamping promises a revolutionary approach to investigating neural organization. By leveraging these synthetic circuit tools, we can start to dissect how position is not only defined but also recorded and fine-tuned over the duration of human neurogenesis, which takes place over many weeks (Sanchís-Calleja et al., 2026; Scuderi et al., 2025). This process facilitates the "timestamping" of signalling events and bridges the gap by mapping early exposure to the morphogen gradient as it orchestrates the late-time organisation of cortical layers and long-distance connectivity patterns, which have been intractable to self-organising models to date (Shi et al., 2025; Yao et al., 2024). In addition, focusing on systems that recapitulate cross-lineage interactions, such as neurovascular and liver-immune organoids, will be key to unravelling how the differential effects of patterned signalling control the co-organisation of different cell types in functional tissue units. Intestinal and liver organoids, owing to their clinical relevance, should be natural targets for experiments that link gradient-driven architecture to clinically relevant functions such as barrier function, cholate metabolism, and pharmacokinetics (Alnasser, 2025; Huang et al., 2021; Xiang et al., 2024; Xu et al., 2025; Yao et al., 2024). The kidney and lung organoid communities present formidable challenges with high rewards, where successful improvements in patterning will enable valuable disease models.

Lastly, field standards for reporting gradient patterning studies would be imperative, such as descriptions of device architecture, morphogen dynamics, strategies for gradient measurement, and cell line-specific responses (Huang et al., 2021; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). Such reporting will allow for better inter-lab comparisons and the development of a knowledge base.

9. 2. Clinical and Translational Implications

From a clinical perspective, synthetic morphogen-gradient engineering is part of the emerging trend towards programmable, precision regenerative medicine. From a regulatory and translational standpoint, a critical consideration is the duration for which synthetic control must be maintained within the graft. While the long-term maintenance of integrated synthetic networks, such as stably expressed optogenetic systems or synthetic receptors, offers prolonged spatial control, it also introduces substantial clinical risks, including insertional mutagenesis, off-target genetic effects, and long-term immunogenicity against foreign proteins. Consequently, transient "hit-and-run" gradient engineering presents a much more viable path toward Good Manufacturing Practice (GMP) compliance. Biologically, transient application utilising mRNA-based circuits or non-integrating tools during the early differentiation phase is likely sufficient to establish stable tissue architecture. Similar to natural embryogenesis, where morphogen gradients frequently act as temporary cues that trigger symmetry breaking, early transient

engineering can establish foundational spatial coordinates. Once cells cross specific morphogen thresholds, endogenous gene regulatory networks and epigenetic memory "lock in" these committed fates. This allows the foundational architecture, such as a neural axis or a crypt-villus boundary, to be set early, enabling the pre-patterned organoids to undergo subsequent maturation in standard, device-free bioreactors prior to transplantation.

Concerning the organ shortage, gradient-optimised organoids may not be the next organ transplants, but they may be viable candidates for patch/substantial grafts, intestinal sleeves for improved digestion, or liver lobules or patches for metabolic backup, or retina patches for local vision (Alnasser, 2025; Huang et al., 2021; Yao et al., 2024). Realistically, gradient-engineered, clinically transferable grafts for such applications are unlikely to be available for at least another decade of trial-and-error experimentation and safety testing, due to the challenges of integrating the synthetic gradient, gene circuits and host system.

For precision medicine, patient-specific patterning correction is an intriguing idea. We can envisage a process in which patients' organoids are standardised using defined gradient programmes to produce standardised tissue structures, which are subsequently used to screen gene therapies or drug treatments under controlled microenvironmental conditions (Alnasser, 2025; Xiang et al., 2024; Xu et al., 2025). If coupled with CRISPR-based historic recording, this approach can capture long-term reactions and new lineages, supplying vital information to plan patient-specific therapies (Pulecio et al., 2017; Shi et al., 2025)

9. 3. Theoretical and Conceptual Implications

From a theoretical point of view, the engineering of morphogen gradients in organoids has an impact on theories of positional information. Synthetic systems allow independent control of gradient noise, geometry and circuit topology, which are not typically accessible in embryos. This provides fresh insights to test theoretical models that quantify the capacity of a gradient to transmit positional information and the extent of this information used by cells (Dupin et al., 2022; Green & Sharpe, 2015; Turing, 1952; Wolpert, 1969). Preliminary studies with synthetic gradients hint that developmental systems might be closer to the information-theoretic limits than once thought but also give rise to redundancy and feedback enabling robust patterning in the face of considerable noise (Dupin et al., 2022; Lam et al., 2022).

Similarly, the new hybrid model, in which exogenous engineering provides boundary conditions and endogenous development provides details, suggests a more sophisticated model of control in biology. Instead of engineering vs self-organisation, organoids seem to be in an intermediate regime where simple, timely interventions can tip the scales towards desired attractors without predetermining details. A deeper understanding of this balance may help us understand the robustness and evolvability of the natural development process and how far such a process can be pushed in synthetic systems.

10. Limitations of this Review

This review is a narrative one, which explores human organoid systems and synthetic morphogen gradient engineering, rather than including invertebrate and plant systems, despite the impressive capabilities of morphogen gradient engineering in these systems. This means that conceptual and technological advances from non-human systems are only included indirectly in this synthesis. Our survey of the literature highlights peer-reviewed publications to late 2025. Although this field is rich with preprints and conference abstracts, which were not extensively surveyed, it may provide further insights.

The perspective offered here is a specific approach to integrating the literature; we focused on studies that explicitly link gradient engineering to organoid patterning rather than tracing a comprehensive coverage of all synthetic biology literature. Variability in technology platforms, cell types, and analysis methods across studies often precludes direct comparisons, as differences may arise primarily from technical aspects and not biological relevance. Lastly, the translational outlook is based on proof-of-concept, early-stage experiments; regulatory, manufacturing and clinical challenges with these new technologies may be greater than expected.

11. Conclusion

From mathematical descriptions of embryonic development to the control of variables for engineering human tissue in the laboratory, morphogen gradients are poised to move from descriptive to predictive biology. Combining chemical and mechanical

gradient generators with biomaterial and microfluidic technologies, synthetic gene circuits and orthogonal receptors can now be used to allow cells to sense, process and store positional information with a new level of control. The key promise of this technology is to enable organoids to evolve from probabilistic self-organising microstructures to deterministic building blocks in regenerative medicine. Recent findings indicate that, while external gradients enhance regional fate specification, the most robust structures are based on hybrid approaches that integrate artificially engineered cues with self-organised information.

To successfully transition these hybrid models into multi-lineage co-cultures, researchers must address the complex dynamic where native immune and stromal cells act as unpredictable sources and sinks for morphogens, potentially destabilising intended spatial patterns. One viable strategy to preserve patterning fidelity is spatiotemporal staging, utilising transient "hit-and-run" gradients to establish the foundational parenchymal architecture before introducing the immune and stromal compartments, thereby decoupling initial symmetry-breaking from inflammatory cross-talk. Alternatively, orthogonal receptor engineering can bypass this noise entirely. By delivering synthetic ligands that only the engineered parenchymal cells can recognise, the gradient remains perfectly insulated from stromal consumption or interference. For models that must rely on native morphogens, the deployment of dual-axis microfluidic systems, where one axis delivers developmental cues and a secondary axis provides chemokine gradients for immune cell homing, or "smart" DNA-based scaffolds capable of dynamically adapting to cellular consumption will be essential for maintaining precise morphogen thresholds in a complex microenvironment.

Developing this potential over the next decade will demand an integrated design approach to optimise biochemical gradients, mechanics, and blood supplies. In the long run, establishing these guidelines for gradient engineering will not only unleash targeted therapies, such as zonated liver lobules or layered retinal tissues, but also provide the underlying principles for building elaborate human tissues on demand.

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