

REVIEW ARTICLE

Temporality, curvature, responsiveness, and beyond: Dimensional expansion in organoid bioprinting

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Abstract

The convergence of organoid technology and three-dimensional (3D) bioprinting has improved control over human tissue models, yet spatial precision at fabrication does not recapitulate the dynamic processes by which tissues develop, remodel, respond to injury, and recover. Here, we frame multidimensional bioprinting by capability rather than as a mature 3D, 4D, 5D, 6D hierarchy. The framework comprises spatial patterning, temporal programming, curvature-aware deposition, environmental responsiveness, and feedback. Direct organoid evidence is strongest for spatially controlled 3D printing and selected temporally programmed systems; multi-axis curvature control is supported mainly by adjacent biofabrication studies, whereas 6D remains a non-standardized and largely conceptual integration of multi-axis fabrication with stimulus responsiveness. We distinguish direct organoid-bioprinting evidence from enabling technologies and conceptual proposals, and evaluate each added capability against matched static 3D controls, longitudinal measurements, and organ-specific functional outcomes. This framework links engineering control to morphogenesis, disease trajectories, and adaptive organoid platforms, while emphasizing that progress should be judged by mechanistic interpretability, predictive performance, and interventional control rather than by dimensional labels alone.

Keywords: Organoid bioprinting; Higher-dimensional bioprinting strategies; Disease modeling; Organoid models; 3D bioprinting; 6D printing

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1. Introduction

Organoids have transformed studies of human development and disease by combining tissue-specific cell states with intrinsic self-organization. Intestinal organoids derived from single Lgr5-positive stem cells established that stem and progenitor populations can generate ordered architectures when provided with permissive biochemical and physical cues.¹ Related principles have since been extended to brain, liver, intestinal, kidney, lung, pancreatic, and other organoid systems derived from pluripotent or adult tissue stem cells.^{2,3} These models support mechanistic studies, drug discovery, precision medicine,

and regenerative research while retaining biological features that are difficult to reproduce in conventional two-dimensional culture.⁴

The self-organizing capacity that makes organoids biologically informative also creates a persistent engineering challenge. Starting cell state, aggregate geometry, extracellular matrix composition, culture history, and stochastic developmental events can alter cellular composition, topology, and maturation. Limited vascularization and mass transport constrain tissue scale and long-term function, whereas inaccessible luminal surfaces and incomplete stromal, vascular, neural, and immune integration limit physiological interpretation.^{3,5} The central challenge is therefore not simply to produce larger or more anatomically elaborate organoids, but to make their developmental and response trajectories reproducible, measurable, and experimentally controllable.

Three-dimensional bioprinting addresses part of this challenge by controlling the initial placement of cells, matrices, and signaling factors. Extrusion, droplet, laser-assisted, and light-based platforms can standardize starting geometry, establish multicellular interfaces, introduce perfusable channels, and impose local mechanical gradients.⁶⁻⁸ In this role, 3D printing defines the initial and boundary conditions within which self-organization proceeds. Most constructs, however, remain specified primarily at fabrication; subsequent maturation, matrix remodeling, deformation, and adaptation are still governed largely by cells and the culture environment. Spatial precision at time zero is therefore necessary but insufficient to control a living tissue trajectory.

Development and disease are path-dependent processes rather than static three-dimensional states.⁹ Folding, cavitation, branching, regional growth, inflammatory injury, and repair arise through temporally ordered cell behaviors, material remodeling, transport, and force generation; the resulting geometry can then redirect polarity, lineage choice, and tissue mechanics.^{10,11} A vascular network, epithelial fold, or pathological interface is therefore both a structure and a record of the process that generated it. Models that reproduce a terminal tissue state without resolving the intervening transitions may be geometrically sophisticated yet mechanistically incomplete.¹²

In this review, we use multidimensional bioprinting as a capability-based rather than purely numerical concept. Temporal programming controls when and in what sequence a construct changes;¹³ curvature-aware or non-planar deposition controls how cells and materials are organized relative to complex surfaces;¹⁴ environmental responsiveness couples material or tissue behavior

to biologically relevant stimuli;¹⁵ and feedback links measurement of tissue state to active adjustment. These capabilities are complementary rather than sequential measures of maturity. Natural post-printing growth is not sufficient evidence of 4D programming, a curved product does not necessarily require multi-axis or 5D fabrication, and stimulus responsiveness is not closed-loop control unless a measured tissue state is used to alter a subsequent input.

1.1. Evidence framework for evaluating multidimensional claims

To avoid conflating adjacent technologies with direct organoid bioprinting, we classify evidence into three categories. Direct evidence requires implementation of the added capability in an organoid or organoid-derived printed construct together with a biological or functional endpoint. Enabling evidence establishes a relevant material, manufacturing, microfluidic, organ-on-chip, or tissue-engineering principle without demonstrating organoid-specific benefit. Conceptual evidence describes a technically plausible integration that has not yet been realized experimentally. Representative studies are evaluated using a common matrix comprising added capability, model, evidence class, longitudinal measurement, functional outcome, and principal limitation. This distinction is essential because an engineering proof of concept does not, by itself, establish biological value in an organoid context.

We further distinguish technological complexity from integration maturity. Level 0 denotes static 3D spatial patterning; Level 1 adds a single dynamic or non-planar capability; Level 2 couples two or more capabilities within a mechanistically linked design; and Level 3 closes the loop by sensing tissue state, interpreting that state, and actuating a corrective or instructive response. At every level, the causal benchmark is a matched static 3D comparator in which cell source, matrix composition, geometry, and culture conditions are preserved while the added capability is removed wherever feasible. These criteria are summarized schematically and tabulated ([Figure 1](#); [Tables 1 and 2](#)).

The review follows a progression from structure to dynamics, biology, disease, control, and translation. **Section 2** defines the controllable variables that extend beyond static 3D patterning; **Section 3** examines how these variables influence morphogenesis and maturation; **Section 4** focuses on disease trajectories rather than disease snapshots; **Section 5** addresses integration and comparative validation; and **Section 6** considers terminology, materials, sensing, computation, regulation, and over-dimensionalization. The central question focused

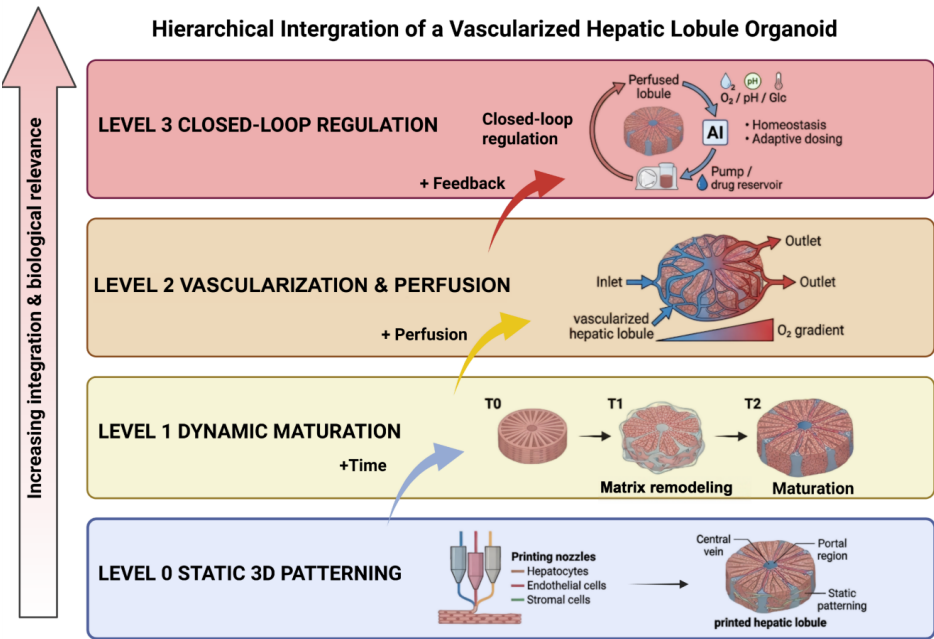


Figure 1. Hierarchical integration of a vascularized hepatic lobule organoid. The framework progresses from static multicellular patterning (Level 0), through dynamic maturation and matrix remodeling (Level 1) and vascular perfusion (Level 2), to sensing–decision–actuation closed-loop regulation (Level 3), with increasing biological integration and relevance. Created in BioRender. Juice, Y. (2026) <https://BioRender.com/eyi6okl>; licensed under CC BY 4.0.

Table 1. Operational distinction between static 3D and multidimensional organoid bioprinting.

Capability	Primary question	Static 3D baseline	Multidimensional extension	Required validation
Spatial patterning	Where do cells, matrices and signals begin?	Initial geometry and composition are specified.	Regional heterogeneity and interfaces are deliberately constructed.	Geometric fidelity, cell viability and matched unprinted or manually assembled controls.
Temporal programming	When and in what sequence does the construct change?	Post-printing evolution is largely uncontrolled.	Degradation, mechanics, shape or signal presentation follow an encoded schedule.	Transformation kinetics linked to lineage progression and organ-specific function.
Curvature control	How does deposition relative to a curved surface affect tissue behavior?	Planar layer-by-layer deposition produces a final 3D shape.	Multi-axis or non-planar paths control orientation, anisotropy and local curvature.	Comparison with a geometrically matched planar-deposition construct.
Environmental responsiveness	How does the construct react to a changing biological state?	A fixed external stimulus is applied to a passive structure.	Material or tissue behavior changes in response to pH, oxygen, metabolites, force or other cues.	Physiological stimulus range, specificity, reversibility and biological consequence.
Feedback control	Can deviation be detected and the trajectory redirected?	Measurements are terminal or observational.	Sensors inform real-time adjustment of perfusion, mechanics or signaling.	Sensor accuracy, control latency, stability, failure modes and improved outcome.

in this review is not how many dimensions a platform claims, but which additional biological processes it enables investigators to measure, predict, or redirect.

2. Expanding the controllable dimensions of organoid bioprinting

A multidimensional bioprinting claim is meaningful only when it specifies a controllable variable beyond the initial spatial arrangement. Conventional 3D printing determines where cells and materials are placed at fabrication. Additional capabilities become relevant when a construct changes, how it is organized along curved surfaces, how it responds to biologically relevant conditions, or how a measured tissue state modifies a subsequent input. These capabilities are complementary rather than hierarchical, and their added complexity must be justified by the biological question.^{16,17}

2.1. Spatial patterning in conventional 3D bioprinting

Spatial patterning remains the foundation of organoid bioprinting. Extrusion, droplet, laser-assisted, and light-based systems can define cell density, matrix composition, channel position, interface geometry, and local stiffness.^{7,8} This control is particularly useful when tissue function depends on ordered interactions among parenchymal, stromal, endothelial, and immune compartments. By standardizing aggregate size and starting geometry, printing can also reduce variation associated with spontaneous assembly.⁶ Thus, 3D bioprinting does not replace self-organization; it defines the initial and boundary conditions within which self-organization proceeds.

However, a spatially accurate starting condition does not constitute a controlled biological trajectory. Printed tissues remain limited by diffusion, incomplete vascular integration, shear-related injury, bioink instability, and trade-offs between printability and cell-permissive mechanics. Higher spatial resolution alone cannot reproduce capillary-scale transport or time-evolving tumor-stroma interfaces, and a mechanically stable ink may become restrictive as cells proliferate and remodel the matrix. Static 3D printing therefore controls where components begin, but generally not when, how, or why their organization changes.

2.2. Temporal programming

Temporal programming requires a designed post-fabrication transition in shape, stiffness, porosity, permeability, degradation, molecular presentation, or cell-matrix coupling.^{18,19} Elapsed culture time alone is not an added dimension. A system is temporally programmed

only when the onset, rate, sequence, or spatial distribution of change is encoded in the material, manufacturing process, or biological circuit and is evaluated against a defined trajectory. This operational criterion distinguishes 4D biofabrication from ordinary maturation after 3D printing.²⁰

Material-driven approaches use swelling, shape-memory behavior, dynamic covalent bonds, light-responsive chemistry or other stimuli to induce predictable changes after printing.^{21,22} These mechanisms can fold sheets into tubes, alter pore architecture, expose cell-adhesive ligands or release instructive factors in a defined sequence. Their value for organoids lies less in visible deformation than in whether the transformation produces a biologically meaningful change, for example, synchronized epithelial bending, improved vascular invasion or stage-appropriate differentiation.²⁰

Biologically driven approaches rely on cell contraction, matrix deposition, fusion and remodeling. In these systems, cells are not passive cargo but active actuators whose forces reshape the printed environment.²³ This can generate tissue emergence that is difficult to pre-fabricate directly, but it also reduces predictability because the response depends on cell state, density and matrix history. Temporal biofabrication therefore requires paired material and biological measurements: deformation kinetics, cell viability, lineage progression and organ-specific function must be tracked together.²⁴

2.3. Curvature and non-planar deposition

Multi-axis printing adds rotational freedom to conventional translational motion, allowing deposition along curved, oblique or continuously changing trajectories.¹⁴ This capability can reduce stair-stepping, improve fiber orientation, limit support-material requirements and align printed features with local anatomical or mechanical axes. For organoid systems, its principal relevance is not that it produces a curved object, but that it controls the direction in which cells and matrices are deposited relative to a curved surface.²⁵

A curved final shape can still be produced by conventional layer-by-layer printing and should not automatically be described as 5D.¹⁴ The more stringent distinction is whether non-planar path planning changes tissue-relevant properties such as epithelial polarity, anisotropic force transmission, tubular continuity or cell alignment. Multi-axis printing is therefore best treated as a curvature-control strategy rather than a higher biological dimension. Its added value must be demonstrated by comparison with a geometrically matched structure fabricated through conventional planar deposition.²⁵

2.4. Environmental responsiveness

Responsiveness extends programmed change from a fixed schedule to a condition-dependent event. Materials may respond to temperature, pH, light, ionic composition, oxygen, glucose, reactive oxygen species, mechanical strain or fluid shear.^{20,26} In principle, this allows a construct to change its mechanics, permeability or molecular output when a disease-relevant variable crosses a threshold. Such behavior is most informative when the stimulus reflects a genuine biological transition rather than a convenient laboratory trigger.

Most current responsive systems remain open loop: a stimulus is applied, the material changes and the biological outcome is measured. A more advanced system would sense the state of the tissue, classify that state and adjust a second variable, for example, increasing perfusion when oxygen falls or releasing a repair cue after barrier disruption.²⁷ The term 6D printing is sometimes used for constructs that combine multi-axis geometry with stimulus-responsive behavior, but this remains a conceptual umbrella rather than a standardized organoid platform.¹⁵

2.5. A capability-based framework beyond numerical labels

The overlapping use of 3D, 4D, 5D, and 6D terminology is best resolved by reporting the underlying operations: spatial patterning, temporal programming, curvature

control, environmental responsiveness, and, where present, feedback.^{15,16,25} A platform may combine several of these capabilities, but their scientific value depends on whether they provide access to a biological process that cannot be reproduced or interrogated adequately in a static 3D system. This capability-based description accommodates different printer architectures and material strategies without implying that every application should maximize the number of nominal dimensions.

Design should therefore be question-driven. Temporal programming is warranted when developmental order matters; curvature control when geometry regulates polarity or force; responsiveness when tissue state changes during the experiment; and feedback when the trajectory must be actively stabilized or redirected. Adding a dimension without a corresponding biological hypothesis increases complexity without increasing explanatory power. The preferred design is the minimal combination of capabilities sufficient to answer the question.

These capabilities should be evaluated as coordinated controls over a single organoid trajectory rather than as isolated technologies. We retain 4D, 5D, and 6D labels where they are used in the literature, but separate those labels from evidence maturity: each biological claim still requires an appropriate static comparator and a trajectory-resolved functional endpoint (Figure 2).

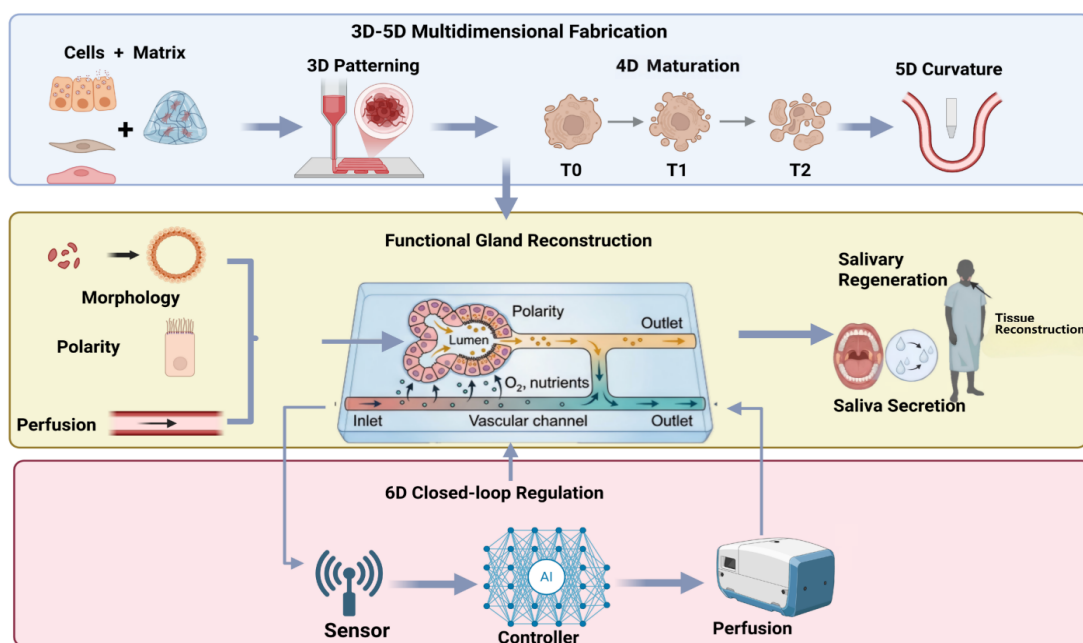


Figure 2. Multidimensional biofabrication for functional gland reconstruction. Spatial patterning of cells and matrices is extended through temporal maturation, curvature control, and sensor-guided closed-loop regulation. These engineering dimensions support tissue polarization, vascular perfusion, and directional secretion toward functional gland regeneration. Created in BioRender. Juice, Y. (2026) <https://BioRender.com/shq9v9sy>; licensed under CC BY 4.0.

3. Translating engineering dimensions into biological processes

Engineering dimensions acquire biological significance only when they alter how cells organize, differentiate, or interact. Development provides a stringent test because proliferation, migration, polarity, lineage allocation, and tissue mechanics evolve together.^{10,11} Multidimensional printing can make selected boundary conditions and perturbations experimentally controllable, but it cannot substitute for the distributed cellular decision-making that drives morphogenesis. Its purpose is therefore to make developmental trajectories tractable without suppressing their emergent character.¹²

3.1. Temporal coordination of differentiation and maturation

Temporal programming is most informative when engineered transitions are synchronized with developmental state. The same ligand or mechanical cue can support progenitor expansion at one stage and impede maturation at another. Sequential changes in matrix properties or signal presentation can therefore separate otherwise conflicting requirements: a scaffold may first support expansion, then soften or degrade to permit rearrangement, and finally present maturation cues.^{28,29} The critical variable is not elapsed time itself, but the correspondence between material transition and cell-state transition.

Spatiotemporally patterned matrices, aptamer-mediated factor presentation, and transformable constructs provide routes to sequence biochemical and mechanical cues.^{29,31} Rather than exposing an entire organoid uniformly, these approaches can generate local developmental waves or stage-specific niches. Their success should be assessed with lineage-resolved trajectories rather than endpoint marker expression, because a similar terminal phenotype can arise through an abnormal or unstable developmental path.

Liver systems illustrate this requirement for temporal coordination. Hepatic function depends on coordinated maturation of parenchymal, endothelial, stellate, and immune compartments under changing oxygen and metabolic conditions. Dynamic matrices and controlled-release systems can separate early tissue assembly from later metabolic maturation or injury induction.³² Coupling these transitions to perfusion and online metabolic measurements can distinguish genuine maturation from transient adaptation or selective survival.^{33,34}

Brain organoids impose even stronger temporal constraints because progenitor expansion, regional specification, neuronal migration, glial maturation, and

network formation occur on different timescales. Bioinks that change stiffness or composition during culture can approximate developmental shifts in the neural extracellular environment, whereas staged assembly can position populations at defined maturational states (Figure 3a).³⁵ Functional validation should therefore extend beyond neuronal marker expression to stable, interpretable circuit activity. Controlled fluid delivery and perfusion provide complementary means of modulating dynamic culture and supporting vascular maturation.³⁶

3.2. Curvature-dependent morphogenesis and epithelial polarity

Curvature control becomes biologically informative when geometry changes cell packing, polarity, force transmission, or lineage behavior.³⁷ Apical constriction, differential growth, and local matrix resistance generate folding and bending; the resulting geometry redistributes stress and, in turn, alters signaling and tissue organization.¹⁰ Multidimensional printing can therefore make curvature an experimentally controlled variable rather than an uncontrolled consequence of organoid growth.³⁸

The distinction between shape replication and curvature-guided morphogenesis is essential. Printing a mature-looking tube demonstrates manufacturing accuracy; creating a boundary that causes epithelial cells to polarize, bend, and form a lumen tests a developmental mechanism.³⁸ Curvature, stiffness, and strain should therefore be quantified together, because an identical geometry can elicit different cellular responses under different mechanical boundary conditions.³⁹

The intestine is an informative testbed because crypt-villus architecture couples curvature to stem-cell maintenance, differentiation, absorption, and directional transport.^{40,41} Conventional closed organoids contain the major epithelial lineages but often present an inaccessible inward-facing lumen and variable topology. Scaffold-guided and photopatterned systems can impose directional growth and establish distinct crypt- and villus-like domains, allowing curvature, polarity, and barrier function to be analyzed within the same construct (Figure 3b).³⁸

3.3. Matrix remodeling and mechanobiological feedback

Temporal material change and mechanobiological feedback are inseparable in living constructs. Cells generate traction, secrete extracellular matrix, degrade crosslinks, and alter local hydration; the remodeled matrix then feeds back on migration, differentiation, and collective behavior.²³ A multidimensional platform should therefore treat the matrix as an evolving participant rather than a fixed

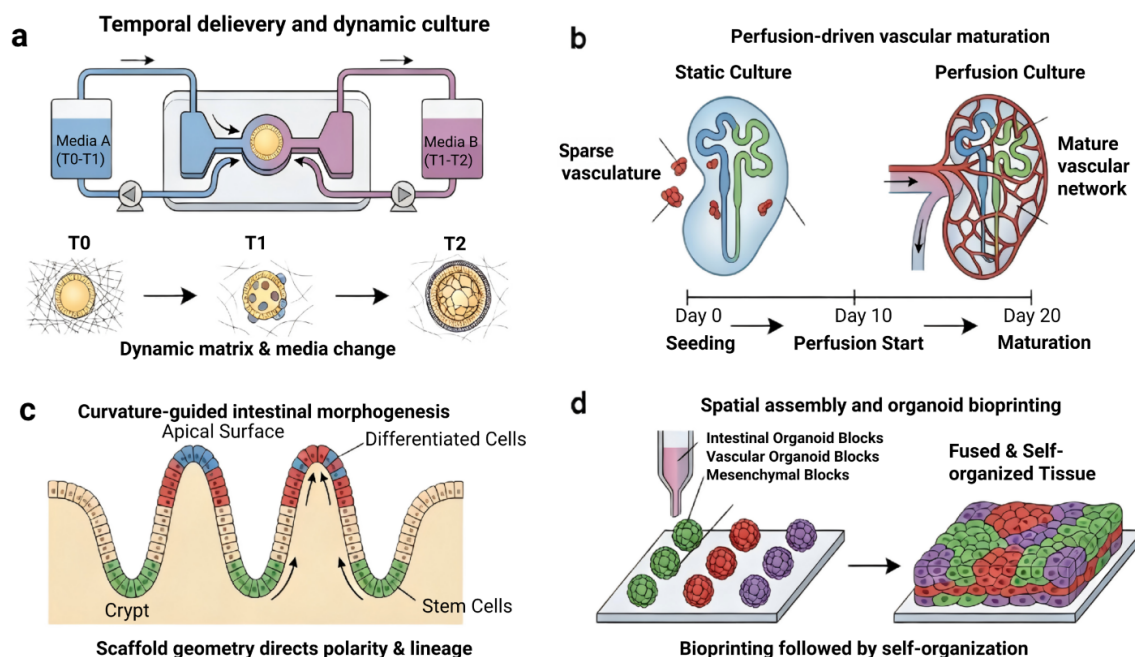


Figure 3. Engineering control of organoid development, maturation, and self-organization. (a) Temporally programmed delivery and dynamic culture coordinate sequential changes in media and matrix properties with successive stages of organoid development. (b) Perfusion promotes vascular expansion and maturation in kidney organoids compared with static culture. (c) Scaffold-defined curvature directs epithelial polarity, crypt-villus organization, and spatial segregation of intestinal stem and differentiated cells. (d) Spatial bioprinting of organoid and supporting-cell building blocks enables their subsequent fusion and tissue-scale self-organization. Together, these strategies illustrate how temporal, transport, geometric, and spatial controls can guide organoid development while preserving cell-driven morphogenesis. Panel (a) is an original schematic informed by Cho *et al.*³⁶ Panels (b–d) were conceptually adapted from Homan *et al.*,⁵⁶ Nikolaev *et al.*,³⁸ and Brassard *et al.*,²⁴ respectively. Created in BioRender. Juice, Y. (2026) <https://BioRender.com/1ype3e5>; licensed under CC BY 4.0.

scaffold. Cell-driven 4D morphogenesis is particularly relevant when tissue form emerges from reciprocal tissue-matrix remodeling rather than from externally imposed deformation.^{42,43}

This reciprocity creates a fundamental design trade-off. A highly stable material preserves printed fidelity but can suppress remodeling, whereas rapid degradation permits self-organization at the cost of geometric control.⁴³ The material may therefore need to shift from a load-bearing template to a permissive developmental niche at a rate matched to cell-generated forces. Quantitative measurements of local strain, degradation, and cell state are required to distinguish intended morphogenesis from structural failure.^{44,45}

Neural tissues demonstrate why material dynamics cannot be reduced to bulk stiffness. Brain-derived matrices, viscoelasticity, and thermoresponsive behavior can influence neurite extension, progenitor fate, and network maturation,⁴⁶ while compositional changes can confound genetic or pharmacological comparisons. Mechanobiological studies should therefore report stress relaxation, degradation, ligand density, and spatial

heterogeneity over time in addition to nominal modulus, thereby resolving reciprocal feedback between matrix mechanics and cell-state transitions.^{47,48}

3.4. Branching, cavitation and regional growth

Branching and cavitation require coupled spatial and temporal control because they depend on repeated, spatially selective decisions. A lung, kidney, or glandular epithelium must initiate a bud, extend it, stabilize a lumen, and determine where the next branch forms.⁴⁹ Static printing can reproduce the terminal network but cannot reveal the feedback that generates it. Time-varying gradients, region-specific compliance, and local cell contractility provide routes to interrogating the sequence rather than the endpoint alone.^{50,51}

Regional growth presents a related challenge because different parts of an organ mature under distinct transport and signaling conditions. In the liver, lobular zonation reflects gradients of oxygen, nutrients, and morphogens across a vascular architecture.⁵² Printed hepatocyte, endothelial, and stromal compartments can reproduce aspects of this organization, but a stronger test is whether

perfusion and adaptive materials generate stable functional zones and whether those zones change appropriately during injury or drug exposure.^{53,54}

Kidney organoids require coordinated nephron patterning, vascular integration, tubular continuity, and outflow. Current systems reproduce nephron-like segments but remain immature and incompletely connected.⁵⁵ Dynamic perfusion and geometrically programmed assembly may help integrate nephron-forming regions with collecting and vascular compartments.^{56,57} The relevant biological test is whether fluid transport and tissue interaction drive maturation beyond that achieved by isolated organoids, rather than whether additional modules can simply be printed (Figure 3c).

3.5. Balancing engineering control with self-organization

Because organoids derive much of their biological value from self-organization, engineering control should be deliberately limited to variables required by the hypothesis. Excessive external constraint can produce a reproducible structure that no longer follows a biologically meaningful developmental program.^{24,37} Printing should define permissible states, interfaces, and perturbations while preserving symmetry breaking, adaptation, and emergent organization.⁴³

This balance favors an iterative design–print–develop–monitor–optimize workflow. Live imaging, embedded sensing, lineage tracing, and computational modeling can identify divergence from the intended trajectory; subsequent design cycles can then adjust geometry, degradation, signal timing, or perfusion.^{58,59} Spatial assembly of printed organoid building blocks can enable subsequent fusion and tissue-scale self-organization (Figure 3d).⁶⁰ Geometrically engineered organoid units and kidney assembloids further illustrate how modular construction may bridge controlled assembly and endogenous integration, although full organ-level connectivity remains unresolved.^{51,61}

4. Capturing disease trajectories rather than disease snapshots

Disease models are commonly judged by whether they reproduce a recognizable endpoint, such as cyst formation, protein aggregation, barrier failure, fibrosis, or inflammatory signaling. Yet disease is path dependent: initiation, adaptation, progression, repair, and treatment response occur on different timescales and in different regions.⁹ Multidimensional bioprinting becomes consequential when it converts these transitions into controllable and observable variables through dynamic

perturbation, longitudinal measurement, and explicit comparison with matched static 3D models.⁶²

4.1. Initiation and progression

Disease initiation requires both spatial definition of the perturbation and temporal resolution of its propagation. Genetic disease makes this distinction explicit: a pathogenic variant may initially affect a single cell type, while tissue-level dysfunction emerges later through paracrine signaling, abnormal mechanics, or altered development.⁶³ Spatially patterned mixtures of mutant, wild-type, and corrected cells can distinguish cell-autonomous from non-cell-autonomous effects and test whether local rescue propagates across a tissue. Patient-derived and immune-enhanced organoids already demonstrate the value of linking genotype and treatment response in a human context.^{64,65}

Multi-material printing can generate mosaics in which normal, mutant, and gene-corrected regions coexist under identical global culture conditions. Such designs are relevant to cystic fibrosis, inherited metabolic disease, and neurodevelopmental disorders,^{66–68} in which tissue context modifies the phenotypic effect of a mutation. Relative positioning of genotypes with respect to vessels, lumens, and supporting cells then makes it possible to determine whether a phenotype spreads, remains locally constrained, or is rescued across tissue boundaries.⁶⁹

Genome editing extends this strategy from reconstruction to intervention. Knockout, base-editing, and prime-editing approaches can generate isogenic controls or repair patient-derived defects.^{69,70} Multidimensional platforms can then assess not only terminal rescue but also the kinetics with which cell behavior, tissue organization, and function recover. Longitudinal imaging and multi-omics are essential because an apparently corrected endpoint may conceal an abnormal intermediate trajectory or persistent compensatory state.⁷¹

Metabolic disease similarly emerges from interacting changes in nutrient exposure, oxygenation, vascular function, inflammation, and matrix mechanics. Liver models should therefore capture the transition from adaptive lipid storage to oxidative stress and inflammatory dysfunction rather than reproduce steatosis at a single time point.⁷² Perfusable, zonated constructs can vary fatty-acid delivery, oxygen tension, and cytokine exposure while resolving region-specific metabolic responses.^{73,74}

Pancreatic and musculoskeletal models extend the same principle. Beta-cell function depends on vascular proximity and fluctuating glucose, whereas osteoarthritis integrates altered loading, inflammation, metabolic reprogramming, and matrix degradation.^{75,76} Dynamic

perfusion, mechanical stimulation, and embedded sensing can distinguish treatments that normalize an acute output from those that alter the longer-term trajectory toward tissue failure.⁷⁷

Across metabolic applications, the decisive readouts are kinetic and functional: glucose-stimulated insulin secretion, oxygen consumption, albumin and urea production, cytochrome activity, matrix synthesis, and recovery after perturbation.⁷⁸ Spatial organization is valuable only insofar as it explains these functions or predicts their decline. Vascularized liver and pancreatic constructs indicate that coupling transport with tissue architecture can improve physiological relevance,^{79,80} but individualized prediction will require standardized longitudinal benchmarks.

4.1.1 Representative scenario: dynamic liver injury and mechanism-resolved toxicity

A static hepatotoxicity assay can detect loss of viability yet miss exposure-order effects, regional susceptibility, and reversible adaptation. A multidimensional liver model should instead combine controlled zonation or perfusion with time-resolved drug exposure and metabolic sensing so that the same construct can be followed from early stress through recovery or irreversible failure.^{72,33} Direct bioprinted liver studies support multicellular spatial organization and perfusable architectures, whereas organoid-on-chip biosensing and dynamic perfusion provide enabling evidence for longitudinal monitoring.⁷⁴ A stronger test will be whether such platforms discriminate mechanisms with distinct kinetics, including mitochondrial dysfunction, phospholipid accumulation, cholestatic transport failure, and delayed inflammatory injury, rather than merely generating a more elaborate endpoint assay.

For drug-development decisions, the relevant benchmark is predictive discrimination. A useful model should reduce false-negative toxicity calls caused by short exposures, reduce false-positive calls caused by transient and reversible stress, and improve ranking of compounds or exposure schedules against known human outcomes. These claims require blinded validation sets and predefined performance metrics; at present, most integrated liver systems provide enabling rather than decision-grade evidence for this use.

4.2. Spatial heterogeneity and pathological gradients

Spatially heterogeneous disease requires explicit control of interfaces and topology, together with longitudinal readouts that determine whether local lesions remain confined, propagate, or resolve. In inflammatory disease,

cytokines, immune cells, vascular activation, and matrix damage concentrate around focal lesions while adjacent regions may remain compensated. Printed gradients and multicellular interfaces can recreate this uneven landscape and expose mechanisms that are obscured when all cells receive the same inflammatory stimulus.⁸¹ Rheumatoid arthritis, inflammatory bowel disease, and osteoarthritis are therefore better represented as evolving tissue ecosystems than as uniformly inflamed cultures.^{82,83}

The intestinal barrier makes topology inseparable from pathology. Crypts, villi, mucus, lumen, immune cells, and stroma determine where microbial products and inflammatory mediators are encountered.⁸⁴ Villus-like scaffolds, centimeter-scale self-organizing tissues, and microfluidic systems can establish directional exposure while measuring permeability, electrical resistance, and repair, thereby converting barrier disruption from a terminal assay into a spatially resolved process.^{41,85}

Combining perfusion, tracer assays, and high-content imaging can resolve the sequence from epithelial injury to immune recruitment and recovery.^{86,87} Independent perturbation of luminal and basolateral compartments makes it possible to determine whether treatment restores barrier function, suppresses inflammation, or merely delays structural failure. Incorporating patient-derived immune cells and microbial components could further support relapse modeling and subtype-specific response analysis.⁸⁸

4.2.1. Representative scenario: intestinal barrier injury, repair and relapse

Conventional 2D monolayers provide accessible apical and basal surfaces but flatten crypt-villus geometry and omit much of the stromal and immune architecture. Closed intestinal organoids retain epithelial self-organization but often conceal the lumen and complicate repeated, side-specific perturbation. A multidimensional design is justified only when the added controls change the inference, for example, by combining curvature-defined epithelial organization, independently addressable luminal and stromal compartments, temporally sequenced inflammatory challenges, and repeated barrier measurements.⁸⁶ Direct organoid-bioprinting studies support controlled organoid placement and tissue emergence, whereas scaffold-guided intestinal morphogenesis and perfused inflammatory systems provide enabling evidence for topology and longitudinal injury readouts.^{38,87} A platform that integrates curvature, repeated inflammatory stimulation, real-time sensing, and adaptive intervention has not yet been demonstrated and should therefore be considered conceptual rather than established 6D organoid technology.

The decisive experiment is a matched comparison with a static 3D construct containing the same cells, matrix, and geometry. Added value would be demonstrated if the multidimensional system identified when barrier failure becomes irreversible, distinguished epithelial from stromal or immune mechanisms of failed repair, or predicted post-treatment recovery more accurately than a single terminal measurement. Remaining bottlenecks include stable long-term luminal access, incorporation of patient-derived immune and microbial components without uncontrolled variability, sensor drift during repeated challenges, and inter-laboratory reference perturbations that define a reproducible injury-repair trajectory.

Immune-mediated and neoplastic diseases add clonal and stromal heterogeneity. Endothelial activation, antigen presentation and immune-cell migration occur across defined interfaces, while tumor and stromal populations generate local metabolic and mechanical niches.⁸⁹ Printed tumor microenvironments and vascularized tissues can place these compartments into interpretable arrangements, provided that spatial complexity is tied to a mechanistic hypothesis rather than reproduced for visual resemblance alone.^{90,91}

Psoriasis, autoimmune vasculopathy and tumor-lymph-node systems illustrate how layered organization can be combined with pathway-specific perturbation.⁹² The field should move beyond asking whether an inflammatory phenotype is present and instead test where it begins, how it propagates and which compartment controls resistance or resolution. Spatially resolved cytokine measurements, clonotype analysis and live-cell tracking are particularly important for this purpose.^{93,94}

4.3. Injury, repair and relapse

Injury-repair models depend on temporal programming and environmental responsiveness because a single exposure captures damage but not the memory of prior episodes. Repeated inflammatory, metabolic, or mechanical insults with controlled intervals can distinguish complete recovery from a primed state or a transition beyond which repair becomes incomplete. Feedback represents a higher level of integration only when the measured tissue state actively modifies a subsequent intervention.

In liver systems, degradable matrices, controlled-release cues, and perfusion can separate reversible metabolic adaptation from inflammatory or fibrotic progression.⁷² Online measurements of metabolism and secreted factors can identify divergence before terminal function is lost.³³ In intestinal models, the same logic applies to barrier injury and repair, where stromal and macrophage states influence whether epithelial regeneration succeeds or stalls.⁸⁷

Kidney models are well suited to repeated-injury paradigms because tubular transport and cell death can be quantified over time. Patient-derived organoids reproduce inherited cystic and Alport-related phenotypes, while nephrotoxics produce dose- and time-dependent injury.⁹⁵⁻⁹⁷ Integrating multiwell printing, perfusion, and automated imaging may distinguish transient stress from maladaptive repair and improve the human relevance of safety testing.⁹⁸

4.4. Immune infiltration and tissue adaptation

Immune infiltration requires spatially defined interfaces, dynamic gradients, and perfusion because recruitment is a sequence rather than a cell-mixing problem. Circulating cells adhere to activated endothelium, cross the vascular barrier, migrate through matrix, recognize antigen, and alter parenchymal function.⁹⁹ Models that begin with immune cells already embedded in tissue cannot resolve these steps. Printed vascular interfaces and chemotactic gradients can therefore make recruitment and tissue entry experimentally separable, whereas closed-loop regulation of recruitment or suppression remains largely conceptual in organoid-bioprinting systems.^{91,100}

Layered skin and psoriasis models link epithelial hyperproliferation to IL-23-IL-17 signaling and T-cell localization, while lymph-node and tumor-lymph-node organoids provide complementary settings for antigen presentation, clonal expansion, and immune suppression.⁹²⁻⁹⁴ Connecting these compartments through perfusable routes and tracking the emergence of response or resistance during treatment would move the models from static co-culture toward dynamic immune-tissue interaction.

Tissue adaptation also depends on stromal, neural, and vascular circuits that are absent from many organoids. Sensory neurons and Schwann cells can modify inflammation and repair in skin, whereas endothelial and fibroblast states shape immune retention and drug access.^{89,101} Additional cell types should therefore be introduced only when their spatial or temporal role is experimentally testable, rather than treating cellular complexity as a surrogate for fidelity.

4.5. Chronic degeneration and treatment response

Chronic degeneration requires longitudinal measurement and controlled perturbation because a static endpoint cannot distinguish progressive decline from acute injury. In neurodegenerative disease, protein aggregation, mitochondrial dysfunction, synaptic failure, neuroinflammation, and blood-brain barrier disruption accumulate over prolonged periods and may progress at

different rates across regions.¹⁰² Printed neural tissues can define spatial relationships among neurons, glia, and endothelium, but repeated functional measurements are required to establish that the system captures degeneration rather than a single injury episode.^{48,103}

Bioprinted cortical and core-shell Alzheimer models reproduce aspects of amyloid and tau pathology, oxidative stress, synaptic impairment, and microglial interaction.¹⁰⁴⁻¹⁰⁶ Their principal advantage is the ability to follow plaque formation, network deterioration, and cell migration within an accessible three-dimensional environment. Regional vulnerability can then be tested by varying genotype, matrix, perfusion, or inflammatory exposure across defined compartments.

Microfluidic perfusion, electrophysiology, reporter lines, and live imaging can convert these systems into repeated-measure platforms.¹⁰⁷⁻¹⁰⁹ Treatment response should be judged by trajectory-level outcomes, such as delayed decline, restored connectivity, reduced barrier leakage, or prevention of pathological spread, rather than by a single terminal biomarker. Related sensory-organ models indicate that the same principles can extend to injury and regeneration beyond the central nervous system.

5. Building integrated and testable multidimensional organoid platforms

The preceding sections establish how individual capabilities can expose developmental and disease processes. The next challenge is integration without conceptual inflation. A platform becomes genuinely multidimensional when its components form a causal chain: a controlled input changes a defined tissue process, that process is measured longitudinally, and the resulting evidence is evaluated against an appropriate static 3D counterfactual. The integration levels introduced in **Section 1.1** provide the corresponding design and validation framework ([Figure 2](#)).

5.1. Matching control dimensions to biological questions

The biological question should determine the added capability. Differentiation order requires temporal control; epithelial polarity requires explicit control of curvature and mechanics; relapse requires repeated perturbation and a measurable memory state; and adaptive therapy requires sensing linked to feedback. This mapping avoids the common failure mode in which responsive materials or multi-axis printing is added without changing the hypothesis, readout, or inference supported by the experiment.

A practical design matrix should specify the target

process, controlled input, expected intermediate state, functional output, and static comparator. A pH-responsive intestinal model, for example, is informative only if pH varies within a disease-relevant range, triggers a defined material or biological response, and improves modeling of barrier injury or drug release. An otherwise identical non-responsive construct is required to show that the response, rather than the underlying 3D architecture, caused the observed effect.

5.2. Levels of multidimensional integration

Level 0 is static 3D patterning: the initial geometry and composition are controlled, but subsequent evolution is not. Level 1 adds a single dynamic capability, such as programmed degradation, non-planar deposition or stimulus-dependent release.^{14,18} Level 2 couples capabilities, for example, curvature with time-dependent deformation, or spatial heterogeneity with local responsiveness. Level 3 is a closed-loop adaptive system in which tissue state is sensed, interpreted and used to modify perfusion, mechanics or signaling.²⁷ These levels describe experimental maturity, not a universal naming convention.¹⁵

Most organoid studies currently occupy Levels 0 or 1. A smaller number of couple dynamic matrices with morphogenesis, perfusion or spatial patterning, approaching Level 2.^{24,32} Closed-loop Level 3 systems remain rare because reliable sensing, state classification and actuation must operate without disrupting the tissue.²⁷ The framework is nevertheless useful because it identifies the missing link in a proposed platform and prevents conceptual claims from outrunning experimental evidence.

5.3. Proving added value beyond static 3D printing

Every multidimensional claim requires a matched 3D comparison. Wherever feasible, the comparator should preserve cell source, matrix composition, geometry, and culture conditions while removing only the dynamic, responsive, or multi-axis feature under study. Without this counterfactual, improved maturation may reflect a different bioink, altered cell density, or more favorable perfusion rather than the claimed capability. Studies of printed kidney organoids, guided brain organoids, intestinal tissues, and macroscale self-organization show that controlled geometry can alter biological outcomes,^{6,35,38} but the source of the gain must be isolated experimentally.

Added value should be assessed across five domains: trajectory fidelity, functional maturity, reproducibility, predictive performance, and interventional control. Stronger systems reproduce the correct temporal order, achieve organ-specific function, reduce batch variation,

predict patient or drug response, and can be redirected after deviation.^{59,110,111} Morphological complexity is not a substitute for any of these outcomes.

The cost of complexity must also be reported. Multi-axis hardware, smart materials, sensing modules, and long-term imaging can reduce throughput and introduce additional failure modes. A multidimensional platform is justified only when the additional information or control cannot be obtained more simply. This constraint is especially important in drug screening, where modest gains in biological fidelity may be outweighed by reduced scalability or poor inter-laboratory reproducibility.

5.4. Evidence hierarchy and maturity of the field

Applying the three evidence classes introduced in **Section 1.1** reveals an uneven maturity landscape. Direct evidence implements the added capability in an organoid or organoid-derived printed construct, whereas enabling

evidence establishes a relevant material, manufacturing, microfluidic, organ-on-chip, or tissue-engineering principle. Conceptual evidence refers to plausible integrations that have not yet been experimentally realized. Validation strength should be assessed separately according to whether engineering, biological, functional, and longitudinal endpoints are reported.

By this standard, automated organoid printing and selected temporally programmed organoid systems have direct evidence, although longitudinal functional validation remains limited.^{6,32} Multi-axis printing has strong engineering and tissue-fabrication evidence but comparatively little organoid-specific demonstration.¹⁴ The 6D label is used inconsistently: the formulation introduced by Georgantzinos and colleagues¹⁵ couples multi-axis fabrication with stimulus-responsive behavior, whereas closed-loop sensing and actuation represent a separate integration-maturity criterion rather than an

Table 2. Evidence matrix for representative studies supporting multidimensional organoid bioprinting

Study	Added capability	Model	Evidence class	Longitudinal measurement	Functional outcome	Major limitation
Lawlor <i>et al.</i> ⁶	Automated extrusion/spatial control	Kidney organoids	Direct	Limited; production/end-point comparison	Improved reproducibility and conformation	Primarily static spatial control
Brassard <i>et al.</i> ²⁴	Bioprinting-assisted tissue emergence	Intestinal/tubular organoid-forming tissues	Direct	Yes; self-organization over time	Macro-scale self-organization and tissue architecture	Dynamic control is largely cell-driven
Cadena <i>et al.</i> ³⁵	Printed scaffold + controlled geometry	Cortical organoids	Direct	Yes; calcium/network monitoring	Developmental patterning and functional readout	Not a closed-loop adaptive system
Urciuolo <i>et al.</i> ³²	Live bioprinting / local hydrogel patterning	Organoids and organotypic cultures	Direct	Yes; growth/remodeling monitored	Spatial guidance of organoid development	Capability depends on application-specific validation
Nikolaev <i>et al.</i> ³⁸	Scaffold-guided curvature + perfusion	Mini-intestines	Enabling/adjacent	Yes	Accessible lumen, crypt-like domains, homeostasis	Not a bioprinting study
Homan <i>et al.</i> ⁵⁶	Dynamic flow/perfusion	Kidney organoids	Enabling/adjacent	Yes	Vascularization and maturation under flow	Organ-on-chip/perfusion rather than bioprinting
Gao <i>et al.</i> ¹¹²	Bioprinted curvature + flow heterogeneity	Human-scale intestinal model	Enabling/adjacent	Yes	Regional epithelial specialization and transport	Not a stem-cell organoid platform
Whitehouse <i>et al.</i> ¹⁰⁶	3D-bioprinted disease architecture	Alzheimer-related neural model	Direct	Yes	Connectivity and neurite phenotyping	Dynamic capability beyond 3D is limited
Georgantzinos <i>et al.</i> ¹⁵	Multi-axis + stimulus-responsive 6D concept	Smart structures	Conceptual	No organoid longitudinal validation	Defines coupled manufacturing concept	No organoid-specific demonstration

alternative definition of 6D. Treating these as separate axes, including capability label and integration level, makes the framework compatible with the literature while avoiding the implication that 6D organoid systems are already mature.¹⁵

Minimum reporting should include printer kinematics, path strategy, material composition, stimulus range, transformation kinetics, cell viability, trajectory-resolved biological measurements, and a static comparator. Closed-loop systems additionally require sensor accuracy, control latency, actuation range, and failure behavior. These parameters allow readers to determine whether an added dimension is mechanistically necessary or merely descriptive.

5.5. Organ-specific testbeds and functional benchmarks

Organ systems differ in the dynamic capability they are best positioned to test (Table 3). The liver is suited to studying zonation, metabolic adaptation and injury–repair transitions because function can be measured through albumin, urea, cytochrome activity and regional metabolism.¹¹³ Perfusable vascular networks and responsive matrices should be evaluated by whether they stabilize physiologically distinct zones and predict drug-induced dysfunction, not simply whether they improve survival.^{33,72}

Brain organoids test temporal patterning, dynamic mechanics and functional connectivity. Printed scaffolds can constrain geometry and support vascular or regional organization,³⁵ while tissue-specific matrices can influence neurite extension and maturation. The decisive benchmarks are electrophysiological activity, synaptic connectivity, region-specific identity and reproducible responses to stimulation.^{36,110} A construct with convincing anatomy but unstable network behavior remains an incomplete neural model.

Intestinal systems test curvature, epithelial polarity and reversible barrier injury. Scaffold-guided morphogenesis, tissue-specific bioinks and multicellular co-culture can establish accessible luminal and stromal compartments.³⁸ Functional benchmarks include transepithelial resistance, permeability, directional transport, lineage balance and recovery after inflammatory challenge.^{86,41} These outputs directly distinguish a dynamic mucosal model from a static epithelial structure.

Kidney organoids test geometric control of nephron formation, perfusion-dependent maturation and longitudinal toxicity. Automated printing improves starting consistency, while assembloid and collecting-system strategies seek to connect nephron, vascular and outflow modules.^{6,56} Acceptance criteria should include segment identity, transport, injury kinetics, reproducibility and predictive nephrotoxicity.¹¹¹ The persistent absence

Table 3. Organ-specific testbeds for multidimensional control and functional validation.

Organoid system	Key dynamic bottleneck	Most informative added capability	Functional benchmarks	Critical static 3D comparator
Liver	Metabolic zonation, vascular transport and transition from adaptation to injury.	Temporal perfusion and metabolic responsiveness coupled to spatial zonation.	Albumin, urea, cytochrome activity, oxygen consumption and regional metabolic profiles.	Same multicellular geometry without dynamic perfusion or responsive material behavior.
Brain	Stage-dependent regionalization, tissue mechanics and network maturation.	Temporal mechanics, curvature-guided patterning and activity-responsive monitoring.	Electrophysiology, synaptic connectivity, region identity and stimulus response.	Same neural populations and geometry in a mechanically static matrix.
Intestine	Crypt–villus topology, accessible polarity and reversible barrier injury.	Curvature control, sequential niche signals and inflammatory responsiveness.	Transepithelial resistance, permeability, transport, lineage balance and repair kinetics.	Same epithelial–stromal composition without programmed topology or dynamic challenge.
Kidney	Nephron maturation, perfusion, tubular integration and outflow.	Geometry-controlled assembly, shear-responsive perfusion and longitudinal injury monitoring.	Segment identity, reabsorption, transport, injury kinetics and nephrotoxicity prediction.	Same progenitors and cell number in a static aggregate or printed geometry without flow.

of mature perfusion and urine outflow remains a more important limitation than the number of printed modules.¹¹⁴

Across organ systems, the most informative platform is not one that applies the same technical configuration indiscriminately, but one that matches each added capability to a tissue-specific bottleneck and evaluates it with a function that matters for that organ. This comparative approach turns organ examples from a second catalogue of applications into experimental testbeds for the general framework.

6. Challenges and outlook

Multidimensional organoid bioprinting is shifting the field from printed form toward controllable tissue trajectories, but progress depends on resolving conceptual, material, computational, sensing, validation, and regulatory constraints. The objective is not to normalize ever-higher dimensional labels, but to establish platforms in which timing, curvature, mechanics, transport, and feedback can be manipulated precisely enough to answer biological questions and reproducibly enough to support cross-laboratory comparison.

6.1. Terminology and conceptual standardization

The field requires definitions based on observable operations. A 4D claim should specify the programmed post-printing change;¹⁸ a multi-axis or 5D claim should specify the additional kinematics and their functional consequence;¹⁴ and use of the term 6D should state explicitly which literature convention is being followed. In its original additive-manufacturing formulation, 6D combines multi-axis fabrication with stimulus-responsive behavior rather than necessarily implying a closed-loop controller.¹⁵ We therefore use 6D only as a literature-facing label for that coupled capability and use Level 3, independently, to denote true sensing–decision–actuation feedback. This distinction is more useful because it separates printer/material capability from control-system maturity and allows different hardware architectures to be compared on what they actually do.

6.2. Predictable and biologically permissive materials

Smart bioprints must satisfy conflicting requirements. They must print reproducibly, protect cells during fabrication, retain shape initially, permit later remodeling, and respond within a biologically relevant range.^{20,115} Their mechanical and chemical evolution must remain predictable after cells begin to secrete matrix and degrade the original scaffold. Tissue-derived matrices offer biological specificity but

introduce compositional variability; synthetic systems offer control but may lack instructive cues.⁴³ Hybrid materials will be useful only if both sources of variation are measured.

6.3. Multiscale computational design

Path planning and deformation modeling should be linked to cell-state data. Existing simulations can predict stress, swelling or shape change,^{14,116} but organoid outcomes also depend on proliferation, migration, matrix remodeling and transport. Multiscale models must therefore connect printer motion and material mechanics to tissue-level morphogenesis and molecular state.⁶⁰ Prospective prediction—not retrospective fitting—should be the benchmark for computational value.

6.4. Real-time sensing and closed-loop control

Longitudinal measurement is the bridge between dynamic design and dynamic biology. Perfusion, embedded oxygen or metabolic sensors, live imaging, electrophysiology, and secreted-factor analysis can detect deviation before terminal failure, whereas single-cell and spatial profiling provide deeper state resolution but are usually destructive and sparse in time.^{33,58} The technical barrier to Level 3 control is not simply adding a sensor. Sensors must remain biocompatible during prolonged culture, resist biofouling and calibration drift, and report within a range that can be related to tissue state.^{86,107} Real-time data processing must distinguish true trajectory changes from noise and combine continuous low-dimensional signals with intermittent molecular measurements. Actuators—such as pumps, valves, release modules, photochemical switches, or mechanical drivers—must deliver stable, reversible, and spatially resolved responses without perturbing the tissue more than the signal they are intended to correct. Control latency, actuator saturation, sensor failure, and fail-safe behavior should therefore be reported as core biological-model parameters rather than engineering details.

6.5. Functional benchmarks and reproducibility

Standardization must extend beyond print parameters to biological acceptance criteria. These should include starting-cell quality, structural consistency, lineage composition, maturation state, functional output, trajectory variance, and response to reference perturbations.^{6,111} Different platforms may produce visually similar constructs through different biological routes; trajectory-resolved benchmarks are needed to detect this divergence. Multi-site studies and blinded replication will be essential before multidimensional systems can support clinical or industrial claims.¹¹⁴

6.6. Translation, manufacturing and regulatory readiness

Translation requires different validation pathways depending on whether the multidimensional construct is intended as a living therapeutic product or as a nonclinical model. For implantable or regenerative products, the defining challenge is that time-evolving performance conflicts with quality-control logic built around a product released at a single time point. In the European Union, tissue-engineered products can fall within the advanced-therapy medicinal-product framework, which places emphasis on manufacturing control, quality, safety, and efficacy; analogous classification in the United States depends on the product's composition, manipulation, intended use, and regulatory pathway.¹¹⁷ For dynamic products, release testing at fabrication is therefore insufficient. Developers will need bounded trajectory specifications—for example acceptable ranges for deformation kinetics, degradation, potency, trigger-response behavior, and failure states—together with non-destructive monitoring that links those critical quality attributes to function.

For organoid models used in drug development, the regulatory question is context of use rather than product release. The U.S. Food and Drug Administration (FDA)'s 2026 draft guidance on new approach methodologies emphasizes validation frameworks, study design, reporting, and evidence that a new approach methodology (NAM) is reliable for the proposed regulatory purpose, while the Organization for Economic Co-operation and Development (OECD)'s second-edition Good *In Vitro* Method Practices (GIVIMP) guidance emphasizes defined test systems, controls, standard operating procedures, performance, reporting, and record retention.^{118,119} The FDA roadmap on reducing animal testing further places organoids and other advanced *in vitro* systems within a broader transition toward validated human-relevant methods.¹²⁰ For multidimensional organoid platforms, this means that inter-laboratory reproducibility, reference compounds or perturbations, prespecified acceptance criteria, sensor and actuator calibration, and locked analysis pipelines are not ancillary requirements; they are prerequisites for regulatory confidence.

Decision-relevant validation should also be explicit. In drug screening, a multidimensional model should be tested for whether it reduces false-positive or false-negative efficacy and toxicity calls, detects mechanism-specific liabilities that depend on exposure duration or tissue region, and improves prediction beyond a matched static model. In precision medicine, temporally sequenced combination therapy is a particularly relevant use case because treatment order, washout, recovery, and relapse

can be studied in the same patient-derived construct. Claims of individualized prediction should nevertheless require prospective or blinded concordance with patient response rather than retrospective biological plausibility. Recent studies on head and neck squamous cell carcinoma (HNSCC) illustrate this translational need: multi-omic profiling has resolved metastatic cell-identity reshaping,¹²¹ patient-derived oral squamous cell carcinoma (OSCC) organoids have enabled pre-chemotherapy drug-response testing,¹²² and patient-derived HNSCC organoid models have been used to evaluate chemotherapy combinations linked to tumor stemness and chemoresistance.¹²³

6.7. Avoiding over-dimensionalization

A dimensional arms race is a genuine methodological risk. Combining multi-axis hardware, smart materials, microfluidics, sensors, and artificial-intelligence control can increase system complexity, reduce throughput, and create new failure modes without improving the biological inference. The correct counterfactual is therefore not a lower-dimensional label but the simplest platform capable of answering the same question. New dimensions are justified when they expose a previously inaccessible state transition, improve causal discrimination, increase predictive performance, or enable intervention; they are not justified by visual sophistication or technological novelty alone. Negative comparisons in which an added dimension fails to improve the relevant endpoint should be considered informative because they define where complexity is unnecessary.

This principle also improves reproducibility and translation. Minimal-sufficient designs reduce the number of parameters that require calibration, make matched controls feasible, and allow failure modes to be localized. Reporting should therefore include not only what capabilities were added but which simpler alternatives were considered, what incremental information each module contributed, and whether that information changed a biological or translational decision.

The broader opportunity is to move organoid bioprinting from reproducing tissue form toward experimentally controlling tissue-state transitions. Static 3D printing established the spatial placement of living components; the next challenge is to determine when those components should change, how curvature and force redirect their behavior, how pathological states emerge, and whether trajectories can be measured and corrected. Multidimensional biofabrication will be scientifically consequential only when it improves interpretability, prediction, or intervention without compromising the self-organization that gives organoids their biological value.

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