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REVIEW ARTICLE

Integrated applications of microfluidics, organoids, and 3D bioprinting in *in vitro* 3D biomimetic models

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Abstract

Biomedical research has long faced challenges in accurately replicating human organ microenvironments and overcoming interspecies biological differences, thereby limiting the in-depth understanding of physiopathological mechanisms and hindering the development of cutting-edge therapeutic approaches. Recently, novel technologies such as organoids, microfluidics, and three-dimensional (3D) bioprinting offer promising solutions, fostering innovation, and accelerating progress in biomedical science. However, none of these technologies alone can serve as a fully representative preclinical model, underscoring the need for integrated approaches. This review provides a comprehensive overview of various strategies combining microfluidics, organoids, and 3D bioprinting to develop more physiologically relevant preclinical models. After briefly introducing each technology, we examine the advantages of their pairwise integrations and discuss their prospects for drug research, disease modeling, and beyond. In addition, we explore the potential of combining all three technologies, including the emerging concept of 4D culture systems, which incorporate the temporal dimension to better mimic dynamic biological processes. We anticipate that these integrated models will propel significant advances in biomedical research and contribute to the transformation of future healthcare.

Keywords: 3D bioprinting; Combinations, *In vitro* 3D biomimetic models; Microfluidics; Organoids

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1. Introduction

Advances in developmental biology, disease mechanism research, and drug discovery continue to be constrained by the limitations of current experimental models. To advance the development of physiologically relevant *in vitro* systems for biomedical applications, there is a pressing need for engineered models that faithfully replicate

critical histological characteristics—including tissue-specific architecture, cellular heterogeneity, and functional microdomains—that mirror the structural organization and core biological activities of native human tissues. The structural and functional development of organisms, along with the dynamics of organ homeostasis, are orchestrated by physical and spatial cellular interactions. These interactions simultaneously modulate the epigenetic landscape and gene expression and are dynamically shaped by environmental cues, establishing a continuous feedback loop that regulates organ function.[1] Organogenesis is governed not only by stimuli from the immediate local microenvironment but also by influences from other tissues and systemic factors, such as fluid flow and mechanical forces.[2,3] Therefore, to develop clinically relevant model systems, it is essential to replicate the microenvironment of human organs or tissues using advanced technologies, such as three-dimensional (3D) cultures, extracellular matrix (ECM) supports, and microfluidic devices. These approaches aim to reconstruct the complex *in vivo* microenvironments that cells inhabit.[4]

Even though creating such advanced experimental models remains technically challenging, research efforts continue to surge in addressing these barriers. Animal models are the most widely used approach; however, due to interspecies differences, they often fail to accurately simulate and predict physiopathological processes and responses to interventions as they occur in humans. With advancements in cell biology and tissue engineering, cell cultures were initially performed on two-dimensional (2D) substrates such as Petri dishes and porous plates, where cells adhere and grow along the flat surfaces. However, traditional 2D culture systems cannot recapitulate the complex *in vivo* environment of human tissues, resulting in altered cell morphology and function, and limiting the development of tissue-specific architecture.[5] Despite these limitations, the monolayer cell culture method remains valuable in the early stages of drug testing and compound screening due to its cost-effectiveness and ease of use.[6] To address the limitations of both 2D models and species-specific differences in animal models, 3D cellular models derived from human cells have emerged. Among these, organoids have gained attention for their ability to self-organize into complex structures and simulate functional tissue states.[7] Organoids more closely resemble *in vivo* organs in terms of gene transcription, protein expression, functional metabolism, and microstructure. Nonetheless, they are limited by issues such as incomplete maturation and functionality, low accessibility, high heterogeneity, and inconsistent model readouts.[8–11] The advent of microfluidic technologies has further revolutionized the ability to recapitulate *in vivo*-like microenvironments by enabling precise spatiotemporal control of fluid dynamics. This facilitates biomimetic regulation of critical parameters such as shear stress, nutrient gradients, and intercellular communication.[12] Microfluidics not only enhances nutrients and metabolic waste transport but also supports the integration of external stimuli (e.g., mechanical forces) and *in situ* monitoring of key parameters (e.g., pH), thereby contributing to the development of more robust human models. However, challenges remain, such as limited spatial control precision, low scalability, and reliance on manual processing. In addition, the integration of novel 3D bioprinting concepts into model fabrication enables more accurate construction of diverse cellular microenvironments. It provides physical boundaries to guide morphogenesis and facilitates signaling regulation across temporal and spatial dimensions. However, these pre-determined model structures may reduce intercellular communication and hinder the efficiency of material transport.[13] Table 1 briefly summarizes key comparisons among these technologies.

Although many novel technologies have made significant progress in addressing the challenges of *in vitro* model construction, several critical issues persist. First, current models still fall short in simulating the biological complexity of real *in vivo* environments, especially in terms of cellular functions, cell–cell interactions, and tissue–tissue integration.[14] Second, technological limitations hinder scalability, throughput, and cost-effectiveness, restricting widespread application. In addition, improving model maturity, functionality, and stability while accurately replicating the human tissue microenvironment remains a top priority.[15] Therefore, integrating organoid culture, microfluidic systems, and 3D bioprinting—each offering distinct advantages—holds great promise for advancing the field of bioengineering. This paper first describes the individual technologies: organoids, microfluidics, and 3D bioprinting. It then focuses on the characteristics and applications of combining these technologies in areas such as drug research, disease modeling, immunological research, clinical therapy, and interdisciplinary integration. Finally, the paper discusses existing studies involving all three technologies and proposes an innovative 4D cultivation model that incorporates the temporal dimension.

2. Brief introduction to microfluidics, organoids, and 3D bioprinting

2.1. Microfluidics

Microfluidics is a system for studying and manipulating fluid flow at the sub-millimeter scale. It enables precise control of key factors such as the physico-mechanical properties and chemical composition of *in vitro* models. This technology primarily utilizes microfluidic chips to

Table 1. Summary of microfluidics, organoids, and 3D bioprinting

Technology	Advantages	Disadvantages	References
 Microfuildics	Replicates key structures and functions; accurate fluid flow control; dynamic mechanical simulation; real-time monitoring; high-resolution imaging	Limited simulation of microscale properties; restricted spatial shaping capability; manual operations	26,200–202
 Organoids	Organ specificity; similarity to primary tissues; natural cellular interactions and secretory functions	Limited maturity and functional; limited accessibility; static culture mode; limited spatial organization; heterogeneity; lack of external control, low readability	40,125,200,203
 3D bioprinting	Superior spatial control; fabrication of complex spatial structures; biomimetic fidelity; automated and high-throughput processes	Reduced cell–cell interaction; difficulties in nutrient/oxygen delivery and waste removal; physicochemical control limitations; discrepancies between bioprinted structures and native tissues; impaired cellular activity	15,93,204

Note: Figures were created in BioRender. Liang, L. (2025) https://BioRender.com/6zt3gft

support cell cultures that more closely resemble *in vivo* conditions.[16] Beyond being a mere tool, microfluidics is a multidisciplinary field that integrates principles from physics, engineering, chemistry, and biology to improve the biological relevance of experimental models and the accuracy of experimental results.[17,18]

The core component of microfluidic technology is the microfluidic chip. The first such chip in human history was the organ-on-a-chip reported by Huh et al., designed to mimic the natural anatomy of the target organ by incorporating key functional components essential for replicating physiological functions. Structurally, the chip successfully recreated a critical anatomical feature—the alveolar–capillary interface–by co-culturing human alveolar epithelial cells with microvascular endothelial cells. Functionally, the system exhibited barrier integrity and permeability, surfactant production, and the ability to mimic physiological respiratory movements through a computer-controlled vacuum system that induced cyclic stretching of the tissue–tissue interface.[19]

With the exponential growth of multidisciplinary research, microfluidics has undergone significant expansion and technological advancement. Improvements in precision and stability of fluid flow control have not only effectively improved its ability to transport a wide range of components (e.g., nutrients, metabolic wastes, oxygen, and immunomodulatory factors), but also permitted it to exert dynamic mechanical forces (e.g., vascular shear stress, intestinal peristalsis, skin tensions) within the cellular microenvironment. These capabilities make microfluidic systems an excellent platform for designing novel bioassays that allow both the precise manipulation of experimental parameters and seamless integration with other techniques.[20]

Models based on microfluidic devices have been widely developed across various biological systems. For example, a lung cancer brain metastasis model consists of two biomimetic units—an upstream "lung" and a downstream "brain"—connected by a functional region mimicking the blood–brain barrier (BBB). By monitoring, in real time, the progression from primary tumor growth to BBB penetration and eventual invasion of the brain parenchyma, researchers identified aldo-keto reductase family 1 B10 (AKR1B10) as a potential serum biomarker for patients with brain metastases of lung cancer. The study also suggested that AKR1B10 may play a role in mediating cancer cell extravasation.[21] Similarly, microfluidic systems have been applied in culturing ovarian cancer spheroids to assess the feasibility of multi-class drug sensitivity assays. The stable transport of substances and controlled

modulation of the microenvironment ensured the stabilization of the cell lines, further demonstrating the technique's ability to regulate spatiotemporal variables and generate precise flow patterns at the microscopic scale.[22] Furthermore, robust and streamlined microfluidic chips enable the incorporation of dynamic mechanical forces—such as fluid flow and shear stresses—into tumor spheroid cultures, providing a valuable paradigm for constructing physiologically relevant *in vitro* models.[23,24]

Undeniably, while microfluidics enables precise control of fluid dynamics at the microscopic scale, the models produced can only replicate key structural and functional features of target organs or tissues and remain biologically distinct from native organs.[25] Additionally, microfluidic techniques alone lack spatial refinement for complex model sculpting and have yet to meet established benchmarks for advanced biomedical applications.[26] In addition, the initial setup and cell seeding processes typically require manual operation, underscoring the urgent need for integration with complementary technologies to automate procedures, streamline data acquisition, and improve system accessibility.[27]

2.2. Organoids

Organoids are 3D structures derived from pluripotent stem cells (PSCs) or tissue-derived adult stem cells (AdSCs). They undergo growth and differentiation processes that mimic the tissue of origin and ultimately consist of organ-specific cell types. These cells self-organize through lineage commitment, cell sorting, and spatial restriction.[28]

In general, organoids derived from PSCs are engineered based on the principle of sequential stem cell differentiation. Successful generation requires the timely and sequential addition of relevant signaling factors to guide cell fate decisions and support self-organization. Growth factors are administered in a sequence that mirrors embryonic development to establish correct regional identities and enable the identification of organ-specific lineages using unique biomarkers or functional assays. In contrast, AdSC-derived organoids are relatively simpler to generate. The process involves isolating tissue-specific stem cell populations, embedding them in an ECM, and providing defined combinations of growth factors to support their proliferation and differentiation.[29,30] While PSC-derived organoids can give rise to complex structures such as vasculature and immune components by incorporating diverse cell types, they often require prolonged development times and present challenges in maintaining stable propagation over multiple passages. These factors limit their immediate application in disease modeling.

In contrast, AdSCs-derived organoids, sourced from patient tissues, retain the genetic background of the donor and demonstrate significant potential for personalized medicine. Notably, PSCs can also be derived autologously.[31] Both types of organoids can effectively mimic the pathophysiological characteristics of patient tissues, offering powerful platforms for developing *in vitro* personalized disease models. Nevertheless, AdSC-derived organoids are generally restricted in their differentiation capabilities and typically recapitulate only specific parts of a given organ. For example, adult intestinal stem cells can differentiate exclusively into intestinal epithelial cells. Furthermore, inter-individual variability and high levels of heterogeneity underlie the instability of these organoids during long-term culture.[29] Organoid technology is firmly rooted in classical developmental biology, drawing from extensive research in cell dissociation and recombination. By imposing rational spatial constraints, this innovative approach effectively guides the fate of progenitor cells, ultimately shaping the morphogenesis and organization of organoids.[32,33] Building on this foundation, organoids formed through the spatial organization of multiple cell types—referred to as assembloids—allow for a more profound exploration of tissue functionality.[34]

Organoids often closely resemble human tissues, exhibiting microscale organization and physiological functions that are more similar to those of natural organs. These attributes were demonstrated by Sheridan et al.,[35] who created embryonic trophoblast organoids. The cultured tissues not only successfully differentiated into syncytial and extrachorionic trophoblast structures but also functionally secreted placenta-specific peptides and hormones. For this very reason, organoids have been widely used in various areas of biological research. Patient-derived organoids have been demonstrated as functional models for predicting the pharmacological effects of anticancer agents. Culture systems established from specimens representing different stages of disease progression and histological grades in bladder cancer effectively maintain the histopathological features and molecular heterogeneity of the original tumors, including their multiclonal genetic characteristics. These culture systems consistently exhibit key genomic variation patterns that mirror those of parental tumors, with dynamic changes corresponding to tumor evolutionary trajectories observed through longitudinal analyses.[36] In addition, the differentiation of human embryonic stem cells into thymic epithelial progenitor cells through precise regulation of developmental signaling molecules, including fibroblast growth factor 8, retinoic acid, sonic hedgehog, noggin, and bone morphogenetic protein 4, has further demonstrated the great potential of organoids

for biomimetic modeling. When transplanted into swine thymus tissue with a supportive microenvironment, these progenitors not only successfully integrated into the grafts but also significantly enhanced thymocyte production efficiency and increased the reconstitution of $CD4^+$ naive T cells in peripheral circulation.[37]

However, organoids also face notable limitations.[38–41] They are often cultured for relatively short durations, limiting robust differentiation into the full cellular diversity available in the target organ and resulting in poor maturation and functionality. Their short life cycle is partly attributed to limited accessibility to nutrients and inefficient removal of metabolic waste.[42] As organoids increase in size, nutrient and waste transport primarily occurs through diffusion, which becomes inefficient, ultimately compromising long-term viability. Moreover, the self-organizing nature of organoid formation and the stochasticity of cell fate decisions contribute to morphological and functional heterogeneity within the cultures.[43,44]

Material limitations and traditional monitoring methods also limit the interpretability of organoid model data. For example, commonly used substrates such as Matrigel lack sufficient biocompatibility and standardization, making accurate replication of the *in vivo* environment challenging.[41,45] Additionally, traditional culture media often fail to deliver oxygen and nutrients uniformly, resulting in cellular inhomogeneity.[46,47] Simultaneously, existing monitoring methods struggle to capture the complex dynamics within organoids in real time, and their 3D structure complicates data acquisition and analysis.[48,49] Furthermore, internal tissue heterogeneity adds another layer of complexity to data interpretation.[50]

2.3. 3D bioprinting

Three-dimensional bioprinting is an innovative fabrication technology that utilizes computer-aided design and control systems to pattern and assemble biological and non-biological materials into defined 2D or 3D organization for constructing complex biological tissue or organ models.[51] Key components of this technology include the development of a digital blueprint using specialized software, the selection of an appropriate printing device compatible with the material and tissue characteristics, and the implementation of layer-by-layer deposition techniques.

Bioprinting devices usually consist of printheads, printing platforms, and control systems that facilitate precise printing and lamination processes. Optimization of the printing procedure involves adjusting printing parameters, determining the layering sequence, and designing support structures to ensure fidelity and functional outcomes.[15,52] This technology enables the reconstruction of complex tissue structures with high precision, customization, personalization, and throughput, while also supporting automation.[53]

Layer-by-layer deposition techniques include nozzle-based bioprinting, light-based bioprinting, and hybrid systems that combine both methods. Within these techniques, key printing parameters in the control system serve as critical determinants for ensuring both high spatial precision and biologically compatible outcomes[54] (see Table 2 for details). In extrusion bioprinting, stable extrusion of thermosensitive materials is achieved by regulating nozzle temperature, pressure, and material viscosity, thereby minimizing cell damage.[55–58] Inkjet printing (drop-on-demand), on the other hand, relies on precise control of nozzle temperature, jet pressure, and ink viscosity to generate uniform droplets while maintaining cell viability.[40,59–62] Cellular electro-writing requires accurate adjustment of voltage, nozzle-to-stage distance, and material viscosity to form stable fibers without compromising cell integrity.[63] For light-based bioprinting techniques, such as digital light printing and volumetric bioprinting, parameters including light intensity, exposure time, material viscosity, and print speed are critical for controlling material curing and achieving high printing resolution.[64–66] Multiphoton lithography attains nanoscale precision through controlled light intensity and scanning speed, while laser-induced forward transfer relies on precise regulation of laser pulse energy and focal distance for efficient material transfer.[67,68] Furthermore, precise thermal regulation and the preservation of cell viability within bioinks are critical for successful bioprinting. Proper management of these printing parameters allows effective integration of material properties, cellular requirements, and printing precision, paving the way for innovative opportunities and expansive applications in the fields of tissue engineering and regenerative medicine.[69,70]

Material selection for bioprinting and tissue engineering requires a systematic evaluation of physical, chemical, and mechanical properties to ensure biocompatibility, functionality, and structural integrity—critical factors for successful tissue formation and integration within the body. Biomaterials are generally classified into two broad categories based on their origin as follows: natural and synthetic. Regardless of their source, all materials must be biocompatible, printable, and cytocompatible (see Table 3 for more details). Natural materials such as chitosan and hyaluronic acid are widely utilized due to their exceptional biocompatibility and degradability.[55] Chitosan exhibits good biocompatibility, and its mechanical strength and stiffness can be optimized by adjusting the degree of cross-linking.[71–74] Its viscosity

Table 2. Introduction to 3D bioprinting technology

Name	Mechanism	Size	Advantages	Disadvantages	References
Nozzle-based bioprinting					
EB	Mechanically actuated or pneumatically extruded polymers	Filament diameter range 100–500 μm	The high degree of customization, control of microstructure, flexibility, the high degree of automation, lower costs	Increased risk of cell damage, limited resolution, not suitable for high-viscosity bioinks, prone to cell clogging, limited choice of biomaterials	[55–58]
DoD	Introducing strand breaks in polymer extrusion jets by means of heating, piezoelectricity, electrostatics, or electrodynamics	Droplet diameter range 5–2000 μm			[40,59–62]
CEW	Stabilized jets of filaments sprayed from polymers controlled by electric fields	Fiber diameter range 5–50 μm			[63]
Light-based bioprinting					
DLP	Layer-by-layer crosslinked resin printing using CAD-projected light voxels	Minimum feature resolution of 20 μm	High resolution, rapid prototyping, suitable for complex structures, biocompatible, precise control	Limited range of printable materials, limited by the focus of the beam and optical system, loss of bioactivity due to light curing, high environmental requirements, toxicity of photoinitiators, equipment limitations, high cost of systems, and long time to fabricate small structures	[64]
VBP	The photocrosslinked material is rotated with the aid of a laser-projected CAD file to create a holographic pattern.	40 μm for positive features and 100 μm for negative features			[66]
MPL	The laser scans the focal point, activating the resin and polymerizing it layer by layer.	Lateral resolution of 100 nm and axial resolution of 300 nm			[67]
Combining 3D bioprinting based on light and nozzle principles					
LIFT	Utilizing a high-power pulsed laser focused on a thin film of ink, the ejected material forms voxels that are transferred to the surface of the receiver substrate.	Resolution of 20 μm	Fine structure and high resolution, fast print speeds, flexibility and versatility, precise control and customization for complex structures	Increased complexity, difficult technology integration, increased cost, print accuracy limitations, material compatibility challenges, and difficult technology debugging	[68]

Abbreviations: CAD, computer-aided design; CEW, cellular electro-writing; DLP, digital light printing; DoD, droplet-on-demand; EB, extrusion bioprinting; LIFT, laser-induced forward transfer; MPL, multi-photon lithography; VBP, volumetric bioprinting.

and rheological properties also vary significantly with molecular weight and concentration.[71,74–76] In contrast, hyaluronic acid is valued for its high hydrophilicity and excellent hydration properties; its cross-linked form can form elastic hydrogels, though it exhibits reduced stiffness when uncross-linked.[77] Synthetic materials such as poly(ε-caprolactone) and the Pluronic series provide distinct performance advantages.[78–82] Poly(ε-caprolactone) is highly biodegradable and easily processed due to its low melting point, remaining solid at body temperature.[78,80,81] Its mechanical strength can be further enhanced through cross-linking or compositing. Poly(ε-caprolactone) also exhibits high melt viscosity, favorable rheological properties, and low thixotropy.[81] The Pluronic series, composed of nonionic surfactants, is known for its biocompatibility and thermosensitive properties.[79] In aqueous environments, these materials form gels or micelles with low mechanical strength but high thixotropy, making them well-suited for temperature-sensitive applications.[81] The integration of 3D bioprinting with micro- and nanotechnology enables precise, site-specific delivery of bioactive compounds at the molecular level. This combination offers innovative solutions, such as incorporating micro- and nanoparticle structures

Table 3. Introduction to 3D bioprinting materials

Material	Attributes	Advantages	Disadvantages	Applications	Improvement	References
Natural polymers						
Alginate	Water-soluble polysaccharide, derived mainly from brown seaweeds	Easy to print to form 3D structures; compatibility with ionic cross-linking; water absorption; low cost	Poor cell adhesion, limited mechanical properties, unstable degradation rate, low molding precision, and limited application range	Bones, muscles, cartilage, skin, nerves and blood vessels, and functional organs, including the heart, liver, kidneys, and bladder	Addition of other cell-attachable biomaterials or modification with adhesion molecule sequences	[205]
Chitosan	Polysaccharide derived from the deacetylation of chitin in crustacean shells and fungi	Relatively inexpensive; good non-toxicity; biodegradable; antimicrobial	Acidic environment unsuitable for cell survival, slow gelation rate, poor mechanical properties for bioprinting	Cartilage regeneration, bone tissue engineering, liver tissue engineering, and vascular tissue engineering, etc.	Adjusting solvent pH to neutral, mixing with other hydrogels, cross-linking with various compounds, and working with other polyelectrolytes	[71–73,206,207]
Agarose	Water-soluble polysaccharide from seaweeds	Inert; good thermal sensitivity	Non-adhesion, biodegradability	Forms cell aggregates and/or supports differentiation of pericytes, "sacrificial biomaterial" for scaffold vascularization	Mixed with collagen	[208]
HA	Glycosaminoglycan found in the ECM	Stimulates inflammatory response; water-soluble; the resulting solution has a high viscosity; tunable physical and biological properties	Time-consuming, possibly toxic to encapsulated cells, poor mechanical properties, and rapid degradation	Dermal fillers for wound healing, auxiliary materials to regulate the viscosity of solutions of other biomaterials in bioprinting, and suitable materials for cell incorporation	Curing methacrylates with UV light	[77]
Collagen	Abundant natural protein in the body	Natural receptor for cell attachment; soluble in slightly acidic aqueous solutions; polymerizes at 37°C and neutral pH in 60 min	Faster degradation, biocompatibility needs to be further improved, higher cost, difficult to control its mechanical properties, poor structural stability	Tissue scaffolds use the widest range of natural materials, including fat, bladder, blood vessels, bone, cartilage, heart, liver, nerves, and skin, among other tissues	Covalent bonding and irradiation cross-linking methods applied with thermal polymerization of collagen solutions; blends of collagen and other synthetic polymers	[209]
Gelatin	Derived from partial hydrolysis of collagen	Biocompatible; non-immunogenic, cell affinity; fully biodegradable *in vivo*	Temperature-sensitive, structurally unstable, and relatively weak mechanical properties	Osteochondral regeneration, wound healing, heart tissue repair, cornea, and blood vessel formation	Chemicals incorporating metal ions, glutaraldehyde, and other printable materials	[210,211]

(*Continued...*)

Table 3. Continued ...

Fibrin	Blood clot-forming protein active in wound healing	Inherent cell adhesion capacity	Low mechanical stability, rapid degradation, and limited viscosity	Creating cell-doped fibrin constructs that can serve as drug delivery systems for wound healing	Use of high concentrations of fibrinogen or thrombin; mixing with other biomaterials with better mechanical stability; addition of protease inhibitors; optimization of printing temperature, calcium concentration, and cell density; premixing of fibrinogen and thrombin solutions	[212–214]
dECM	ECM biomaterial obtained via tissue decellularization	Contains a variety of biologically active molecules and proteins that promote cell growth and function.	Weak mechanical properties	Compatible with multiple technologies for tissue engineering, including heart, kidney, and liver	Use of other polymers as frames	[215,216]
PBH	Synthetic peptides self-assembled into hydrogels	High biological activity	Poor printability, poor mechanical stability	Tissue engineering, drug delivery, cell culture, and building biosensors	Modifying elastin-like polypeptides to make them photosensitive; layering dipeptides with oppositely charged terminal residues to promote electrostatic interactions and form stable structures	[217–219]
Synthetic polymers						
PCL	Biodegradable polyester degraded by hydrolysis	Low melting point (60 °C); hydrophobicity; slow degradation; thermoplastic behavior; considerable mechanical strength; hydrolysis-induced biodegradation	Melting temperature too high to maintain cell viability	Formation of mechanically stable 3D structures with good structural fidelity	Cell inoculation after printing with another hydrogel bioink during the scaffold fabrication and impregnation process	[77]
PBP	Composed of PEG and PEO via ethylene oxide polymerization	Water soluble; metabolically inert; biocompatible; high permeability	Limited support for protein binding and cell adhesion	Reduced immunogenicity after implantation, used to encapsulate cells for cell delivery	Modification with peptides with enhanced cell adhesion capacity	[220,221]
Pluronic	PEO-PPO-PEO triblock copolymer based on PEO and PPO arrangement	Heat-sensitive, amphiphilic properties, surfactant properties	Limited cell adhesion, limited degradation, uncertain cytocompatibility	Used as a sacrificial bioink for creating molds, channels, containers, or vascular systems for 3D bioprinting, or as a temporary support structure	NA	[222]

(*Continued...*)

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Table 3. Continued ...

Composite polymer						
HFP	Combination of two or more natural and/or synthetic polymers with synergistic properties	Enhanced mechanical and biological properties and rheological and indentation characteristics	Lower mechanical strength, water solubility, shrinkage, and strict reaction conditions	Complex of type I collagen and ECM protein; complex of alginate and type I collagen	NA	[55]
CHIF	Inorganic ceramics combined with natural or synthetic polymers	Superior strength and bioactivity; printable	Inhomogeneity, reduced mechanical properties, interfacial issues, increased cost, and biocompatibility issues	Provides scaffolding support and release of bioactive substances to promote bone regeneration and repair	NA	[223–225]

Abbreviations: CHIF, composite hydrogels incorporating fillers; dECM, decellularized extracellular matrix; ECM, extracellular matrix; HA, hyaluronic acid; HFP, hydrogel forming polymers; NA, not available; PBH, peptide-based hydrogels; PBP, polyethylene-based polymers; PCL, poly-epsilon-caprolactone; PEG, polyethylene glycol; PEO, polyethylene oxide; PPO, polypropylene oxide.

that effectively minimize non-specific interactions with blood components and the mononuclear phagocyte systems, thereby facilitating targeted therapy for localized lesions.[83] As 3D printing research advances, a wide range of smart materials has emerged, including shape memory polymers, hydrogels, liquid crystal polymers, shape memory alloys, dielectric elastomers, piezoelectric materials, magnetically active materials, and biologically functional particles or fillers—each offering diverse biomedical applications.[84,85] Notably, shape memory polymers and hydrogels have gained prominence due to their superior printability, inherent biocompatibility, and capacity for structural reconfiguration, making them ideal for fabricating complex tissue architectures.[85–87] Compared to traditional biological preparation methods, which often struggle to simulate complex biological tissue structures, 3D bioprinting enables precise layer-by-layer control of material placement and tissue architecture, thereby achieving a high degree of biomimicry. By fine-tuning printing parameters and material properties, tissue and organ models can be customized to reflect individual anatomical and physical characteristics.[88] Moreover, automated printing systems and optimized protocols enable the rapid and efficient production of large quantities of biological models.[89]

Three-dimensional bioprinting has revolutionized the biomedical field through its advantages in customization, precision, efficiency, and versatility, laying a robust foundation for individualized medicine and advanced biomedical research. For example, Roth et al. utilized high-throughput automated inkjet bioprinting to generate complex multicellular patterns through the precise deposition of collagen. This printing technique not only ensures the high resolution of cell patterns but also reduces experimental contamination associated with manual handling.[90] Hafa et al.[91] engineered biologically active full-thickness skin tissue with high printing speed (0.66 mm^3/s) and resolution (9 μm). The constructs retained key histological features of the epidermis and dermis and maintained structural integrity and metabolic activity for 41 days, demonstrating the promise of this technology in the field of tissue regeneration and organ transplantation. In another study, a homogeneous *in vitro* 3D model of colorectal cancer and its liver metastasis was efficiently constructed through 3D bioprinting of patient-derived primary tumor cells and bioinks. Notably, this model not only effectively preserved parental tumor biomarkers and mutation profiles but also exhibited substantial tumor heterogeneity in chemotherapeutic response, showcasing its utility in precision oncology and preclinical testing.[92]

Overall, 3D bioprinting enables the accurate replication of complex tissue structures while offering high degrees of personalization and customization for individual needs. Its high degree of automation and productivity makes it ideal for the rapid generation of tissue and organ models, accelerating advancements in biomedicine. However, certain limitations remain, including reduced cell–cell interactions, challenges in nutrient and metabolic waste exchange, difficulty in simulating and precisely controlling physicochemical factors, and discrepancies between the printed constructs and native tissue *in vivo*.[93,94]

3. Pairwise integration of microfluidics, organoids, and 3D bioprinting

3.1. Microfluidics and organoids

The integration of microfluidic technology into organoid models enables fine-tuned regulation of the microenvironment, which not only facilitates the incorporation of system-level parameters but also enhances vascularization and prolongs model viability. This dual-technology approach significantly enhances data interpretability and supports real-time imaging through synergistic functional enhancement.

3.1.1. Integration of system-level parameters

In vitro organoid cultures, derived from stem cells, recapitulate the cellular composition, microstructure, and vital functions of native tissues, making them highly valuable for clinical applications. However, achieving clinically translatable models requires organoids that more closely mimic physiological conditions. Fortunately, with the introduction of auxiliary technologies such as microfluidics, organoid culture will no longer be limited to the intrinsic self-organization of cells. Instead, it can be externally regulated to a controllable extent by manipulating the microenvironment to adjust system-level parameters (e.g., fluid flow, mechanical force, and tissue space dimensions), thereby enabling the construction of biologically authentic organoid models. For example, under static culture conditions, most models remain avascular and immature. In contrast, renal organoids cultured under flow conditions on a millifluidic chip developed a vascular network with perfusable lumens surrounded by mural cells, exhibited more mature pedunculated cells and tubular compartments, and showed enhanced cell polarity and adult gene expressions (Figure 1A).[95] Similarly, for stomach organoids, integrating organ-on-a-chip systems with microfluidic modulation enabled the simulation of fluid flow and peristaltic-like motions within the luminal space. These features recapitulated physiological gastric functions and effectively reduced the risk of bacterial overgrowth, intestinal obstruction, and inflammatory bowel disease associated with impaired peristalsis (Figure 1B).[96] Likewise, colon tumor organoids cultured on microfluidic chips successfully mimicked the *in vivo* mechanical stimulation of intestinal muscles through cyclic pressure-channel contractions, illustrating the potential of engineering approaches that integrate biophysical factors into organoid cultures.[97]

3.1.2. Vascularization of organoid cultures

Vascularized organoid technology—arising from the integration of microfluidic principles with organoid models—is essential for replicating organ growth and function and for advancing this important field. The core of this technology is self-assembly, which stimulates the formation of microvascular networks through angiogenesis, while microfluidic systems enable dynamic perfusion.[98,99] A common technique for generating vascularized organoids involves inducing the self-organization of PSCs to form the desired organoid and initiating vascularization following the application of specific inducing factors. In addition, shear stress introduced via increased fluid flow during organoid self-organization enhances vascularization by triggering mechanotransduction pathways in vascular endothelial cells. This stimulation promotes the secretion of pro-angiogenic factors, which drive endothelial cell migration and proliferation.[95] Another strategy involves co-culturing stem cells with endothelial cells under specific conditions. This method facilitates the self-assembly of vascular networks through the intrinsic bioprogramming of both cell types.[100] For example, Shin et al.[101] utilized injection-molded microfluidic chips to co-culture spheroids derived from induced neural stem cells with human umbilical vein endothelial cells. This setup led to the development of a 3D model resembling a vascularized brain organoid (Figure 1C).

A further method entails the engineered assembly of organoids with microfluidic channels lined with endothelial cells. In this approach, organoids are formed from stem cell differentiation, while vascular networks develop by seeding endothelial cells into porous microfluidic channels. These cells then invade the adjacent ECM via angiogenesis, forming a perfusable vascular network. The geometry (diameter and length), density, branching, perfusion, stability, and permeability of these networks are highly dependent on biophysical (e.g., interstitial and intraluminal flow and matrix stiffness) and biochemical (e.g., vascular endothelial growth factor) cues provided by the surrounding matrix and stromal cells.[102]

The ultimate goal of vascularized organoid technology is to generate tissue-specific microvascular systems and to connect multiple organoids through dynamic vascular networks, creating integrated systems capable of

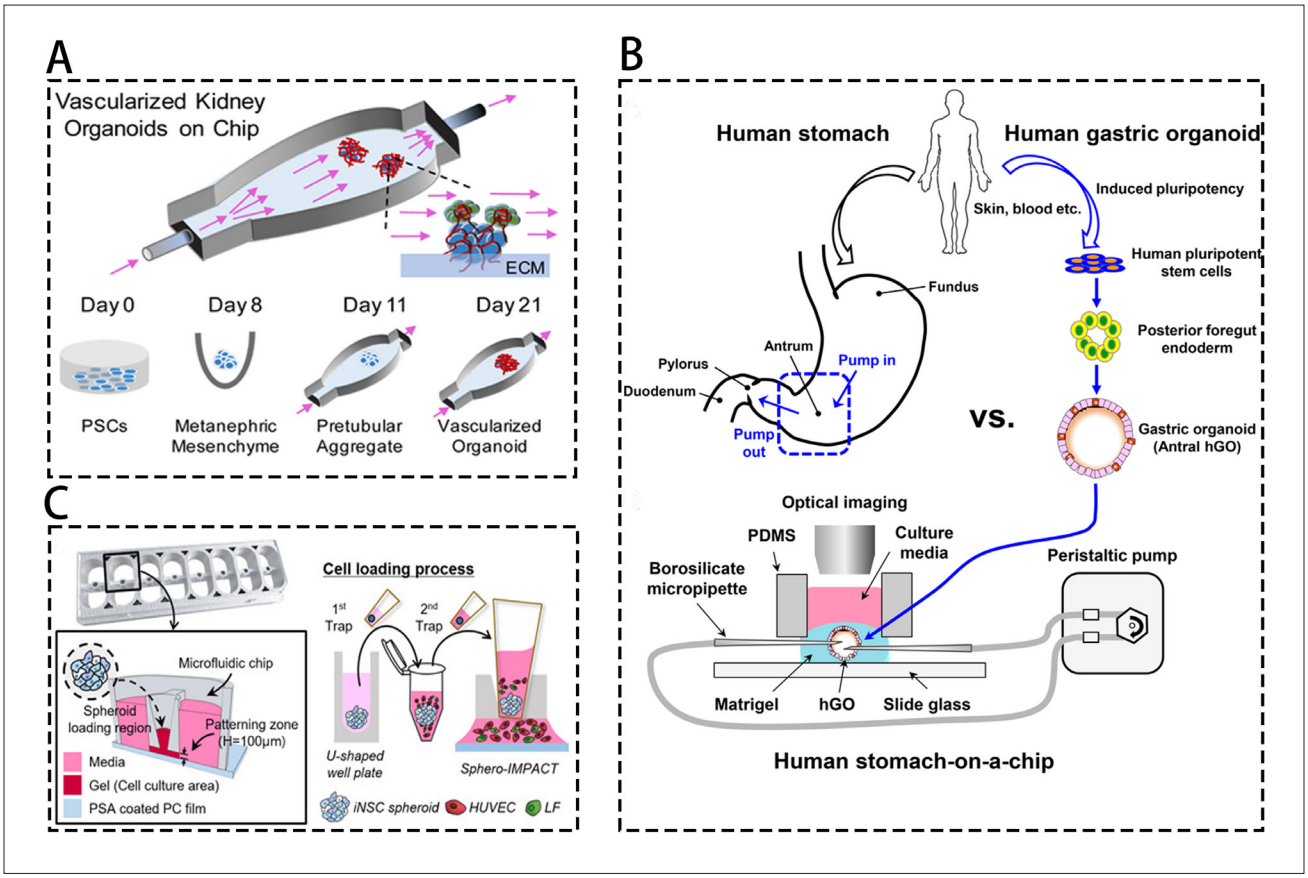


Figure 1. Microfluidics and organoids—Part I. (A) Kidney organoids cultured on a dynamic microfluidic chip showed more mature podocytes and renal tubular compartments, along with a large network of perfusable luminal vessels. Adapted with permission from ref.[95], Copyright © Nature 2019. (B) Gastric organoids placed in a microfluidic device were exposed to rhythmic stretching and contraction, realistically simulating peristaltic-like stomach motions. Adapted with permission from ref.[96], Copyright © Royal Society of Chemistry 2018. (C) Vascularized brain organoids were generated by co-cultivating iNSC-derived spheroids with perfusable blood vessels in an injection-molded microfluidic chip. Adapted with permission from ref.[101]. Copyright © Wiley 2022. Abbreviations: ECM, extracellular matrix; hGO, human gastric organoid; HUVEC, human umbilical vein endothelial cell; iNSC, induced neural stem cell; LF, lung fibroblast; PC, polycarbonate; PDMS, polydimethylsiloxane; PSA, pressure-sensitive adhesive; PSC, pluripotent stem cell.

responding to physiological changes. Currently, successful examples of such techniques have been reported in organoids representing the bone marrow, brain, heart, liver, pancreas, and intestines.[103–108]

3.1.3. Survival enhancement

Microfluidic devices can also be utilized to prolong the survival time of organoids. This benefit primarily stems from the ability of microfluidic systems to mimic the *in vivo* microenvironment and provide precise biological and physiological regulation, thereby creating a more favorable growth environment for organoids (Figure 2A).[20] Through the use of microchannels and fluid control, microfluidic devices enable precise spatiotemporal regulation and delivery of nutrients, oxygen, and metabolites, which is essential for maintaining organoid cell viability.[109,110] In addition, microfluidic systems promote inter-tissue interactions and cellular signaling, facilitating communication and coordination among cells. These interactions contribute to the maintenance of tissue structure and cellular function within the organoid. Consequently, microfluidic platforms effectively reduce cell death in the core regions of the culture, thereby enhancing cell proliferation and differentiation, and ultimately extending the survival time of the organoid (Figure 2B).[111]

In summary, while organoids are capable of recapitulating key generational characteristics of primary tissues, they are limited in their ability to regulate the extracellular physicochemical microenvironment using conventional technologies. Therefore, the development of physiologically and pathologically relevant homeostatic organ models necessitates the incorporation of technologies

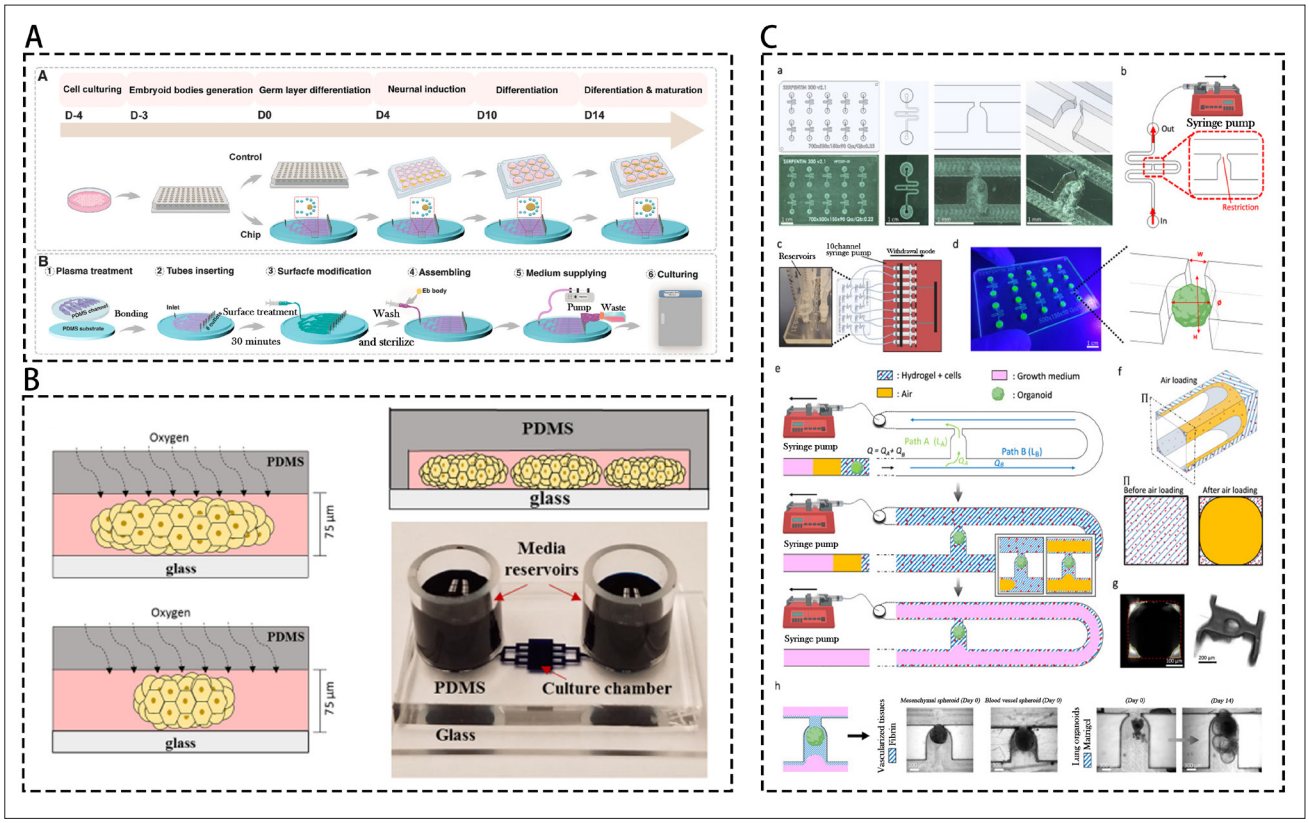


Figure 2. Microfluidics and organoids—Part II. (A) A brain organoid–microfluidic platform with dynamic fluidic perturbation and oxygenation enabled sustained culture for over 50 days. Adapted with permission from ref.[20], Copyright © Elsevier 2023. (B) Bladder cancer organoids maintained a long-term culture using a microfluidic device or microchamber. Adapted with permission from ref.[111], Copyright © Nature 2017. (C) Visualization of organoid vascularization using a microfluidic device. Adapted with permission from ref.[98], Copyright © Nature 2024. Abbreviation: PDMS, polydimethylsiloxane.

such as microfluidics to support and enhance organoid growth and maintenance.

3.1.4. Improved data readability and real-time imaging

Organoid culture is a complex and dynamic process, and maximizing the utility of organoid technology requires enhanced data readability and dynamic monitoring capabilities. Given its current limited data readout (e.g., geometry, quantity, oxygen concentration, pH) and monitoring capabilities, there is a critical need for precise and accurate functional readouts and real-time imaging to maximize the benefits of organoid technology. The integration of microfluidic devices into organoid culture platforms enables real-time monitoring and computational imaging of physicochemical factors within the microenvironment, facilitated by embedded sensors.[112] A representative example of this concept is the organoid-on-a-chip system. These microchip platforms replicate both the structural and functional aspects of biological systems, allowing for more accurate tracking of key biological and physiological parameters compared to conventional methods.

For example, Schneider et al.[113] utilized hydrostatic self-assembly of induced PSC (iPSC)-derived cardiac spheroids to form aligned myofibers and integrated fluidic electrodes with an open-source pulse generator for electrical stimulation. A resin-embedded microfluidic chip with real-time optical oxygen sensing enabled dynamic environmental monitoring. This multimodal regulation and monitoring system provides a novel solution for automated high-density tissue fabrication. Furthermore, image-based analysis is indispensable for assessing the physiopathological parameters of organoids, and its effectiveness is greatly enhanced through integration with microfluidics. For example, Quintard et al.[98] developed a microfluidic platform to dynamically image the formation of endothelial networks around mesenchymal and pancreatic islet spheroids, as well as the generation of vascular organoids from PSCs (Figure 2C).

3.2. Microfluidics and 3D bioprinting

The combination of microfluidics and 3D bioprinting enables *in vitro* models to incorporate fluid flow dynamics and spatial control, allowing cultures to more closely mimic the anatomical structure and functional states of *in vivo* organs.[114,115] The implementation of this integrated technological platform supports the precise calibration of biomimetic system parameters through iterative optimization protocols.[116] Additionally, this technology not only improves sterility standards during the culture process but also significantly enhances the consistency of experimental procedures due to the highly automated nature of bioprinting.[27,117]

3.2.1. Modeling complex organizational structures

Three-dimensional bioprinting integrated with microfluidics enables flexible and precise manipulation of small-scale biological components and fluids, facilitating the simulation of complex microstructures found in natural tissues. In traditional *in vitro* models, replicating the layered structure of native myocardium poses a substantial challenge in engineering functional cardiac tissues. Zhang et al.[118] address this by directly bioprinting a vascular bed within a microfibrous hydrogel scaffold using a composite bio-ink, onto which cardiomyocytes were seeded. This resulted in an aligned myocardial tissue capable of spontaneous and synchronous contraction. The endothelialized myocardium was then embedded into a specially designed microfluidic device, which not only partially restored the *in vivo* myocardial structure but also enabled toxicity assessment of relevant cardiovascular drugs (Figure 3A).

Similarly, Silvani et al.[119] reconstructed a compartmentalized polymorphic glioblastoma microenvironment consisting of a functional BBB and adjacent 3D perivascular tumor ecotopes by selectively mimicking physiological shear stress and mechanical interactions, including cell–cell and cell–matrix communication. Specifically, they bioprinted brain endothelial cells encapsulated in gelatin methacryloyl (GelMA) and fibronectin into a ring structure, then deposited a GelMA-alginate hydrogel loaded with glioblastoma cells at the center of the ring, followed by layered bioprinting and external perfusion. This novel integration of microfluidics and 3D bioprinting recapitulates the complex structure of glioblastomas, providing a valuable biological tool for studying cancer mechanisms and therapeutic interventions (Figure 3B).

3.2.2. Forming a vascular network

Vascular network formation is a diverse area of bioengineering, and the approach discussed here differs from the organoid vascularization presented in the **Section 3.1.2**. This method refers to engineered vascularization using 3D bioprinting to replicate the native vascular topology of *in vivo* tissues, followed by the application of microfluidic principles to simulate blood flow through perfusion.

For example, Fritschen et al.[120] integrated on-demand positioned bioprinting with robotic manipulation of microfluidic chips to develop an automated platform capable of precisely constructing three tissue models on-chip within 60 s, while achieving continuous, unmanned fabrication of multi-organ chips. The core innovation lies in the creation of sealable post-printing microfluidic chips compatible with mainstream bioprinters and perfusion systems. When validated using a vascularized liver cancer model, complete 3D vascular networks formed within 14 days, with HepG2 cells exhibiting significant spheroidal proliferation and sustained albumin secretion—demonstrating the functional viability of the engineered tissue. However, coaxial bioprinting remains challenging for vascularizing large constructs with 3D interconnecting channels. This challenge can be partially addressed by applying void-free 3D bioprinting techniques to hydrogel-based customized microfluidics. Specifically, sacrificial bioinks containing endothelial cells are deposited layer-by-layer alongside matrix bioinks to generate void-free, multimaterial structures. The sacrificial material is subsequently removed to create well-defined templated flow channels, which were then connected to a peristaltic pump using a polydimethylsiloxane sleeve for controlled perfusion. This system enables the fabrication of large 3D vascularized constructs with interconnected channels while autonomously maintaining stable perfusion and cell viability.[121]

3.2.3. Optimizing bioprinting parameters

The integration of microfluidics with 3D bioprinting facilitates the optimization of key parameters, including print concentration, resolution, and stability. Traditional extrusion bioprinting techniques are limited by the predetermined concentration of cell-laden bioinks. However, Serex et al.[122] developed a microfluidic-based printhead capable of adjusting cell concentration in real time. This system can deliver up to 10 million fibroblasts per milliliter, enabling the bioprinting of highly concentrated cells. This approach yields cell densities that more closely resemble those found in living tissues, reducing intercellular distances and promoting cell–cell communication. In addition, microfluidic enhancements to 3D bioprinting nozzles can significantly improve printing resolution and stability. Highley et al.[123] created a droplet-based T-shaped microfluidic system that generates microgels capable of further crosslinking to enhance structural stability. These

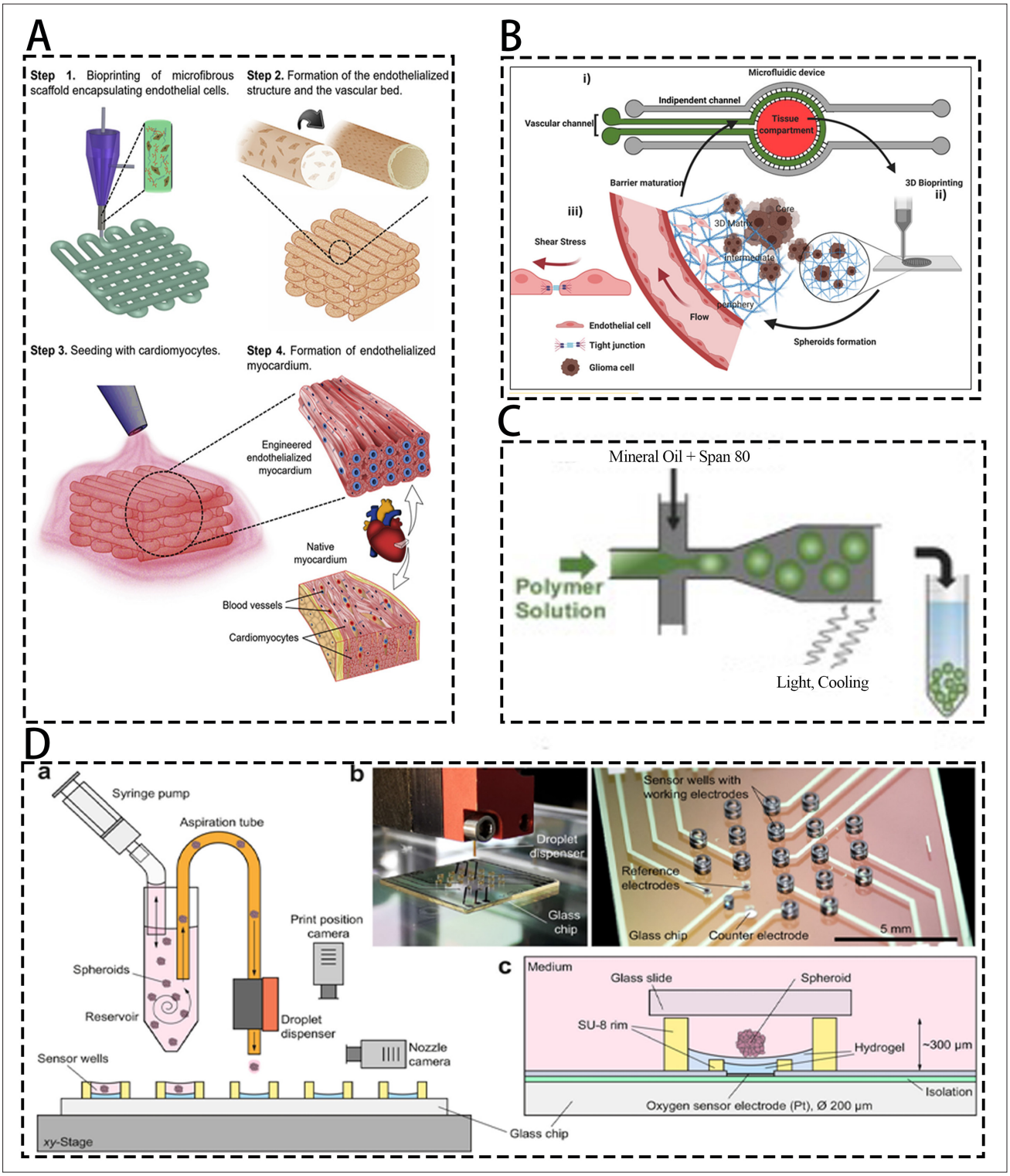


Figure 3. Microfluidics and 3D bioprinting. (A) 3D bioprinted endothelialized myocardium embedded in a specially designed microfluidic device for cardiovascular toxicity testing. Adapted with permission from ref.[118], Copyright © Elsevier 2016. (B) Integration of 3D bioprinting and microfluidics to simulate the blood–brain barrier and adjacent 3D perivascular tumor microenvironment. Adapted with permission from ref.[119], Copyright © Wiley 2021. (C) Microfluidic printhead for real-time regulation of printed cell concentration. Adapted with permission from ref.[123], Copyright © Wiley 2018. (D) Automated bioprinting of tumor spheroids into chip-based electrochemical oxygen sensor microvias. Adapted with permission from ref.[117], Copyright © Royal Society of Chemistry 2022.

inks exhibit shear-thinning behavior—flowing under external force but rapidly recovering their mechanical properties post-extrusion. This approach minimizes discrepancies between bioprinted structures and their computer-aided designs while maintaining cell viability, thereby overcoming major limitations related to stability and resolution in 3D bioprinting (Figure 3C).

3.2.4. Automation and dynamic monitoring

The combination of microfluidics and 3D bioprinting enables automated handling and dynamic monitoring of biological models. Traditionally, cells are introduced into microfluidic devices manually using pipettes—a process that increases the risk of contamination by laboratory personnel and introduces variability. Using a 3D bioprinter in a sterile environment minimizes human interference, reduces contamination risk, and enhances reproducibility.[27] In addition, while conventional bioprinting methods lack the capacity to deliver real-time data on cell metabolism and cell culture reliability, embedding 3D-printed constructs into sensor-integrated microfluidic devices allows for dynamic monitoring. Dornhof et al.[117] demonstrated that combining bioprinting with an on-demand drop-processing microfluidic system can ensure environmental sterility, reliable data acquisition, and seamless sensor integration during model cultivation. Their device demonstrated accurate and stable electrochemical oxygen sensing across atmospheric to hypoxic conditions. Such engineered systems, with high automation and scalability, offer broad potential for future biomedical applications (Figure 3D).

3.3. Organoids and 3D bioprinting

The combination of organoids with 3D bioprinting technology enables the precise spatial placement of cells and biomaterials to construct localized stem cell microenvironments in a targeted manner. This approach not only effectively reduces the randomness and non-reproducibility of traditional culture processes but also facilitates the establishment of highly biomimetic models.[124–126] In addition, integrating these techniques transforms conventional bioprocessing methods and significantly improves the throughput and fidelity of organoid production.[127,128]

3.3.1. Enhancing structural and functional complexity of organoid models

The combination of organoid and 3D bioprinting enhances the architectural complexity of tissue models, modulates intrinsic biological properties such as cell migration and proliferation, and improves the scalability and applicability of composite cultures. For instance, in the context of bone tissue engineering, 3D bioprinting enables the precise fabrication of osteochondral defects with layered structures. Based on the bilayered nature of native osteochondral cartilage, a study precisely prepared an anisotropic bicellular living hydrogel embedding articular chondrocyte progenitor cells and bone mesenchymal stem cells using dual-channel extrusion bioprinting. This construct demonstrated effective cartilage–bone–vessel crosstalk during testing, successfully reconstituting the harmonious cartilage–bone interface and offering insights into the fabrication of anisotropic living material for complex organ reconstruction.[129]

Furthermore, the synergy between organoids and 3D bioprinting enables the precise fabrication of biomimetic corneal architectures, as validated by Sorkio et al.[130] They generated physiologically relevant 3D corneal constructs using corneal limbal epithelial stem cells and adipose tissue-derived stem cells for epithelial and stromal components, respectively. Recombinant human laminin and human collagen I were used in bioinks, combined with laser-assisted bioprinting (LaBP) to fabricate corneal tissues. The resulting constructs included stratified corneal epithelium, stroma, and the epithelium–stroma interface (Figure 4A). These tissues showcased structural integrity, proper physiological function, and expression of relevant marker proteins, confirming the feasibility of LaBP with human stem cells for corneal applications. The integration of 3D bioprinting into organoid fabrication is expected to yield structures with physiological resemblance to host tissues and robust functional performance.

3.3.2. Engineering stem cell microenvironments for reproducible organoid development

The combination of organoid and 3D bioprinting allows for precise spatial control of stem cells and biomaterials, facilitating the targeted generation of specific structures. This convergence provides unprecedented control over 3D spatial deposition, enabling tailored tissue engineering approaches. For instance, stem cells used as base materials for organoid construction can be deposited in positions conducive to spontaneous extracellular mesenchymal self-organization. By precisely regulating spatial location and cell density, researchers have fabricated centimeter-scale tissues exhibiting self-organizing features, such as tubular lumens, branching vascular networks, and tubular epithelia (Figure 4B).[131]

In addition to cell deposition, local modulation of biophysical or biochemical cues can direct organoid development and reduce variability. Gjorevski et al.[39,132] demonstrated this by using a locally softened hydrogel to guide the geometry and patterning of intestinal organoids. Crypt-like buds formed preferentially within softened regions, while none developed outside them. These pseudo-buds extended into crypt-like structures, indicating that

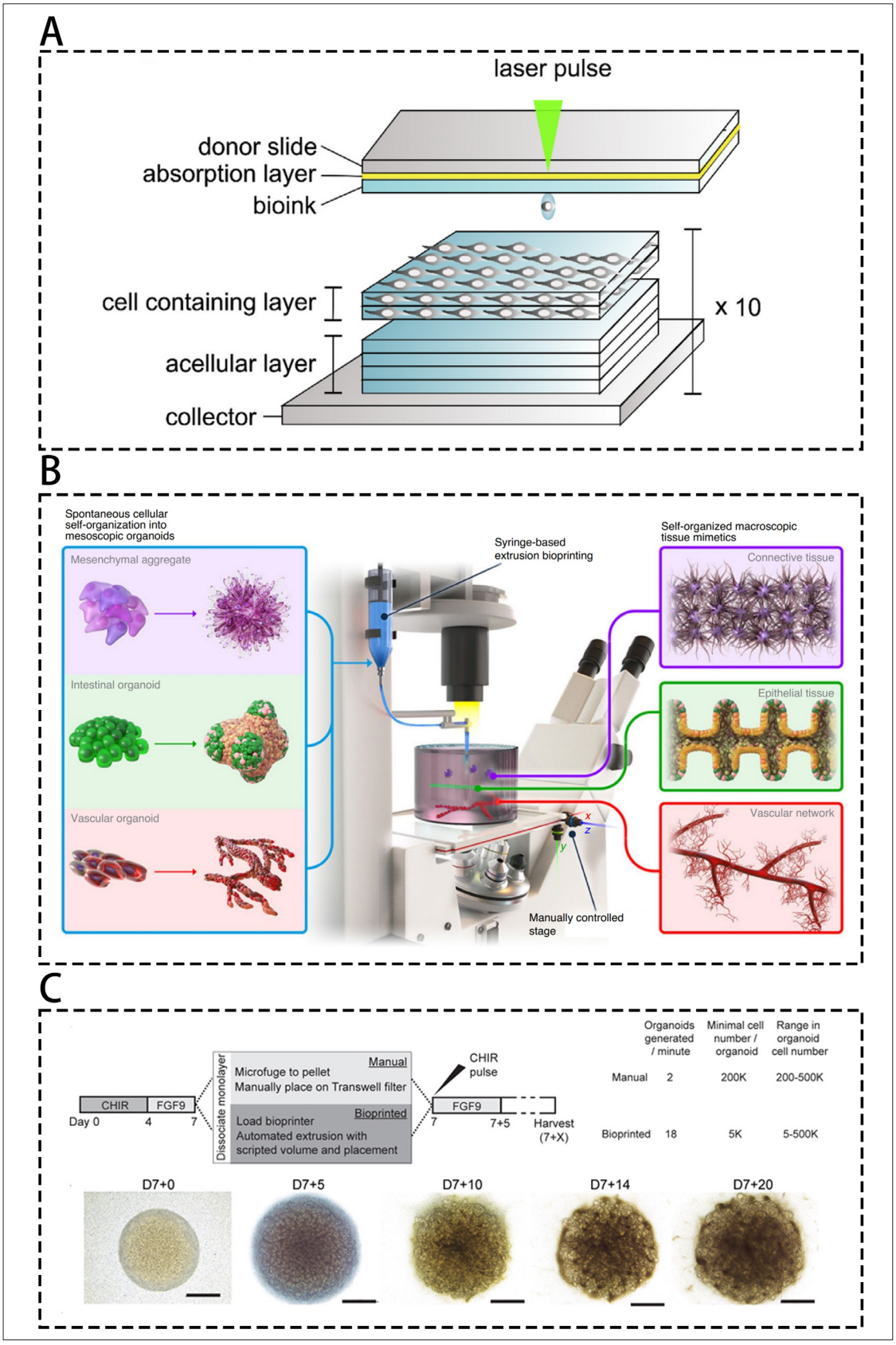

A
laser pulse
donor slide
absorption layer
bioink
cell containing layer
acellular layer
collector
x 10
B
Spontaneous cellular self-organization into mesoscopic organoids
Mesenchymal aggregate
Intestinal organoid
Vascular organoid
Syringe-based extrusion bioprinting
Self-organized macroscopic tissue mimetics
Connective tissue
Epithelial tissue
Vascular network
Manually controlled stage
C
Dissociate monolayer
Manual
Microfuge to pellet
Manually place on Transwell filter
Bioprinted
Load bioprinter
Automated extrusion with scripted volume and placement
CHIR
FGF9
Day 0
4
7
CHIR pulse
7+5
Harvest (7+X)
Organoids generated / minute
Minimal cell number / organoid
Range in organoid cell number
Manual 2 200K 200-500K
Bioprinted 18 5K 5-500K
D7+0
D7+5
D7+10
D7+14
D7+20

Figure 4. 3D bioprinting and organoids. (A) Laser-assisted bioprinting of layered 3D tissues using human stem cells to mimic natural corneal tissue. Adapted with permission from ref.[130], Copyright © Elsevier 2018. (B) Control of self-organization from millimeter to centimeter scales by combining 3D bioprinting and organoid technologies. Adapted with permission from ref.[131], Copyright © Nature 2021. (C) Automated extrusion-based bioprinting improves the throughput, quality, scalability, and structure of kidney organoid production. Adapted with permission from ref.[127], Copyright © Nature 2021. Abbreviations: CHIR, CHIR99201; FGF9, fibroblast growth factor 9.

localized ECM mechanics and hydrogel topology can be used to control organoid size, shape, and developmental trajectory. This advancement addresses organoids' inherent variability and low reproducibility, enhancing their utility in both basic and translational research.

3.3.3. Fabricating high-fidelity, high-throughput, and high-efficiency organoids

Organoids derived from PSCs offer promising models for disease research and drug screening due to their structural and functional similarity to native tissues. However, their development and application in both basic and clinical medicine are hindered by limitations such as high variability, low throughput, and small-scale production inherent to conventional methods. Stem cell-based 3D bioprinting enables efficient and reproducible fabrication of high-throughput organoid cultures with biomimetic fidelity. For example, Lawlor et al.[127] employed extrusion-based 3D cell bioprinting to rapidly generate kidney organoids with highly consistent cell numbers and viability. They demonstrated that manual organoid generation could be replaced by bioprinting in 6- or 96-well formats, with the device capable of printing approximately 200 organoids in just 10 min. Experimental results further showed that the bioprinted kidney organoids exhibited mature renal structures. By leveraging 3D bioprinting's ability to precisely manipulate biophysical properties—including organoid size, cell number, and geometry—the platform enables structural fidelity, quality control, improved throughput, and scalability, thereby facilitating both *in vitro* and *in vivo* applications of stem cell-derived human kidney tissue (Figure 4C). In addition, this dual-technology approach facilitates the automation of high-throughput bioprocessing for patient-derived tumor organoids, thereby circumventing the risk of organoids adhering to the sides of multiwell plates and forming cultures confined to two-dimensional growth.[133]

3.4. Pairwise integration challenges among microfluidics, organoids, and 3D bioprinting

The integration of microfluidics and 3D bioprinting presents several challenges due to conflicts between fluid dynamics and material properties. Microfluidics relies on precise fluid control (e.g., laminar flow and pressure gradients), whereas 3D bioprinting requires bioinks with specific rheological properties (e.g., shear-thinning behavior and rapid gelation).[134,135] The dynamic fluid environment in microfluidics may interfere with bioink stability, resulting in the collapse of printed structures or reduced resolution.[136,137] For example, the shear stress within microfluidic chips can disrupt the crosslinking process of bioinks, directly affecting printing accuracy.[138]

In addition, a mismatch exists between the scale of microfluidic channels and the resolution of 3D bioprinting, making it difficult to seamlessly integrate complex printed structures (e.g., vascular networks) with microfluidic systems. This often leads to leakage or pressure imbalances.[139,140] Another major issue involves the conflict between dynamic fluid environments and static scaffolds.[141] Periodic perfusion in microfluidics can become uneven due to obstruction by printed scaffolds, causing local nutrient or oxygen gradient disparities and negatively affecting cell viability. For instance, printed scaffolds may block microfluidic channels or hinder uniform fluid diffusion.[142]

A critical challenge in integrating microfluidics with organoids lies in the contradiction between the dynamic culture environment provided by microfluidic systems and the stable conditions required for organoid self-organization.[143] Dynamic perfusion can disrupt the mechanical stresses and chemical gradients essential for organoid development, leading to structural abnormalities or functional deficiencies.[144] Material compatibility is another significant concern. Common microfluidic materials, such as polydimethylsiloxane, tend to absorb small molecules critical for organoid culture (e.g., growth factors, lipids), resulting in imbalanced media composition and impaired organoid development.[145] Furthermore, the implementation of real-time monitoring remains limited. The dense 3D architecture of organoids often obstructs optical imaging signals, reducing data readability within microfluidic systems. Invasive sensors, while potentially useful, may damage organoid integrity and further hinder functional analysis.[146,147]

The fundamental conflict in combining 3D bioprinting with organoid technology stems from the mismatch between printing resolution and the native structural complexity of organoids.[148] Current 3D bioprinting technologies may lack the resolution necessary to replicate the fine, intricate architecture of organoids, potentially resulting in a loss of physiological function.[149] For instance, the bioprinted liver organoids may fail to form functional bile ducts due to

the absence of subcellular-level topographical features.[150] The mechanical properties of bioinks also pose a major constraint: scaffolds that are too stiff may hinder organoid self-organization—such as lumen expansion in intestinal organoids—and current technologies for dynamically tuning scaffold stiffness remain immature.[151] Additionally, pre-designed vascularization strategies (e.g., sacrificial material-based methods) may not synergize well with angiogenic signals secreted by organoids, resulting in poor perfusion efficiency.[152]

These core challenges stem from mismatches in physical scale, imbalances between dynamic and static environments, incompatibility at the material–biological interface, and interference from monitoring tools with the native microenvironment.[136,143,145] Addressing these incompatibilities will require innovations in biomaterials, multi-scale interface engineering, and interdisciplinary approaches.

4. Combination of microfluidics, organoids, and 3D bioprinting

While the integration of dual technologies has introduced new perspectives in the biomedical field, there remains a need for a third technology to complement and enhance their capabilities. Each of the three technologies—microfluidics, organoids, and 3D bioprinting—offers unique advantages that play irreplaceable roles in high-precision modeling, making their integration essential. As technological convergence advances, an emerging research paradigm is actively investigating the combined application of these three approaches. This integration aims to achieve higher-quality model construction and to develop more accurate biomedical research protocols, ultimately advancing the understanding of complex biological systems and enhancing the efficacy of biomedical applications.

4.1. Endothelialized myocardial fabrication and its application to cardiovascular toxicity assessment

Zhang et al.[118] proposed an innovative strategy that integrates microfluidics, organoids, and 3D bioprinting to successfully engineer endothelialized myocardial tissue. In this integrated approach, endothelial cells were encapsulated within a microfiber lattice created through 3D bioprinting, while a functional vascular bed was formed by utilizing a microfluidic system to guide human umbilical vein endothelial cells to migrate to the periphery of the microfiber structure. This microarchitecture not only allowed for precise control over cell distribution but also provided an optimal supportive environment for the implantation of human iPSC (hiPSC)-derived cardiomyocytes. Building on this foundation, 3D bioprinting further enabled the precise construction of myocardial tissues by controlling cellular arrangement and tissue structure. This resulted in the formation of cardiomyocytes capable of spontaneous, synchronized contractions. Such cellular organization not only enhances the accuracy of functional simulation within the tissue but also significantly improves its physiological relevance.

Combined with a specially designed microfluidic perfusion bioreactor, this model demonstrated significant advantages in cardiovascular toxicity screening. The microfluidic system orchestrated the microenvironment of the endothelialized myocardial chips by regulating nutrient delivery and waste removal—processes essential for maintaining healthy tissue growth. This enhanced environmental control promotes optimal cellular function and offers a more physiologically relevant platform for assessing the effects of compounds on cardiovascular health, thereby improving the accuracy and reliability of toxicity assessments.

Compared with rat-derived myocardial organoids, hiPSC-derived myocardial organoids exhibited slightly lower endurance at all time points, underscoring the benefits of using organoid models to minimize species differences. This comprehensive model addresses limitations associated with individual technologies and enhances myocardial tissue simulation and function through the synergistic combination of all three. Through the strategic convergence of microfluidics, organoids, and 3D bioprinting, this integrated platform establishes an innovative paradigm for accelerated drug discovery and pathophysiological modeling, facilitating precise interrogation of human-specific biological responses while enhancing translational fidelity in preclinical evaluations.

4.2. Vascularized glioblastoma-on-a-chip model and its application in mechanobiological studies of brain tumors

Pioneering 3D organoid models that incorporate dynamic flow and volumetric cues have emerged as transformative platforms for bridging the current gap in effective cancer treatment within *in vitro* exploration platforms. These models are crucial for accurately mimicking the tumor microenvironment and facilitating studies on intercellular interactions under tumor-specific pathophysiological conditions. Silvani et al.[119] successfully reconstructed the complex brain tumor microenvironment, including a functional BBB and surrounding 3D perivascular tumor microhabitat, by faithful recapitulating physiological shear stress and the mechanical interactions between cells and the matrix. A glioblastoma (GBM) model was assessed using a microfluidic chip under simulated microgravity conditions. The results indicated significant changes in cellular morphology and mechanotransduction

responses, highlighting the critical role of gravity in the mechanoregulation of GBM.[119]

This model was developed to characterize a novel 3D microfluidic bioprinting system for vascularized GBM-on-a-chip constructs, designed to comprehensively replicate the pathophysiological conditions of tumors and their surrounding vascular microenvironment. *In vivo*, GBM typically manifests as a dense, spherical structure with distinct morphological characteristics associated with various regions of the brain tumor microenvironment. These regions include a necrotic core, a perivascular zone with a severely compromised BBB, and adjacent healthy brain tissue with an intact barrier that effectively restricts drug diffusion. A bioprinting strategy employing dual bioinks was used to engineer vascularized tissue constructs with perfusable lumens. Initially, a GelMA–fibronectin mixture encapsulating brain endothelial cells was printed as a ring-shaped outer region, followed by a GelMA–alginate core bioink loaded with GBM cells. Experimental results demonstrated that under gravity-free conditions, GBM cell invasiveness and aggregation were significantly suppressed.

This integrated model showcases the highly synergistic effects of microfluidics, organoids, and 3D bioprinting, offering a robust platform for simulating complex tumor microenvironments and investigating GBM pathophysiological.

4.3. Microfluidic printhead-based bioprinting with high cell concentration and its application to bladder-like organ fabrication

Organoids have become essential tools in preclinical research due to their remarkable ability to closely mimic human tissues. However, for applications such as drug screening, it is essential to ensure not only high fidelity but also methodological reliability and reproducibility. 3D bioprinting has emerged as a viable strategy to meet these criteria, offering precise control over tissue morphology and architecture. Nonetheless, conventional extrusion bioprinting often struggles to replicate the intricate tissue complexity found in native organs. This limitation arises because the technique typically involves dispensing cell solutions at predefined concentrations through a needle. To minimize cell lysis or loss in dead volume, researchers tend to employ diluted cell solutions—concentrations lower than those found in living tissues—which diminishes bioactivity and increases cost.[122,153]

Recognizing the importance of cell concentration for organoid formation, Serex et al.[122] developed a microfluidics-based printhead capable of real-time adjustment of cell density. Their system achieved fibroblast concentrations of 10 million cells/mL with precise volumetric dispensing and was successfully applied to the generation of bladder organoids with preserved urothelial functionality. Hematoxylin and eosin staining confirmed that these organoids retained their original morphology, including a distinct central lumen and multilayered cellular structure.

Immunostaining further confirmed the expression of key urothelial markers such as CD44, CK13, and CK5, affirming cellular coherence and functional relevance. These results indicate that the microfluidic printing technology can generate organoids with multicellular layers and complex structures. Such advancements not only enhance the physiological relevance of organoids but also improve the controllability and reproducibility of the production process, offering an innovative and efficient solution for large-scale organoid generation. This approach significantly reduces variability caused by manual manipulation and standardizes the organoid formation process, thereby promoting the application of organoids in biomedical research. As a result, they represent powerful tools for disease modeling and drug screening.

4.4. Fabrication of 3D tumor spheroids via bioprinting and sensor integration for cellular metabolism monitoring

Three-dimensional cellular agglomerates, such as microtissues, organoids, and spheroids, are increasingly recognized as pivotal modeling tools in biomedical research. These structures are capable of accurately mimicking the functions of *in vivo* tissues under *in vitro* conditions and are increasingly utilized in cancer research and organ-on-a-chip systems. To further enhance the utility of these models, microsensors can provide crucial real-time information about cellular metabolism and the reliability of culture conditions. However, 3D cell cultures, particularly individual spheroids, still face persistent challenges related to reproducible formation, precise localization, and the acquisition of meaningful biosignals when integrated with sensors. These challenges become even more pronounced when working with high cell volume ratios in close proximity to sensing elements.[117]

To address these challenges, Dornhof et al.[117] successfully automated the precise printing of tumor spheroids into the microvias of a chip-based electrochemical oxygen sensor array using advanced 3D bioprinting technology. This innovative approach overcomes issues of shape instability and culture failure in organoid fabrication caused by operator inexperience, achieving highly accurate and reproducible spheroid generation. The diameters of the spheroids can be controlled to approximately 200 μm, with

a deposition accuracy of up to 25 μm and a volume of 22 nL per droplet.

Additionally, the microstructure and hydrogel-coated micropores are designed to precisely position individual MCF-7 breast cancer spheroids near the sensor electrodes. The microelectrode pores are encapsulated to facilitate rapid oxygen concentration measurements in a volume of 55 nL. The system exhibited excellent stability and accuracy as the electrochemical oxygen sensor transitioned from atmospheric to hypoxic conditions. Experimental results demonstrated that the cellular respiration rate of individual tumor spheroids could be measured within a range of 450–850 fmol/min, revealing significant changes in cellular metabolism upon drug exposure.

The study marks the first successful integration of 3D bioprinting with real-time monitoring technologies in 3D cell culture systems. It demonstrates an efficient process for parallelization, sensor integration, and drug delivery in both 3D cell culture and organ-on-a-chip platforms. The system achieves full automation and scalable manufacturing through the transition from conventional microfluidic architectures to a digitally programmable droplet manipulation system, thereby establishing a paradigm shift in liquid handling precision and operational flexibility. This advancement supports greater flexibility in spheroid formation and capture.

The potential applications of this technology are extensive, ranging from basic metabolic studies to standardized cell culture and toxicology experiments, as well as personalized medicine, such as patient-specific chemotherapy.

4.5. Challenges and prospects of combining microfluidics, organoids, and 3D bioprinting

Although the integration of microfluidics, organoids, and 3D bioprinting holds great promise for biomedical applications, their synergistic development still faces multiple challenges. First, the complexity of technological integration lies in achieving compatibility across scales. For example, dynamic perfusion in microfluidic systems and the structural precision of 3D bioprinting must be coordinated within a sub-millimeter to centimeter range, while the heterogeneous growth behavior of organoids may compromise the stability of printed structures.[148,154] Second, the functional design of bioinks has yet to fully meet the demands of multi-technology coupling. Bioinks must not only support cell viability and provide a microenvironment conducive to organoid development during printing but also accommodate the fluid dynamics of microfluidic perfusion. This places increased demands on material rheology, degradation kinetics, and the ability to transmit biochemical signals.[145] In addition, significant bottlenecks remain in the real-time acquisition and analysis of multimodal data. For instance, *in situ* monitoring of organoid functional evolution under dynamic culture conditions requires the development of novel biosensors with enhanced signal sensitivity, improved spatial resolution, and greater integration compatibility with hybrid microfluidic–bioprinting systems.[155] Finally, the lack of standardized frameworks limits the scalability and translational potential of these integrated technologies. Unified standards are urgently needed for cross-platform workflow harmonization, quality assessment metrics, and clinical validation protocols.[156]

Despite the challenges, the deep integration of microfluidics, organoids, and 3D bioprinting presents a unique and promising future. By leveraging the precise microenvironmental control of microfluidics, the biological fidelity of organoids, and the complex structural fabrication capabilities of 3D bioprinting, this technological convergence has the potential to overcome many limitations of traditional *in vitro* models. For instance, dynamic perfusion enabled by microfluidics can enhance the maturation of vascular networks within bioprinted tissues, while high-resolution bioprinted biomimetic scaffolds can spatially guide organoid self-assembly, resulting in multifunctional tissues with physiologically relevant vascular–parenchymal interfaces.[120,157] In terms of functional enhancement, this tri-technology integration allows simultaneous achievement of topological control over cell alignment (e.g., directed contraction in cardiac tissue), simulation of mechanical microenvironments (e.g., shear stress response in the BBB), and metabolic zonation (e.g., oxygen gradients in tumor spheroids), thereby significantly improving the pathophysiological relevance of the resulting models.[119,154,158] Moreover, the combination of microfluidic-driven real-time cell density regulation with feedback-controlled bioprinting parameters could enable a closed-loop manufacturing platform for organoids. Such a system would support both high cell viability and high-throughput production, facilitating the standardized fabrication of patient-specific models for personalized medicine.[122]

Looking ahead, as interdisciplinary technical barriers are progressively overcome, the organic integration of these three technologies is expected to drive a paradigm shift—from static structural mimicry to dynamic functional biomimicry in *in vitro* models. This advancement will

provide a more powerful technological engine for disease mechanism studies, drug development, and regenerative medicine.

5. Advances in the application of combined technology in biomedical fields

Conventional modeling systems face significant limitations in clinical translation, including the inability to adequately simulate the complex physiological environment of the human body, discrepancies in biomarker expression, genetic and metabolic differences, and the lack of disease-specific environmental cues. These shortcomings often hinder the direct application of experimental findings to human patients. The emergence of microfluidic, organoid models, and 3D bioprinting technologies provides new opportunities to overcome these barriers. However, given the inherent biological complexity, no single technology can fully replicate all aspects of human physiology. Consequently, the integration of multidisciplinary technologies to faithfully recapitulate human pathophysiological complexity has emerged as an imperative in contemporary biomedical research. This technological convergence has been widely applied in multiple biomedical research domains, including drug research, disease modeling, immunology, clinical treatment, and the integration of other advanced technologies. Its potential application value is substantial and will inevitably provide an important impetus for the advancement of the biomedical field.

5.1. Pharmaceutical research

The demand for pharmaceutical research—closely tied to human health and survival—is ever-increasing. In developing *in vitro* test models, drug research must address target tissue functionality, monitor drug metabolism, and improve both data readability and model throughput.

Although pharmacokinetics (PK) represents a critical discipline for drug development, conventional models often fail to simulate or predict human-relevant metabolic parameters with sufficient quantitative accuracy. Herland et al.[159] constructed the first human physiological PK model based on the integration of microfluidic and organoid technologies. This model simulates the complete physiological processes of drug absorption, metabolism, and excretion and accomplishes an accurate prediction of PK parameters. At the core of the system is a fluid-coupled organoid chip that connects fluid streams via endothelial channels and shares a common arterial–venous mixing reservoir. This design not only ensures physiological stability and accurate data collection but also accomplishes the quantitative translation of PK parameters from *in vitro* to *in vivo* contexts (Figure 5A).

Similar to PK models, toxicology studies require high data fidelity and the ability to reproduce human-specific scenarios. Focusing on the liver, an area of high relevance for drug toxicity, Vurat et al.[160] combined 3D bioprinting and microfluidic technology to construct a multicellular microtissue model mimicking the periodontal ligament–alveolar bone interface. The bioprinted constructs demonstrated stable mechanical, thermal, and rheological properties. Structures embedded with hydroxyapatite-coated magnetic nanoparticles exhibited enhanced compressive strength. Under dynamic culture conditions sustained over 10 days, the constructs retained their morphological integrity and supported uniformly distributed, viable cells. Immunofluorescence analysis revealed that various cell types expressed characteristic markers within their respective matrices and established direct interactions, indicating robust cellular permeability. Subsequent drug interaction studies demonstrated the platform's capability to assess both drug uptake and toxicity, highlighting its potential application in periodontal disease research.

Drug screening—a critical step in pharmaceutical pipelines—has long been hindered by the inability of traditional platforms to accurately recapitulate human tissue architecture and to support clinically relevant, scalable screened. Tebon et al.[161] developed bioprinted tumor organoids related to time-resolved imaging via machine learning-based single-organoid analysis and high-speed live-cell interferometry. These models allowed precise, parallel mass monitoring of multiple cultures, enabling identification of organoids with transient or sustained sensitivity or resistance to specific therapies. This approach effectively reflects drug efficacy and facilitates targeted drug screening (Figure 5B). In addition, the application of a 3D acoustic assembly device to cellular spheroid fabrication enabled large-scale production of viable cellular aggregates. Rigorous validation confirmed high-efficiency spheroid formation with sustained viability, underscoring the translational potential of integrating organoid technology into robust, high-throughput drug screening pipelines.[162]

In summary, the combined application of microfluidics, organoids, and 3D bioprinting enables the dynamic construction of physiological models, the accurate transformation of pharmacological parameters, and efficient high-throughput drug screening. This integration helps modern drug research surpass the limitations of isolated culture systems and move toward a more comprehensive, systematic drug evaluation framework.

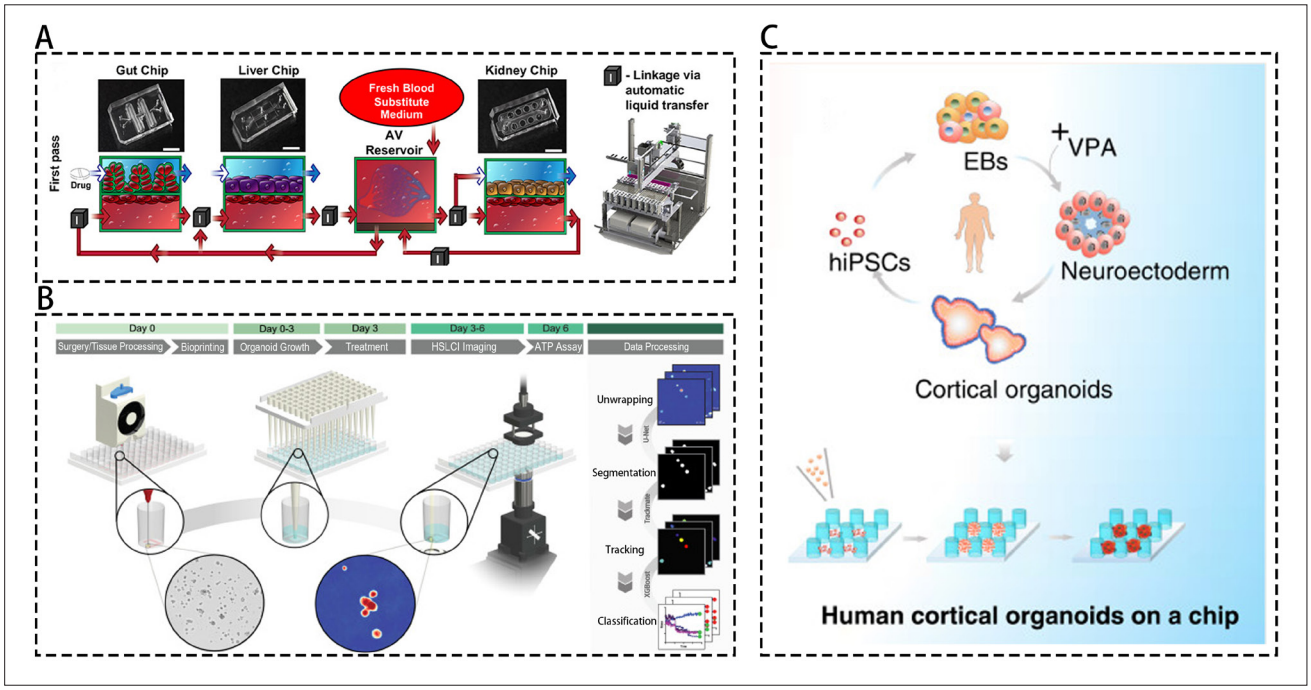


Figure 5. Application of dual-technology combinations—Part I. (A) A physiologically relevant pharmacokinetics model of drug absorption, metabolism, and excretion using microfluidics and organoids. Adapted with permission from ref.[159], Copyright © Nature 2020. (B) High-throughput tumor organoid drug screening using bioprinting. Adapted with permission from ref.[161], Copyright © Nature 2023. (C) Microfluidic culture of cortical organoids derived from hiPSCs used to model early brain development and VPA exposure. Adapted with permission from ref.[163], Copyright © Nature 2020. Abbreviations: AV, arteriovenous; ATP, adenosine triphosphate; EB, embryoid body; hiPSC, human induced pluripotent stem cells; HSLCI, high-speed live-cell interferometry; VPA, valproic acid.

5.2. Disease modeling

In disease modeling, researchers are particularly concerned with the degree of biological fidelity with which *in vitro* cultures replicate *in vivo* processes. The development of ideal human physiological models is essential for exploring the complex and diverse mechanisms of the human body and is critical for building a comprehensive understanding of biological systems. Furthermore, studying disease-specific mechanisms across different organs requires the modeling of key structures and functions that accurately reflect corresponding physiological properties and adaptations.

Cui et al.[163] developed a cortical organoid model using a micropillar array to mimic human brain development in a controlled manner. This model captures the physiological characteristics of early prenatal brain development and reveals the neurodevelopmental effects of the antiepileptic drug valproic acid, thereby expanding the breadth of research on brain development and neurological diseases (Figure 5C).

Yin et al.[164] integrated microfluidic bioprinting with coaxial flows of polymer and crosslinker to fabricate thin, biocompatible, and reproducible hydrogel structures that mimic the epithelial features of the salivary gland. The platform supports the following two printing modes: solid hydrogel fibers with diameters below 100 μm, which can be arranged into millimeter-scale grids, and hollow tubular structures with outer diameters ranging from 0.6 to 2.2 mm and wall thicknesses between 45 and 80 μm, both exhibiting confirmed luminal patency. In both configurations, salivary gland cells were printed at high densities while maintaining their phenotype and viability, highlighting the potential of this strategy for multiscale hydrogel patterning and microscale tissue engineering applications.

Despite substantial advances in *in vitro* model development, there remains a dearth of models that can simultaneously recapitulate the full complexity of the tumor microenvironment, including its diverse cellular components and genetic properties. A vascularized lung cancer model integrating organoid and 3D bioprinting technologies addresses this limitation. This model incorporates idiopathic pulmonary fibrosis-derived lung fibroblasts and a novel porcine lung-derived decellularized ECM hydrogel, effectively replicating the local tumor

microenvironment. Such a system enables the development of targeted therapies and the identification of biomarkers for lung cancer patients with fibrosis.[165]

In addition, a microfluidic intestinal organoid chip, modeled after the natural intestinal villus structure, successfully addresses the *in vitro–in vivo* oxygen dynamics mismatch by improving oxygen exchange efficiency and supporting complex and diverse biological responses. This system sets a paradigm for next-generation, multi-system-interacting organoid chips aimed at precision therapeutic applications.[166]

For cardiovascular toxicity evaluation, Zhang et al.[118] proposed a strategy integrating three technologies to construct a vascularized cardiac tissue model. This model utilized 3D bioprinting to generate microfiber scaffolds and employed a microfluidic system to precisely control the spatial distribution of endothelial cells, thereby mimicking the vascular bed structure and providing functional support for hiPSC-derived cardiomyocytes. The engineered tissue not only exhibited synchronous contraction but also maintained a stable microenvironment through a perfusion system, thereby enhancing the sensitivity and accuracy of toxicity screening.

5.3. Mimicking immune interaction

The mammalian immune system, an extraordinary sensory system for detecting and neutralizing pathogens, plays a critical role in tumor development. The precise recapitulation of intercellular communication networks and faithful reconstruction of tissue-specific immune niches constitute fundamental imperatives in advancing contemporary immunological research paradigms. For example, hepatocellular carcinoma organoid microarrays constructed by co-culturing mesenchymal stromal cells with peripheral blood mononuclear cells partially replicate the tumor microenvironment and provide a promising platform for predicting immunotherapeutic responses in hepatocellular carcinoma patients (Figure 6A).[167]

Similarly, a co-culture tumor immunity model containing patient-derived gastric cancer organoids and tumor-infiltrating lymphocytes mimicked the migration and functional activation of tumor-infiltrating lymphocytes, providing preliminary insights into the mechanisms of both active and passive cell migration.[168]

In addition, a study combining 3D bioprinting and microfluidics demonstrated the potential of fusion technologies for immunoassays. Aimed at accelerating lateral chromatography immunoassays for novel coronavirus screening, the study used microfluidics and 3D bioprinting for protein deposition and shell unit formation, respectively, thereby optimizing and expediting the assay process.[169]

5.4. Personalized therapies

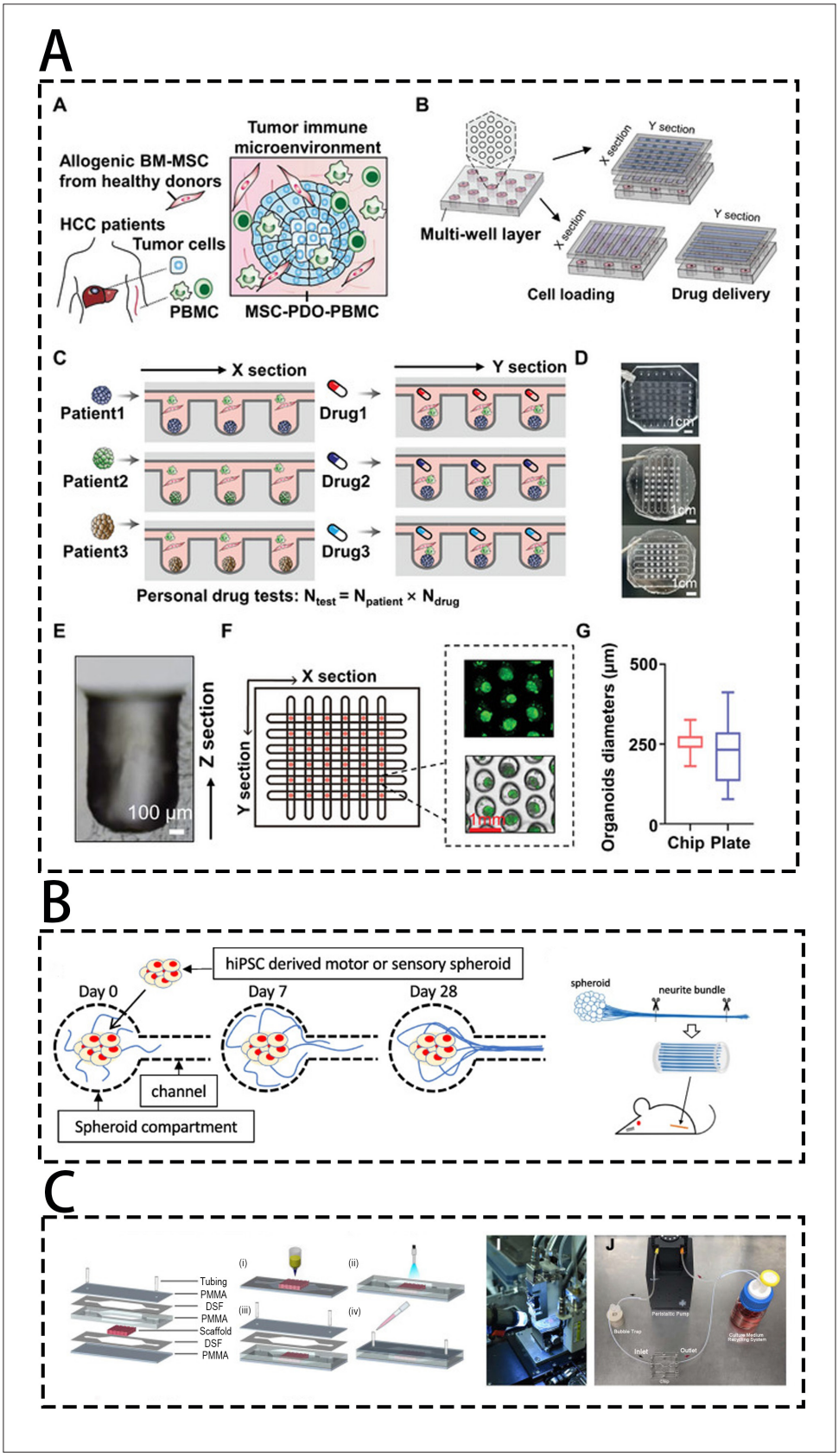
Individualized clinical therapy programs often provide interventions tailored to a patient's specific condition and needs. These personalized therapies are innovative not only in their integration of emerging scientific and technological advancements but also in their use of bioinspired models to assess clinical feasibility and facilitate clinical application.

For example, in cardiovascular disease treatment, the combination of microfluidic molding and coaxial bioprinting technologies enables the fabrication of freestanding, perfusable vascular structures. These structures support the precise integration of relevant vascular cells in multilayered and biomimetic configurations. Mechanical evaluations in tension and bending confirmed the model's feasibility in percutaneous coronary intervention, demonstrating its significant potential in vascular bionics.[170] Similarly, in cases of severe peripheral nerve injury requiring autologous nerve grafts, specific microfluidic devices have been employed to promote the generation of artificial nerve synapse bundles derived from hiPSCs, with physiologically favorable correlations and functional states. This approach represents a promising, safe, and effective peripheral nerve treatment (Figure 6B).[171]

In addition, a study utilizing digital light processing printing successfully engineered and printed bone-healing tissue organoids. The resulting models exhibited stage-specific gene expression patterns reflecting biological processes such as endochondral ossification, underscoring their potential for rapid regeneration and recovery from bone injuries.[172] Overall, the combination of organoids, 3D bioprinting, and microfluidics holds great promise for the development of sustainable and environmentally friendly medical solutions, contributing significantly to the future of medical innovation.

5.5. Cross-disciplinary applications

The current era is marked by the co-evolution of multiple disciplines, fostering innovation, and driving integration across fields. Within this broad context, advanced technologies such as organoids, microfluidics, and 3D bioprinting are playing increasingly pivotal roles, especially in their fusion with other advanced technologies. The synergistic integration of these technologies transcends conventional disciplinary boundaries in biomedicine, establishing a catalytic framework that propels interdisciplinary innovation across precision medicine, biomimetic engineering, and advanced material science. Specifically, cross-disciplinary


A
A
Allogenic BM-MSC from healthy donors
HCC patients
Tumor cells
PBMC
Tumor immune microenvironment
MSC-PDO-PBMC
B
Multi-well layer
X section
Y section
Cell loading
Drug delivery
C
X section
Y section
Patient1
Patient2
Patient3
Drug1
Drug2
Drug3
Personal drug tests: $N_{test} = N_{patient} \times N_{drug}$
D
1cm
E
100 μm
Z section
F
X section
Y section
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Organoids diameters (μm)
500
250
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Chip Plate
B
hiPSC derived motor or sensory spheroid
Day 0
Day 7
Day 28
channel
Spheroid compartment
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neurite bundle
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(i)
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I
J
Peristaltic Pump
Bubble Trap
Inlet
Outlet
Culture Medium Recycling System

Figure 6. Application of dual-technology combinations—Part II. (A) Co-culture of MSCs and PBMCs to construct HCC organoids mimicking the primitive tumor microenvironment. Adapted with permission from ref.[167], Copyright © Wiley 2023. (B) Neural organoids derived from hiPSCs cultured in a specialized microfluidic device to generate artificial synapse bundles for transplantation. Adapted with permission from ref.[171], Copyright © Springer Nature 2024. (C) Liver-on-a-chip model constructed by bioprinting hepatocyte extracellular vesicles onto a microfluidic chip to evaluate the metastatic behavior of reprogrammed triple-negative breast cancer cells in an *in vitro* 3D liver microenvironment. Adapted with permission from ref.[173], Copyright © IVYSPRING 2023. Abbreviations: BM-MSC, bone marrow-derived mesenchymal stem cell; DSF, dual-scale flow; HCC, hepatocellular carcinoma; hiPSC, human induced pluripotent stem cells; PBMC, peripheral blood mononuclear cell; PDO, patient-derived organoid; PMMA, polymethyl methacrylate.

integration not only compensates for the limitations inherent in any single technology but also facilitates a more accurate representation of physiological and pathological processes *in vivo*, thereby accelerating clinical translation. For example, Lu et al.[173] demonstrated the feasibility of reprogramming triple-negative breast cancer cells into hepatocytes using a simplified reagent preparation method, integrated with microfluidics and 3D bioprinting technologies. Through single-cell sequencing analysis and liver-on-a-chip modeling, the study confirmed both the reprogrammed phenotype in a hepatic microenvironment and the ability to inhibit cancer cell metastasis, offering a novel therapeutic approach for triple-negative breast cancer liver metastasis (Figure 6C).

In the field of biomics, Bues et al.[174] used DisCo microfluidics to address the challenge of low-input samples in single-cell RNA sequencing. By combining machine vision with microfluidics, this approach efficiently captured single-cell samples with high throughput and precision. Their analysis of intestinal organoids and mouse intestinal crypts demonstrated the innovative application of DisCo technology in revealing organoid heterogeneity and cellular composition.

In materials science, Qazi et al.[175] developed a photosensitive polymer hydrogel enabling *in vivo* 3D bioprinting within living mouse tissues. They successfully localized complex structures such as the dermis, skeletal muscle, and brain, and demonstrated muscle fiber formation within mouse muscle tissue, opening a promising avenue for minimally invasive surgical organ repair. Similarly, Daly et al.[176] applied bioprinting to transfer organoids into self-healing support hydrogels, facilitating the scalable production of high-cell-density microtissues that mimic cardiac scar tissue post-myocardial infarction. This technological combination enables both *in vivo* 3D bioprinting and the large-scale fabrication of functional microtissues, advancing applications in organ repair and disease modeling.

As the cross-application of organoid, microfluidic, and 3D bioprinting technologies progresses, their cross-disciplinary application marks the advent of a transformative era in regenerative medicine and biomedical engineering.

5.6. Obstacles to transformation and future prospects for technology integration

At the translational level, the widespread implementation of microfluidics, organoids, and 3D bioprinting faces multiple practical challenges. The complexity of integrating these technologies results in high development and maintenance costs. Additionally, the need for precision manufacturing and specialized materials raises the industrial entry threshold, limiting accessibility for small- and medium-sized institutions.[177]

A major barrier lies in the absence of unified technical standards and quality assessment systems. Significant variability in culture environments, printing parameters, and detection methodologies across platforms impedes the development of reproducible, standardized workflows, severely restricting inter-laboratory data sharing and translational efficiency.[178] Clinically, long-term stability of organoid models, functional validation of vascularized constructs, and the immunocompatibility of bioprinted tissues remain critical concerns that require large-scale animal studies and long-term safety evaluations. Regulatory guidelines for approving such emerging integrated technologies remain underdeveloped, significantly extending the timeline from laboratory research to clinical implementation.[179]

Looking ahead, deepening interdisciplinary collaborative innovation is expected to drive breakthroughs across multiple dimensions. Advances in materials science and engineering will facilitate the development of low-cost, highly biocompatible bioinks, and microfluidic chips. When combined with automated control technologies, these innovations can facilitate the construction of intelligent platforms that significantly reduce operational complexity and enhance production efficiency.[180]

The establishment of standardized technical systems will be accelerated through collaboration among industry associations, research institutions, and enterprises. This will lead to comprehensive frameworks covering model construction, performance evaluation, and clinical application, offering clear guidance for technology

translation.[181] In terms of application expansion, integrated technological platforms will significantly empower precision medicine—for example, enabling the development of personalized tumor organoid models for targeted drug screening or the use of bioprinted vascularized tissues for organ repair—thus transitioning clinical practice from empirical approaches to precision-based strategies.[119] The incorporation of artificial intelligence and biosensing technologies will enable real-time monitoring and dynamic regulation of the culturing process, making tissue engineering more intelligent and controllable. This provides technical support for the precise fabrication of complex organ models.[182] In the long term, building an innovation ecosystem that deeply integrates government, industry, academia, research institutions, and clinical practice—along with a robust ethical and regulatory framework—will accelerate the translation of technological advancements into healthcare benefits. This will create a seamless pipeline from laboratory research to clinical application, bringing revolutionary breakthroughs to regenerative medicine and precision healthcare.[183]

6. 4D culture

While the integration of microfluidics, organoids, and 3D bioprinting facilitates the creation of tissue models, significant differences remain compared to the developmental trajectories of real tissues and organs *in vivo*. These discrepancies primarily stem from the lack of precise and targeted induction of key biological processes such as cell differentiation, migration, proliferation, and apoptosis. Although current systems can support *ab initio* culture, they lack the auxiliary induction and precise regulation required at critical physiological developmental stages, resulting in a substantial gap between *in vitro* models and native tissue development.

Introducing time as a variable—transforming 3D into 4D culture—may further optimize the simulation of tissue growth and development. This approach could help narrow the gap between cultured models and the formation of functional, physiologically accurate tissues. Achieving this goal hinges on a thorough understanding of the regulatory factors orchestrating spatiotemporal induction, allowing for targeted interventions at key developmental checkpoints in accordance with the principles governing tissue development. Some experiments have already successfully demonstrated the precise induction of directed cell differentiation and development by modulating controllable factors. These findings provide valuable references for further optimizing tissue model development.

Specific growth geometries significantly influence directed cell differentiation. By shaping organoids to mimic organ-specific morphogenetic processes, stem cells can be guided to differentiate along predefined spatial boundaries and form tissues with distinct structural features. This strategy not only replicates the 3D architecture of various organs but also allows for precise control over stem cell fate and tissue morphogenesis. For instance, research by Liberali and Schier[33] demonstrated how cystic gut-like organoids can be shaped into the crypt and villus structures characteristic of the intestinal tract. Through the synergistic integration of tissue engineering platforms and principles of cellular self-organization, intestinal stem cells were guided to recapitulate tubular epithelial morphogenesis with a patent lumen, resulting in physiologically relevant intestinal architectures. These organoids not only spatially reproduce the *in vivo* structures of crypts and villi but also exhibit key physiological features and strong regenerative capacity. Notably, specialized cell types rarely seen in conventional organoids were successfully induced, enhancing physiological relevance.

Further studies revealed that the emergence and formation of crypt structures in organoids can be predicted and controlled simply by manipulating their initial geometry. Researchers utilized localized light exposure to soften specific areas of the substrate, creating initial geometries that directed where crypts would form. Crypt formation occurred exclusively in the softened regions, while unaffected regions showed no structural changes. Additionally, the timing of substrate softening proved critical, initiating softening 2 days after the onset of stem cell differentiation significantly reduced crypt development.[39]

Regulation of signaling pathways is also a crucial factor in the process of directed cell differentiation. Specifically, various signaling pathways guide stem cell differentiation by being selectively activated or inhibited at precise time points. For instance, cardamonin has demonstrated the potential to induce osteogenic differentiation in human amniotic mesenchymal stem cells through targeted modulation using small-molecule compounds. At non-toxic concentrations, cardamonin significantly enhanced alkaline phosphatase activity and calcium deposition, while upregulating both early and late osteogenic markers. Pathway analysis revealed that this flavonoid regulates cell fate by increasing the phosphorylation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3). Inhibition experiments further confirmed that its effects are mediated through the JAK2/STAT3 pathway, highlighting its potential as a small-molecule osteoinductive agent for regenerative medicine applications.[184] Similarly, Cai et al.[185] induced the differentiation of hiPSCs into valvular endothelial cells and

valvular interstitial cells through the sequential activation of the Wnt, bone morphogenetic protein 4 (BMP4), vascular endothelial growth factor (VEGF), and nuclear factor of activated T cells 1 signaling pathways. They employed CHIR-99021, BMP4, VEGF-165, and forskolin to achieve this modulation. Comparative analyses revealed that the differentiated cells closely resembled primary heart valve endothelial cells and mesenchymal cells in both gene expression profiles and functional characteristics. Furthermore, sequencing technology indicated that the transcriptional trajectories during differentiation closely mirrored those observed in embryonic valve development.

In addition to biochemical cues, the physicochemical environment also plays a critical role in regulating cell differentiation. Oxygen concentration, for example, has a profound effect on erythropoiesis. While hypoxia is typically viewed as a limiting factor in vertebrate embryonic development—due to the need for sufficient oxygen to support metabolism—recent studies suggest that hypoxic conditions can actively induce stem cell differentiation and promote developmental processes. Red lineage differentiation begins early in embryogenesis through oxygen delivery; however, the specific mechanisms regulating this process remain to be fully elucidated.

One study revealed that the *vgll4b* gene in Zebrafish exhibits a sensitive response to oxygen levels.[186] Researchers established a *vgll4b* mutant zebrafish model using CRISPR/Cas9 technology, which exhibited impaired heme synthesis and disrupted terminal differentiation of erythrocytes. This defect underscores the role of *vgll4b* as a critical regulator of HIF1α-dependent erythropoiesis. Moreover, it was discovered that the Vgll4 protein inhibits the negative regulation of *alas2* expression and heme biosynthesis by binding to Irf2bp2. This mechanistic insight elucidates the pivotal role of oxygen tension in erythropoietic regulation and delineates how metabolic oxygen dynamics orchestrate cellular differentiation trajectories through discrete signaling cascades. Another vital regulatory component is mechanical signaling, which plays diverse roles in cell fate transitions, migration, and morphogenesis. Cells dynamically sense and respond to their external physical environment, including stimuli such as substrate mechanical properties and shear stress. Concurrently, the mechanical characteristics of the cell surface modulate intracellular signaling through feedback mechanisms. This bidirectional regulation underscores the profound influence of mechanical signaling on stem cell fate and further illustrates the complexity of cell differentiation processes.[187]

In summary, current *in vitro* factors known to induce directional cell differentiation include external signaling molecules, physical stimuli, and growth geometries. These factors activate specific signaling pathways in various ways, effectively guiding cellular processes. To achieve precise directed differentiation, a deep understanding of the activation mechanisms and regulatory conditions governing these signaling pathways is essential. Technological advancements have revolutionized experimental methodologies, gradually unveiling biological phenomena that were previously challenging to observe using traditional techniques.[188] This progress demonstrates the interdependence between the exploration of signaling pathways and technological innovation—each enhancing the other, jointly driving the advancement of scientific frontiers.

The three technologies highlighted in this discussion—organoids, microfluidics, and 3D bioprinting—are pivotal to this endeavor. Organoids offer a physiologically relevant platform that supports the differentiation of stem cells into 3D structures closely resembling native tissues. Microfluidic systems provide spatiotemporally resolved regulation of exogenous signaling gradients and mechanical cues, while dynamically modulating physicochemical parameters to establish precisely controlled experimental milieus. Meanwhile, 3D bioprinting enables the customization of spatial structures and optimization of the culture environment. The integration of these three technologies not only incorporates diverse factors influencing directed differentiation but also facilitates stage-specific activation of key signaling pathways, enabling precise control of the cell differentiation process. Experimental evidence has already demonstrated the feasibility and synergistic benefits of combining these three approaches.[95,189]

Against this favorable backdrop, we introduce the novel concept of "4D culture," which integrates organoids, 3D bioprinting, and microfluidics. The 4D culture model extends beyond spatial control to include temporal dynamics. In this system, 3D bioprinting engineers the spatial structure, organoids mimic physiologically relevant differentiation processes, and microfluidics generates a dynamic microenvironment necessary for sustaining these processes. Specifically, microfluidics allows for dynamic regulation of the microenvironment by simulating blood flow, removing necrotic cells, delivering and monitoring nutrients and oxygen, supplying growth factors, and introducing hydrodynamic forces.

This 4D culture paradigm emerges as a synergistic framework for next-generation biomedical technologies, establishing physiologically relevant translational platforms that bridge the gap between preclinical modeling and clinical implementation. Such innovations hold the potential to revolutionize disease management

by enhancing predictive diagnostics and therapeutic development, thereby establishing new frontiers in precision medicine.

7. Conclusion

Microfluidics, organoids, and 3D bioprinting are expected to play a central role in modeling systems that require the structural and functional mimicry of natural human tissues. The successful construction of such models depends on the coordinated regulation of various factors, including stem cell proliferation and differentiation, ecological microenvironment control, and spatial precision. It is worth noting that no single technology can overcome all limitations independently; instead, the integrated application of multiple technologies is essential for developing optimal *in vitro* models.

Recent studies have frequently employed dual-technology integration, and the resulting fusion models can be broadly categorized into three groups. The first category involves the combination of microfluidics and organoids—an approach that effectively modulates the microenvironment of cultures, facilitates the integration of system-level parameters, enhances vascularization and nutrient delivery, and improves data readability and real-time imaging capabilities. The second category encompasses the combination of microfluidics and 3D bioprinting; such technological integration significantly advances the microscale shaping of pathophysiological tissue structures and culture microenvironments, the formation of engineered vascular systems, the optimization of parameters such as resolution and structural stability, and the ability to automate and dynamically monitor cultures through the interplay of fluid flow control and spatial patterning. The final category consists of the integration of organoids and 3D bioprinting, which enhances the functional characterization of cultures, enables spatial manipulation, and facilitates high-fidelity, highly replicable, and high-throughput bioprocessing.

With continued exploration of microfluidics, organoids, and 3D bioprinting, combinatorial technologies are gaining traction for their clinical translational potential. Initial successes have been observed in areas such as drug evaluation, disease modeling, immunological exploration, therapeutic development, and broader multi-technology integration.

While dual-technology integration introduces novel paradigms for biomedical innovation, the absence of a third synergistic technological modality limits the full optimization of modeling systems. The unique advantages offered by the three technologies are indispensable for constructing physiologically relevant models, and thus, their combined use is essential. As the field evolves, a small number of studies have been geared toward exploring the tri-technology integration of organoids, microfluidics, and 3D bioprinting to achieve more optimal model selection and development.[118,190] However, notable discrepancies persist between *in vitro* models developed through current fusion technologies and the developmental trajectories of real tissues and organs *in vivo*. Much of this discrepancy stems from the lack of precise, targeted chemotactic guidance for cell fate decisions, including differentiation, migration, proliferation, apoptosis, and the establishment of tissue morphology. Although current fusion systems can initiate culture from scratch, they often lack intentional auxiliary induction and temporal regulation at key physiological checkpoints. As a result, the developmental course of *in vitro* cultures diverges significantly from that of native tissues. To address this gap, introducing a temporal variable into the culture process offers a promising strategy to better simulate tissue growth and developmental dynamics, thereby narrowing the gap between *in vitro* models and natural tissue formation. This approach holds potential for broader application in related research fields. It is important to emphasize that classical studies have clearly demonstrated that the temporal activation of signaling pathways is critical for stem cell differentiation, migration, and morphogenesis. For instance, precise regulation of signaling pathways such as Wnt and transforming growth factor-beta not only optimizes the direction of cell differentiation but also facilitates the formation of tissue structures at specific developmental stages.[191,192] Additionally, the mechanical properties of the ECM and the optimization of the physical microenvironment further enhance the physiological relevance of *in vitro* models.[193,194]

The integration of these factors lays the groundwork for constructing a 4D dynamic culture system, transforming *in vitro* models from static structures into multidimensional dynamic developmental systems. By introducing the time dimension, we aim to significantly narrow the gap between *in vitro* cultures and *in vivo* tissue development, thereby enhancing the physiological fidelity of these models. Such technological advancements not only open new avenues for precise cell differentiation research but also broaden the application potential in disease modeling and drug discovery. We propose, for the first time, a culture approach that adds a temporal dimension to the integrated use of organoids, 3D bioprinting, and microfluidics, and define this system as 4D culture.

In this model, 3D bioprinting is responsible for shaping the spatial architecture; organoids mimic the developmental process of primitive cells differentiating into mature cells; and microfluidics dynamically modulate the

microenvironment of organ development and replicate the functions of blood flow, including the removal of necrotic cells, the transport and monitoring of nutrients and oxygen, the delivery of growth factors, and the application of fluidic forces. In addition, 4D culture can serve as a platform for incorporating various emerging technologies aimed at creating improved models for preclinical studies, ultimately enhancing the prediction, diagnosis, and treatment of diseases to better serve biomedical research.

The integration of multiple technologies in *in vitro* testing methods has profound implications for resource efficiency, data accuracy, and experimental reproducibility. Automation and standardized workflows reduce manual intervention, minimize the risk of human error, and—through the use of data visualization techniques—enable real-time monitoring of experimental data with enhanced resolution, thereby reducing both time and resource consumption. In the long term, these integrated technologies facilitate more accurate simulations of patient-specific biological characteristics, enhancing the precision of drug screening and preclinical trials. This not only increases the translational value of research but may also reduce failure rates in future clinical trial stages.[195,196] With respect to data accuracy, microfluidic technology improves the imaging and data monitoring capabilities of 3D bioprinting and organoid technologies, enhancing the data readability of cell cultures—an essential factor in ensuring the accuracy of experimental results. Automated experimental procedures further minimize human error, significantly improving experimental reproducibility. Moreover, precise control over key external factors, such as growth factor concentrations and initial geometric configurations, supports consistent cell growth and differentiation, thereby strengthening the uniformity of experimental conditions and the reliability of repeated experiments. Although these methods offer clear advantages in long-term cost-effectiveness, the initial investment in equipment and technology can be substantial, potentially posing financial challenges for some research institutions and laboratories.[197,198] Additionally, the integration of these technologies requires higher levels of technical expertise. Effective coordination between platforms, as well as the unification of data analysis workflow for technologies such as microfluidics, organoids, and 3D bioprinting, still requires further research and optimization.[199]

Dual-, triple-, and prospective 4D cultures—incorporating a temporal dimension alongside microfluidic, organoid, and 3D bioprinting technologies—offer unprecedented opportunities to mimic the developmental processes of human tissues. These integrated classes of technologies aim to accurately reproduce cell fate decisions and tissue morphogenesis during culture construction and can dynamically regulate the culture environment across temporal and spatial dimensions to more closely replicate organ development *in vivo*. This innovative paradigm holds transformative potential for redefining the frontiers of biomedical research by establishing physiologically relevant platforms for drug efficacy testing, pathomimetic disease modeling, and therapeutic regimen optimization. In doing so, it advances precision medicine initiatives through the development of high-fidelity, clinically translatable model systems.

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Author contributions

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