ACCSCIENCE PUBLISHING

International Journal of Bioprinting

REVIEW ARTICLE

# Advances in biomanufacturing and medical applications of three-dimensional-printed organoids: A review

**Lingzi Liao[1, 2, 3†], Qiushi Feng[1, 2, 3†], Xiaofeng Shan[1, 2, 3], Zhigang Cai[1, 2, 3*], and Shang Xie[1, 2, 3*]**

[1]Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing, China

[2]National Center for Stomatology, Beijing, China

[3]National Clinical Research Center for Oral Diseases, Beijing, China

## Abstract

The emergence of organoid technology has bridged critical gaps between conventional two-dimensional cell cultures and *in vivo* systems by offering self-organized three-dimensional (3D) microtissues that recapitulate organ-specific architecture, cellular heterogeneity, and functional dynamics. However, traditional organoid models face inherent limitations in structural precision, scalability, and physiological relevance, particularly in replicating vascular networks, mechanical microenvironments, and multicellular interactions. Recent advancements in 3D bioprinting have enabled unprecedented spatial control over cellular and extracellular matrix organization, unlocking new frontiers in engineering organoids with enhanced biomimicry and functionality. This review systematically examines the integration of bioprinting technologies with organoid science, spanning biomaterial innovations, vascularization strategies, and dynamic microenvironmental cues that drive functional maturation. By synthesizing interdisciplinary advances in stem cell biology, materials science, and computational modeling, the work highlights applications across regenerative medicine, disease pathophysiology, and personalized drug screening. Key challenges, including immunogenicity, long-term stability, and clinical scalability, are critically evaluated alongside emerging solutions such as four-dimensional bioprinting, organ-on-chip integration, and artificial intelligence-driven bioink optimization. Through a comprehensive analysis of bioprinted organoids for physiology and 3D disease modeling, this review aims to establish a translational roadmap for leveraging spatially programmed organoids to address unmet clinical needs, revolutionize therapeutic development, and advance precision medicine.

***Keywords:*** High-throughput screening; Patient-derived organoids; Regenerative medicine; Spatiotemporal control; Three-dimensional-bioprinting; Vascularization

†These authors contributed equally to this work.

***Corresponding authors:**
Zhigang Cai
(c2013xs@163.com)

Shang Xie
(xs2013@hsc.pku.edu.cn)

**Citation:** Liao L, Feng Q, Shan X, Cai Z, Xie S. Advances in biomanufacturing and medical applications of three-dimensional-printed organoids: A review. *Int J Bioprint*. 2025;11(4):66-98. doi: 10.36922/IJB025190184

**Received:** May 7, 2025
**Revised:** May 20, 2025
**Accepted:** May 28, 2025
**Published Online:** June 10, 2025

## 1. Introduction

Organoid technology has emerged as a paradigm-shifting innovation in biomedical research, enabling the *in vitro* reconstruction of self-organized three-dimensional (3D) microtissues that recapitulate structural, functional, and developmental features of native organs.[1] Originating from breakthroughs in stem cell biology and developmental signaling pathways, organoids are typically derived from induced pluripotent stem cells (iPSCs) or various tissue-derived cells, including adult stem cells, differentiated cells, or cancer cells, through spatially controlled differentiation protocols. These models have transcended the limitations of conventional two-dimensional (2D) cultures by preserving cell polarity, cell–cell interactions, and tissue-specific functionality, which are critical for modeling organogenesis, disease progression, and therapeutic responses.[2,3] Additionally, they are particularly useful in cancer research due to their ability to preserve genetic and phenotypic stability, as well as their compatibility with cryopreservation techniques.[4,5] Despite being a relatively nascent field, organoid technology continues to face considerable challenges in both construction and cultivation. For instance, the processes of manual passaging and cultivation are both labor-intensive and financially burdensome.[6] Organoids exhibit a lower level of complexity compared to native tissues, primarily due to the absence of immune and vascular systems. Meanwhile, the integration of organoids with tissue engineering scaffolds and the mass production of organoids, along with their application in drug development, remains a complex endeavor. In response to these challenges, researchers have been actively exploring various biofabrication techniques, including 3D bioprinting, to enhance the development of organoids.

Concurrently, 3D bioprinting has evolved from prototyping tools into a sophisticated biofabrication platform, enabling precise deposition of cell-laden bioinks with microscale resolution. This technology leverages additive manufacturing principles to construct spatially organized tissues through layer-by-layer assembly of living cells, biomaterials, and bioactive cues.[7,8] Modern bioprinting modalities, including extrusion-based, laser-assisted bioprinting, and digital light processing systems, offer unparalleled control over tissue architecture, permitting the engineering of vascular channels, heterogeneous cellular zonation, and mechanically graded matrices (Figure 1).[6] Unlike traditional organoid culture, which relies on self-organization within undefined matrices like Matrigel, bioprinting imposes engineered self-organization, a hybrid approach combining bottom–up biological principles with top–down spatial guidance. This synergy addresses critical gaps in organoid technology. Bioprinting provides structural fidelity and reproducibility, while organoids contribute physiological relevance through inherent cellular differentiation programs.

The strategic combination of these modalities creates a transformative framework for precision tissue engineering. On that basis, bioprinting can spatially arrange organoid-forming progenitors within vascularized scaffolds, overcoming the diffusion-limited growth of conventional organoids.[9] Advanced bioinks functionalized with decellularized extracellular matrix (dECM) components or synthetic mimetic peptides further enhance organoid maturation by replicating tissue-specific biochemical niche. From a translational perspective, automated bioprinting platforms enable scalable production of standardized organoid arrays, facilitating their integration into high-throughput drug screening pipelines and personalized disease modeling. Recent demonstrations include bioprinted hepatorganoids with zonated metabolic activity for toxicity testing, and patient-derived tumor organoids incorporating immune cells for immunotherapy evaluation.

This review examines how the strategic integration of 3D bioprinting and organoid technologies is redefining the frontiers in regenerative medicine and pathophysiological modeling. It analyzes the technological synergies that resolve historical limitations of both fields: the capacity of bioprinting to impose architectural control on organoid self-organization and the ability of organoids to confer innate biological complexity to bioprinted constructs. Emerging applications in multiscale vascularization, neurovascular interface engineering, and immune-competent tumor modeling are critically evaluated. By delineating current achievements and persistent challenges, this work aims to chart a roadmap for next-generation biofabricated organoid systems that bridge the gap between *in vitro* models and clinical translation.

## 2. Functional bioprinted organoids for physiology and regenerative medicine

Bioprinted organoids hold transformative potential for regenerative medicine. However, clinical translation requires addressing vascularization deficits, functional maturation, and standardization of bioink formulations.

### 2.1. Engineered hard-tissue organoids

Bone defects are frequently observed in orthopedic clinical research. Traditional treatment strategies often fall short of expectations, due to the distinctive architecture of hard tissue. Bone organoids—3D tissue constructs cultivated *in vitro*—aim to mimic the structure and function of native bone tissue for research and regenerative purposes.[10] The integration of cutting-edge biomaterials and additive manufacturing techniques enables the creation of 3D

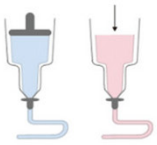
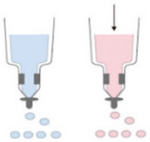
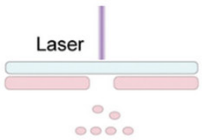
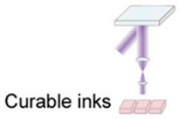
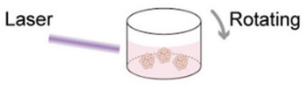
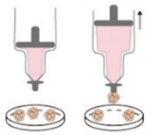
| Bioprinting technology | Advantages | Disadvantages | Types of organoid |
|---|---|---|---|
| Extrusion bioprinting  | • Versatility<br>• Large-scale printing<br>• High cell survival rate<br>• Lower cost | • Lower resolution<br>• Slower printing speed<br>• Material limitations<br>• Post-processing requirements | • Bone<br>• Liver<br>• Cardiac<br>• Kidney<br>• Cancer |
| Droplet-based bioprinting  | • High resolution<br>• High controllability<br>• Low cost<br>• Rapid prototyping | • Material limitations<br>• Low cell viability<br>• Limited printing speed<br>• Nozzle clogging | • Bone<br>• Intestinal<br>• Kidney<br>• Assembloids |
| Stereolithography (SLA)   | • High resolution<br>• Materials options<br>• Smooth surface<br>• High precision | • Material limitations<br>• Low cell survival rate<br>• Printing speed<br>• Complex post-processing | • Cancer |
| Digital light processing (DLP)   | • High resolution<br>• High speed<br>• Good surface quality<br>• High flexibility | • Material limitations<br>• Cell viability<br>• High equipment cost<br>• Resin processing | • Bone<br>• Intestinal |
| Volumetric bioprinting   | • Fast speed<br>• High resolution<br>• Cell-friendly<br>• Uniformity | • Material complexity<br>• Equipment complexity<br>• Technology maturity<br>• Light scattering issues | • Liver |
| Magnetic-assisted bioprinting  | • Contactless printing<br>• High precision<br>• Good compatibility<br>• Strong repeatability | • Magnetic particle influence<br>• Complicated equipment<br>• Material limitations<br>• Early stage of technology | • Assembloids<br>• Cancer |
| Aspiration-assisted bioprinting (AAB)  | • High precision<br>• Cell-friendly<br>• Material diversity<br>• High flexibility | • Slower speed<br>• Technology maturity<br>• Equipment complexity<br>• High material consumption | • Assembloids |

**Figure 1.** Organoid three-dimensional bioprinting technologies and their advantages, disadvantages, and applications. Adapted with permission from Hu et al.[6] Copyright© Wiley 2025.

tissues that can self-assemble into cell aggregates and organoids in response to physiological and induced signals.[11,12] They also help in evaluating the effectiveness of pharmacological treatments aimed at improving bone density and reducing fracture risk.

Bioprinting has been instrumental in engineering hard tissue, where the fabrication of a bone extracellular matrix (ECM) analog has been achieved to properly mimic the 3D-mineralized ECM components. This approach enables precise spatial control over osteogenic cell populations, thereby inducing specific cell fates and functions[13,14] and facilitating the maturation of bone organoids.[15] Bioprinting enables precise manipulation of biophysical properties, including organoid size, cell number, and conformation, with modifications in organoid conformation substantially increasing secreted yield per initial cell number.[16] Rooted in synthetic biology, recent advancements in light-based 3D printing of DNA hydrogels offer promising potential to revolutionize current ECM-assembling approaches. These hydrogels offer key advantages, including resistance to enzymatic degradation, programmability, precise structural control, and desirable mechanical properties.[17]

Recent advances in bioink design, including gelatin methacrylate/alginate methacrylate/hydroxyapatite (GelMA/AlgMA/HAP) composites, have facilitated the creation of self-mineralizing scaffolds that support long-term maturation of bone organoids.[18] Utilizing cutting-edge 3D bioprinting technology and bone matrix-inspired bioink formulations, researchers have developed a platform for generating bone organoids from bone marrow-derived mesenchymal stem cells.[19] This method involves combining bone marrow-derived mesenchymal stem cells with hydrogels to create bioinks, which are then employed in light-curing 3D bioprinting to produce bone organoids (Figure 2A). Upon implantation into an animal model, these organoids exhibited spontaneous mineralization and maturation processes, leading to the formation of fully developed and vascularized bone tissue (Figure 2B).[20,21] Li et al.[22] added hydroxyapatite nanowires to osteoblast precursor cell spheroids, providing numerous material exchange channels for internal cells by interpenetrating them into cell spheroids. The incorporation of nanowires enhanced the osteogenic phenotype and effectively improved the biological activity of core cells in spheroids, which can potentially be used as building blocks for the construction of large, high-density biomimetic tissues and organoids using 3D bioprinting technology (Figure 2C).[22] Fang et al.[23] successfully printed highly vascularized bone organoid tissues using a granular aggregate-pre-vascularized bioink and found that the pre-vascularized mesenchymal spheroids developed an interconnected vascular network through angiogenic sprouting.[23] These constructs promote osteogenic differentiation and support fracture repair mechanisms.

However, challenges persist in replicating the hierarchical mechanical properties of native hard tissues, particularly in achieving stiffness values that match those of human bones (10–30 GPa) while maintaining cell viability.[24] Innovations in nozzle-free bioprinting techniques, which eliminate shear stress on encapsulated cells, have improved organoid post-printing viability (>95%) and preserved multicellular polarization.[25,26] De Leeuw et al.[27] found that elevated cellular density increases the rates of mineralization and enhances the mechanical stiffness of 3D-bioprinted patient-derived bone organoids when exposed to dynamic loading conditions.[27] The present research on bone organoids is in its early developmental phase. These organoids currently emulate only limited aspects of bone tissue functionality. Beyond the standard functionalities and essential characteristics of organoids, bone organoids must replicate both the micro- and macro-architectures of bone tissue. They should also offer sufficient mechanical support and incorporate a bone marrow microenvironment capable of hematopoietic activity. Furthermore, bone organoids are expected to produce immune cells and establish functional interactions with the nervous, immune, lymphatic, and vascular systems. These attributes will facilitate the ability of bone organoids to more accurately replicate the physiological functions of native bone tissue.[28] Other directions include integrating patient-specific stem cells, osteogenesis under compressive stimulation, and computational modeling to optimize printed scaffold porosity and load-bearing capacity for clinical translation.[10,29–31]

### 2.2. 3D reconstruction of biomimetic cardiovascular organoids

A 3D culture system for cardiomyocytes can replicate physiological and dynamic conditions effectively for cardiovascular assessment.[32,33] The 3D system addresses limitations seen in 2D monolayer setups, such as inadequate spreading size, excitation-contraction coupling (T-tubules), mature calcium ion channels for active force stimuli, and efficient energy conversion through oxidative metabolism.[34] Cardiomyocytes and other cardiac cells are cultured within solid biomaterials (like scaffolds or hydrogels) in this microenvironment to enhance cardiac tissue formation and simulate the heart's physiological conditions.[35,36] The geometric morphology of cardiomyocytes, myofibril expression, and junction protein formation differ significantly between 3D and 2D cell cultures.[37] Furthermore, 3D culture offers cardiomyocytes protection against drug-induced mechanical stress and apoptosis.[38] Recently, organoids have been developed

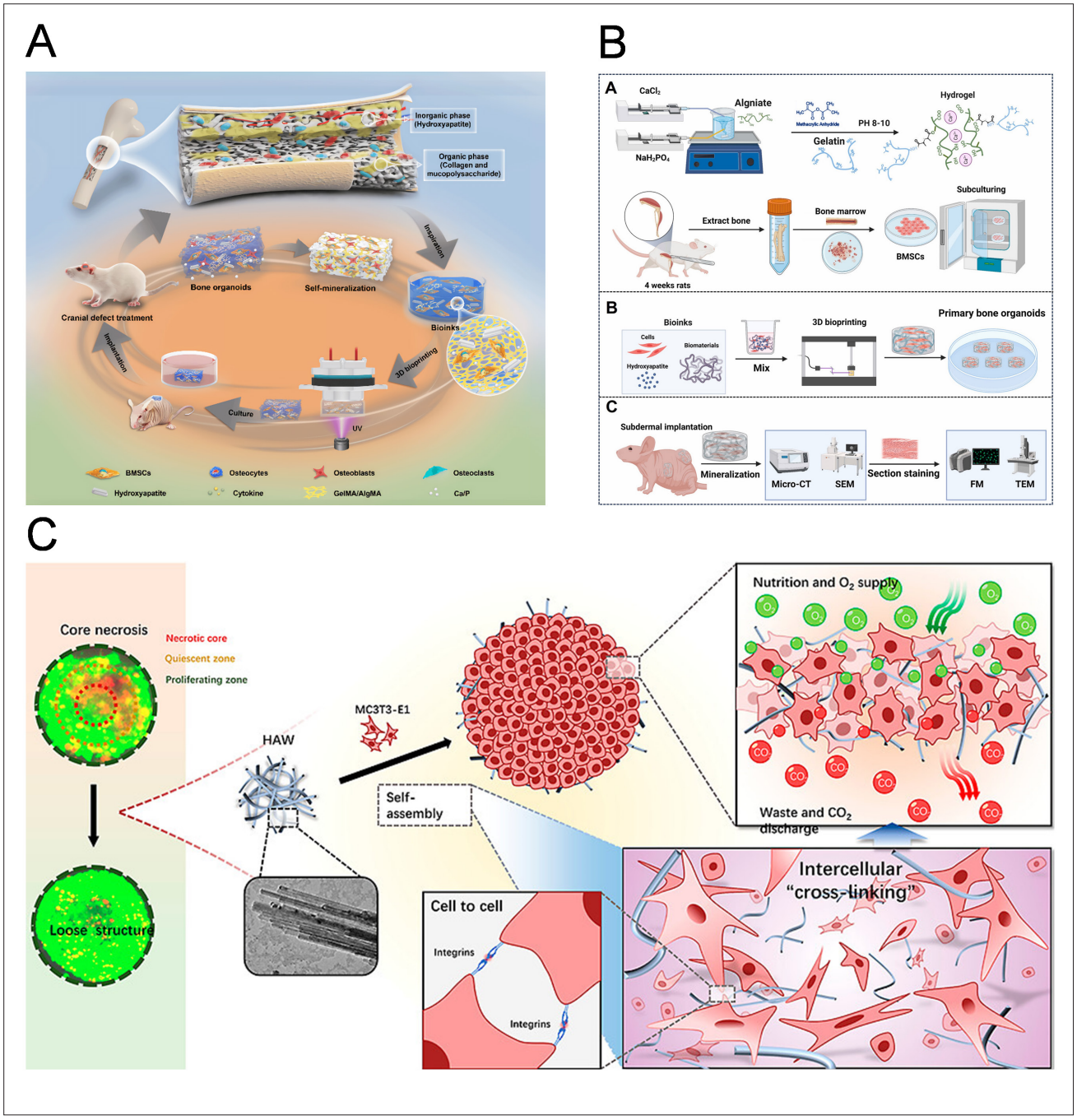



**Figure 2.** Representative advances in bioprinted bone organoids. (A) Large-scale bioengineered self-mineralizing bone organoid platform printed by bone matrix-inspired hydroxyapatite hybrid bioinks. Adapted with permission from Wang et al.[19] Copyright© Wiley 2024. (B) Bioprinted bone organoids exhibit great mineralization and vascularization properties *in vivo* without external stimuli. Adapted with permission from Wang et al.[21] Copyright© Elsevier 2025. (C) Bioprinted bone organoids exhibit great mineralization and vascularization properties *in vivo* without external stimuli. Adapted with permission from Li et al.[22] Copyright© American Chemical Society 2024.

as artificial cardiac tissues to mimic cardiac structures and functions by adjusting their size, shape, and configuration. The reconstruction of functional cardiac organoids requires biomimetic architectures that emulate the electromechanical coupling and vascularization of native heart tissue.[39] Human cardiac organoids are created through the self-assembly of differentiating cardiomyocytes from human pluripotent stem cells.[40] Consequently, cardiac organoids serve as promising models for replicating native cardiac elements, including inflow-outflow territories, cardiac chamber architecture, and heart-related regulation.[41]

Recent breakthroughs in multi-material bioprinting have allowed for the recreation of complex cardiac structures, enabling the fabrication of ventricle models with perfusable vascular networks, a feat unattainable with traditional 3D printing methods.[42] Fang et al.[42] have successfully developed Sequential Printing in a Reversible Ink Template technology, enabling the fabrication of ventricle models with hierarchical vascular networks by combining sacrificial ink printing with microgel-enhanced bioinks.[42] This approach allows sequential deposition of structural bioinks to form cardiac chambers, followed by sacrificial ink removal to create perfusable channels. The resulting constructs exhibit synchronized contractions and action potential propagation, mimicking the helical fiber orientation critical for ventricular ejection dynamics (Figure 3A). Despite structural progress, achieving electrophysiological maturity comparable to adult myocardium remains challenging. Current cardiac organoids often lack the ion channel density and calcium handling capacity required for sustained rhythmic activity. To overcome this obstacle, 4D bioprinting with shape-memory hydrogels has been employed to spatiotemporally align cardiomyocytes into anisotropic architectures in response to physiological stimuli, enhancing action potential propagation velocity by 2.3-fold compared to static cultures.[43] Additionally, the incorporation of organoid-derived pacemaker cells into bioprinted patches has enabled arrhythmia modeling, demonstrating abnormal conduction patterns under β-adrenergic stimulation.

Intriguingly, Zhang et al.[44] have further advanced vascular integration using six-axis robotic bioprinters, which enable omnidirectional cell deposition on complex arterial scaffolds. By employing a mineral oil-based suspension system, endothelial cells adhere to vascular scaffolds without shear stress, forming confluent monolayers that subsequently sprout capillaries under angiogenic factors. This "print-culture" iterative strategy, where alternating layers of cardiomyocytes and endothelial cells are cultured to promote vascular network maturation, has yielded myocardial tissues that maintain rhythmic contractions for over 6 months *in vitro* (Figure 3B). By combining cardiomyocytes, endothelial cells, and conductive bioinks, researchers have achieved synchronized contractions and action potential propagation in bioprinted cardiac patches. These models replicate the helical myocardial fiber orientation critical for ventricular ejection dynamics.[45]

Nevertheless, limitations in scalability and electrophysiological maturity remain, as current cardiac organoids lack the structural complexity of adult myocardium. Emerging strategies, such as 4D bioprinting with shape-memory hydrogels, aim to dynamically align cells into anisotropic tissue geometries under physiological stimuli.[46,47] Additionally, the integration of organoid-derived pacemaker cells could advance arrhythmia modeling and personalized drug testing.[48] This synthesis of bioprinting and organoid technologies represents a paradigm shift in cardiovascular research, offering unprecedented opportunities to model congenital heart defects, ischemic injury, and pharmacogenomic responses with physiological fidelity.

### 2.3. Brain organoids

Conventional self-assembly methods often yield structurally inconsistent neural spheroids with limited vascularization and incomplete regional specification.[49,50] Pioneered in 2009, early cerebral organoids derived from embryonic stem cells and iPSCs demonstrated self-organized cortical regions, neural progenitor zones, and rudimentary laminar organization.[51] These primitive models laid the groundwork for investigating neurodevelopmental trajectories, interspecies divergence, and pathological mechanisms.[52] The convergence of 3D bioprinting with cerebral organoid technology has revolutionized our capacity to replicate human neurodevelopment *in vitro*. Cadena et al.[53] printed a high-throughput, adjustable, and repeatable scaffold for precisely controlling the development and patterns of brain organoids, achieving real-time monitoring of calcium signaling and synaptic plasticity.[53] It was confirmed that the fabricated scaffold exhibited stiffness values comparable to the developing human brain. The organoids cultured long-term within the bioprinted scaffold remain healthy and exhibit expected neuroectodermal differentiation. In addition, the endothelial cells within the printed channel structures demonstrated the ability to migrate and infiltrate the embedded brain organoids. Advanced bioprinting strategies enable the spatial orchestration of neural progenitor cells within tunable ECMs, achieving unprecedented control over cortical layering and synaptic connectivity patterns.[54,55] Innovative bioink formulations combining decellularized brain ECM with thermo-responsive hydrogels have demonstrated enhanced neurite outgrowth (2.3-fold increase versus Matrigel

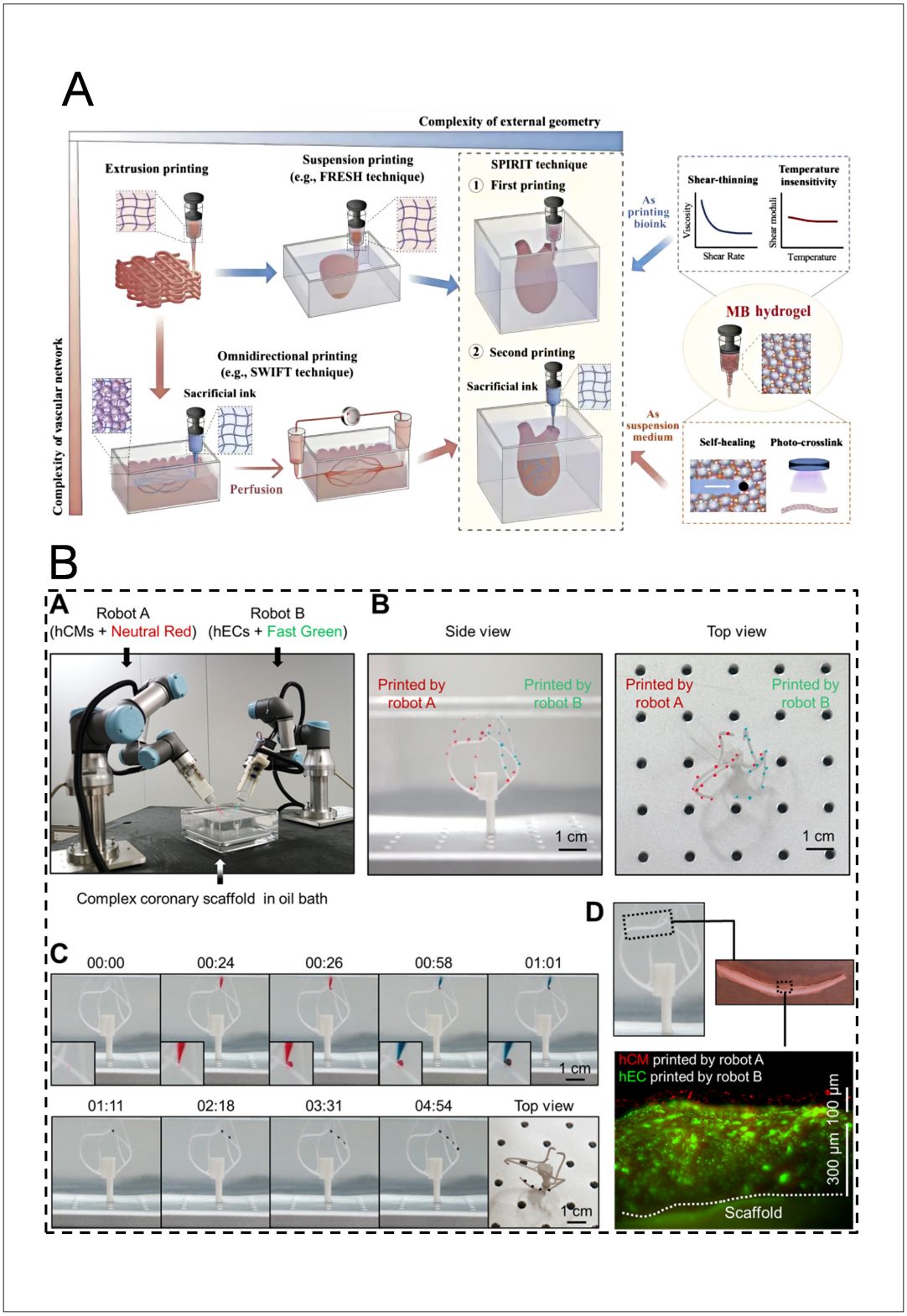



**Figure 3. Recent advancements in bioprinting technologies for vascularized organ construction.** (A) Expanding embedded three-dimensional bioprinting capability for engineering complex organs with freeform vascular networks. Adapted with permission from Fang et al.[42] Copyright© Wiley 2023. (B) Two-robot platforms, enabling cooperative bioprinting of different bioinks onto complex-shaped scaffolds, are jointly bioprinting on a vascular scaffold resembling major coronary vessels using two bioinks. Scale bar: 1 cm. Adapted with permission from Zhang et al.[44] Copyright© Elsevier 2022.

controls) while maintaining stem cell pluripotency during the printing process.[56] Such matrices permit dynamic stiffness modulation from 0.5 kPa (emulating embryonic neuroepithelium) to 8 kPa (mimicking adult parenchyma), guiding targeted differentiation into specialized subtypes, including dopaminergic neurons and Bergmann glia.[50,57] Yan et al.[58] engineered functional neural tissues using iPSC-laden viscoelastic bioinks, demonstrating synaptically active neuronal networks with electrophysiological signal propagation.[58] Furthermore, hierarchical cell assembly strategies permit the integration of differentially matured neural populations, thereby enhancing functional maturation beyond conventional culture constraints.[59]

Cutting-edge protocols now incorporate vasculature-mimicking channels within organoids, addressing limitations in nutrient diffusion and long-term viability.[60] Recent breakthroughs employ multi-material extrusion systems to co-print endothelial cells alongside iPSC-derived neuroectodermal populations, generating perfusable vascular networks that sustain organoids beyond 150 days *in vitro*.[59] This vascularization breakthrough addresses the critical limitation of necrosis in traditional models, allowing the maturation of functional gamma-aminobutyric acidergic and glutamatergic circuits detectable through calcium imaging and patch-clamp electrophysiology.[54,61]

While bioprinted organoids exhibit basic electrophysiological activity, they lack the cytoarchitectural sophistication (e.g., six-layered neocortex, glial diversity) of native cerebral tissue. Additionally, challenges center on achieving single-cell resolution printing for accurate synaptic connectivity and integrating optogenetic components for real-time circuit modulation.[55] Generative artificial intelligence-assisted optimization of bioink rheology and machine learning-driven printing parameterization could enhance structural fidelity. Microfluidic organ-on-chip systems may further simulate interregional brain connectivity. The emergence of clustered regularly interspaced short palindromic repeats (CRISPR)-edited reporter cell lines promises to overcome these limitations, paving the way for clinically transplantable neural constructs.[62] The development of immunoevasive bioinks with tunable mechanical properties and neuroimmunomodulatory functions will be pivotal for transitioning from investigational models to implantable therapeutics.

### 2.4. Bioprinting for hepatorganoids

Liver diseases represent a leading cause of global morbidity and mortality. Due to the scarcity of donor organs and complications like immune rejection, liver failure remains a critical challenge. 3D bioprinting offers a transformative approach, surpassing traditional 2D cell cultures, animal models, and organoids by enabling the fabrication of vascularized tissues and organs in terms of hepato–pancreato–biliary models.[63,64] Vascularized hepatorganoids generated via high-throughput bioprinting systems exhibit improved metabolic activity and drug response compared to static cultures.[65–67] Shrestha et al.[68] separately differentiated epithelial cell adhesion molecule$^{+}$ endodermal progenitor cells and mesoderm-derived vascular progenitor cells from the same human iPSC line, which were then mixed in a 2-mercaptoethanol matrix on a pillar plate platform and concurrently differentiated into vascular human hepatorganoids.[68] Remarkably, this 3D-bioprinted expandable model exhibited significantly superior maturity than vasculature-free hepatorganoids, as demonstrated by increased coagulation factor secretion, albumin secretion, drug-metabolizing enzyme expression, and bile acid transportation, likely due to enhanced nutrient and signaling molecule diffusion. Scientists developed a 3D bioprinting technique using GelMA hydrogel to create hepatorganoids resembling hepatic lobules, exhibiting reduced hypoxia, increased albumin and urea secretion *in vitro*, and supporting angiogenesis post-implantation. By incorporating vascular endothelial growth factor and human umbilical vein endothelial cells, vascularized hepatorganoids with enhanced vascularization were produced.[69,70] Upregulation of growth arrest-specific protein 6/AXL and laminin beta 3/integrin subunit alpha 3 pathways in vascularized hepatorganoids promoted vascularization and proliferation. Orthotopic implantation of vascularized hepatorganoids showed prolonged survival, elevated biomarkers, and increased vascularization in grafts. These studies highlight the efficacy of orthotopic implantation of hepatorganoids for enhanced vascularization, benefiting transplantation, drug screening, and therapy.[70]

Bioinks, typically hydrogel-cell composites, serve as a foundational material.[71,72] A key innovation is the use of spheroid-based bioinks, which preserve hepatic polarity and zonation patterns during printing.[25,73] This technology encompasses four principal methods: inkjet-based, extrusion-based, laser-assisted, and vat photopolymerization bioprinting. In hepatorganoids, hepatocytes are often combined with non-parenchymal cells such as hepatic stellate cells, sinusoidal endothelial cells, and Kupffer cells.[74] Hydrogels derived from dECM are commonly utilized in bioprinted hepatorganoids due to their ability to mimic the native tissue microenvironment and retain essential growth factors and cytokines.[71,75] This characteristic renders them highly biocompatible for 3D bioprinting applications. In the field of hepatorganoids bioprinting, these hydrogels, in combination with liver-

derived cell types, can serve as suitable *in vitro* hepatic models for drug efficacy testing and studies on liver metabolism.[76] Moreover, these hydrogels can be viewed as compliant with good manufacturing practices for expanding liver cells and organoids, offering a safer alternative to basement membrane extracts derived from tumorigenic cell lines commonly used in research. Despite challenges such as weak mechanical properties and limited printability that impede the direct use of dECM hydrogels as bioinks, various modifications to both the dECM and the bioprinting process have been implemented to address these issues. Challenges in scaling up and manufacturing dECM hydrogels include undefined manufacturing standards, variable production methods affecting reproducibility, and considerations for animal-derived sources, such as variability, harvesting conditions, and bioburden reduction.

Bioprinted hepatorganoids have demonstrated remarkable progress in recapitulating hepatocytic functions, including albumin secretion, cytochrome P450 activity, and bile canaliculi formation.[77,78] These advancements hold significant potential for disease modeling, pharmaceutical development, and regenerative therapies.[79,80] Nevertheless, challenges persist, including the need for diverse fabrication techniques, real-time monitoring of drug responses in perfusion cultures, the ability to replicate intricate microenvironments of live sinusoids, and the achievement of long-term functional stability (>30 days) *in vitro*. Despite these hurdles, 3D bioprinting remains a groundbreaking strategy for hepatorganoid engineering. Advances in machine learning-guided parameter optimization and perfusion bioreactors are addressing these limitations, enhancing the scalability of hepatorganoids for transplantation.[78,81,82]

## 2.5. Recapitulation of glandular properties via bioprinting

Glandular organoids, such as pancreatic islets and salivary glands, demand precise biomaterial cues to maintain secretory functions and ductal morphogenesis. The ability of 3D bioprinting to spatially define cellular architectures and ECM microenvironments has revolutionized the engineering of glandular tissues, enabling precise replication of secretory epithelia, ductal networks, and functional polarization.[83] In parallel, bioprinting technology has successfully recreated branching ductal networks in mammary and prostate organoids using hyaluronic acid-based bioinks functionalized with growth factor gradients.[6,84] Recent advances highlight its potential in reconstructing salivary, pancreatic, and mammary gland models with native-like secretory functions and structural hierarchies.

### *2.5.1. Structural recapitulation of glandular units*

Glandular tissues, characterized by intricate acinar-ductal networks, demand bioprinting strategies that mimic their branched morphology. For salivary glands, magnetically guided 3D bioprinting enables precise assembly of epithelial spheroids using iron oxide nanoparticles, replicating acinar-ductal polarity and neural responsiveness.[85,86] Similarly, coaxial microfluidic bioprinting generates cell-laden microfibers and tubules, bypassing xenogeneic matrices like Matrigel to create hierarchical ductal systems.[85] In sweat gland regeneration, GelMA-printed pore-structured matrices (300 μm diameter) guide epidermal progenitors to self-organize into lumenized glands, a process absent in non-printed controls.[87] These findings underscore the critical role of bioprinted scaffolds in dictating morphogenesis through topographical and mechanical cues.

### *2.5.2. Functional maturation and secretory capacity*

Functional maturation of bioprinted glands relies on dynamic biochemical signaling and perfusion. For endocrine glands, the encapsulation of stem cell-derived β-cells within alginate/gelatin scaffolds has been shown to restore glucose-responsive insulin secretion in diabetic models.[88] Another pancreatic organoid demonstrated that mouse insulinoma 6 β-cell constructs, printed using the freeform reversible embedding of suspended hydrogels technique and embedded in vascularized hydrogels, exhibited glucose-stimulated insulin secretion under perfusion, achieving a 4.6-fold increase in insulin output compared to static cultures.[89] In salivary glands, magnetically assembled organoids demonstrate acetylcholine-responsive fluid secretion, mirroring native neuroepithelial interactions.[85]

Bioink design is pivotal for maintaining cell viability and glandular functionality. Chitosan-based bioinks blended with polycaprolactone or glycine methacrylate enhance printability and mechanical strength while supporting acinar cell proliferation.[90] Shear-thinning nanocellulose-alginate composites enable high-resolution printing of cartilage-like structures, with chondrocyte viability exceeding 86% after 7 days, a principle adaptable to glandular applications.[91] These advancements highlight the interplay between bioink rheology, crosslinking kinetics, and glandular morphogenesis.

A critical challenge lies in mimicking the dynamic ECM remodeling of native glands, which regulate cell differentiation through stiffness gradients and protease-sensitive linkages.[92] Recent work leveraging 4D bioprinting with enzyme-responsive polymers has enabled time-dependent ECM degradation, guiding self-organization of acinar and ductal structures.[46,93] Notably, 4D bioprinting

with light-responsive bioinks (e.g., GelMA/poly[ethylene glycol] dimethacrylate) introduces temporal control over scaffold remodeling, enabling post-printing structural anisotropy to support secretory epithelial polarization.[12] Such innovations bridge the gap between structural fidelity and functional integration.

#### *2.5.3. Vascularization and nutrient transport*

Vascularization poses a key hurdle for glandular bioprinting, with strategies such as multi-material bioprinting integrating endothelial cells with glandular parenchyma to establish perfusable networks. Evidence shows that pancreatic constructs combining mouse insulinoma 6 cells and vascular bioinks develop anastomosed capillaries under bioreactor perfusion, enhancing nutrient delivery and insulin secretion.[89] A novel tissue-specific bioink was formulated by blending pancreatic ECM with hyaluronic acid methacrylate.[94] The 3D-printed islet organoids created using this bioink can replicate the pancreatic microenvironment, preserving islet cell adhesion and morphology via the Ras-related C3 botulinum toxin substrate 1/rho-associated protein kinase/myosin light chain kinase signaling pathway (Figure 4A). This approach enhances islet function and activity. Moreover, the 3D-printed structures enhance the formation of vascular networks, while the hyaluronic acid methacrylate/pancreatic ECM hydrogel supports the adhesion and proliferation of new blood vessels, thereby augmenting the density of vascular structures. Nanocellulose-alginate bioinks further improve mechanical stability and osteogenic differentiation, suggesting adaptability for vascularized glandular models.[91] Emerging platforms like the Human Islet-like Cellular Aggregates and Vasculature platform simulate native pancreatic vascular niches, promoting β-cell survival and functional maturation.[95]

Traditional organoids lack precise structure and scalability, whereas bioprinting overcomes these limitations by facilitating the efficient production of millimeter-scale constructs with specific geometries. Bioprinted mammary tumor models incorporate cancer-associated fibroblasts (CAFs) and endothelial cells, recapitulating chemoresistance mechanisms absent in 2D cultures (Figure 4B & C).[96,97] Despite advancements, several challenges remain. Functional longevity is a concern as printed glands often display temporary secretory activity due to inadequate innervation or vascular integration. Co-printing with neuronal progenitors or utilizing optogenetic stimulation could potentially improve sustained functionality. Immunogenicity is another obstacle, which can be addressed by employing patient-specific iPSC-derived cells and dECM bioinks to reduce the risk of immune rejection. Achieving multi-scale resolution is crucial, and this can be accomplished by integrating microfluidic bioprinting with two-photon polymerization to replicate sub-10 μm ductal features.

Future applications should include patient-specific glandular models for autoimmune disease studies and hormone replacement therapies.[98,99] As bioink formulations and multi-omics validation evolve, bioprinted glands will likely transition from disease modeling to clinical transplantation, addressing critical shortages in organ replacement therapies.

## 3. Disease modeling based on three-dimensional bioprinted organoids

3D bioprinted organoids offer a unique advantage in disease modeling due to their ability to replicate the heterotypic organization of multicellular solid organs and the nanoscale precision of pathological processes. It provides a platform for studying the complex microenvironments that regulate cell behavior in various diseases, offering insights into fundamental biological processes and potential therapeutic strategies. A significant advantage of organoids in disease modeling and drug development is the ability to create personalized models using patient-derived cells, which has been studied extensively. Early in 2015, Freedman et al.[100] developed kidney tubular organoids from human pluripotent stem cells with drug responses similar to those found in clinical settings.[100] The model was also amenable to CRISPR/Cas9-based genome editing, which was used to induce polycystic kidney disease characteristics in the organoid. Votanopoulos et al.[101] conducted a study using patient-specific immune-enhanced tumor/node organoids for immunotherapy screening. The responses of the organoids to various immunotherapy drugs were very similar to the clinical response, showing the feasibility of these organoids for patient-specific drug testing.[101] However, further studies are needed to fully understand and widen the applications of organoids. This section discusses advancements in modeling inflammatory, genetic, metabolic, immune, and neurodegenerative disorders using bioprinted organoids.[4]

### 3.1. Inflammatory-related disorders

Chronic inflammatory conditions like rheumatoid arthritis, inflammatory bowel disease, neuroinflammation, liver inflammation, and osteoarthritis (OA) are intricate disorders characterized by persistent inflammation and tissue damage.[14,102,103] The pathophysiology of these ailments involves a blend of genetic, environmental, and immunological factors, leading to diverse patient responses to conventional therapies, necessitating a more personalized treatment approach.[104,105] Recently, the fusion of 3D bioprinting technologies with patient-

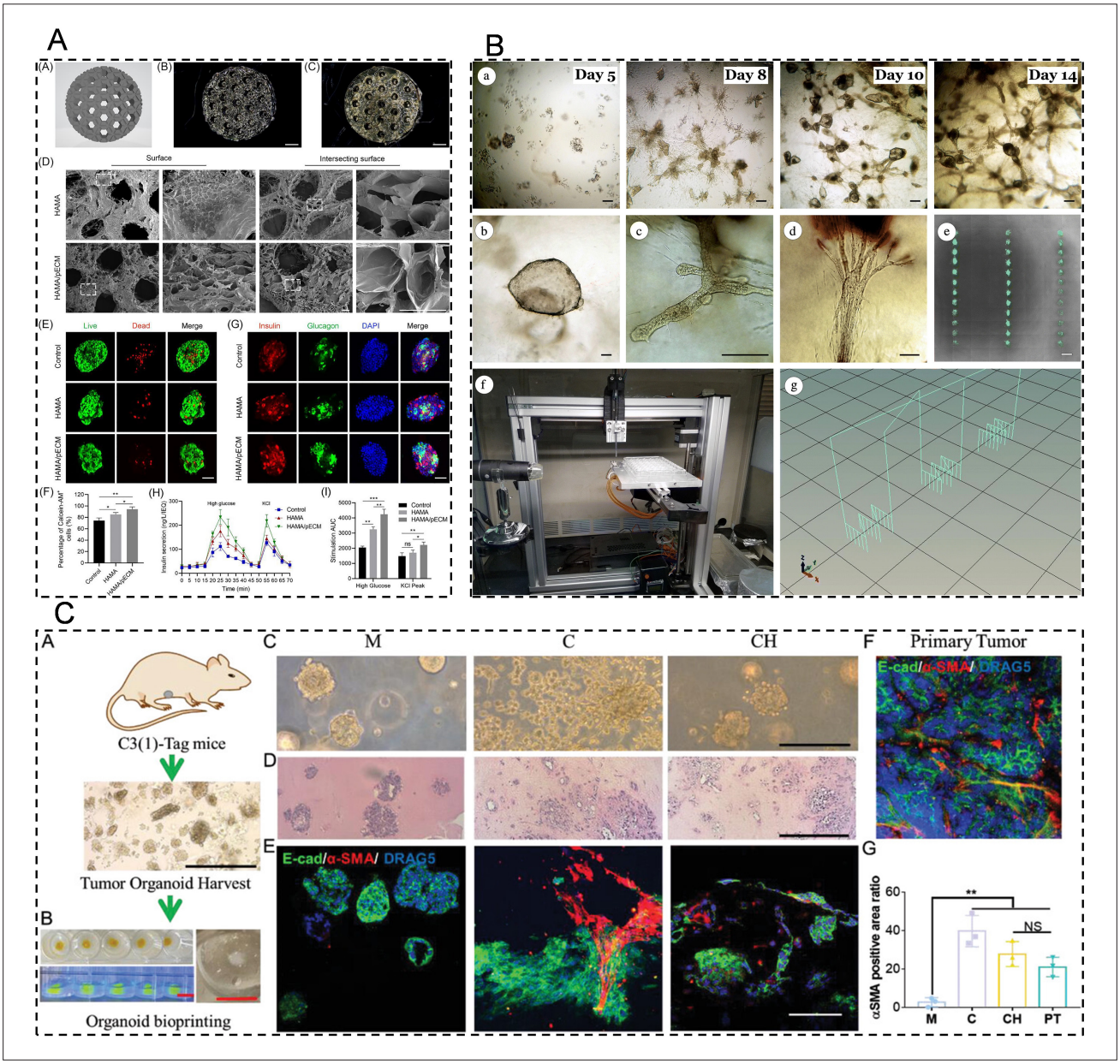



**Figure 4. Bioprinting strategies for engineering functional and disease-specific organoids.** (A) Design and construction of three-dimensional (3D)-printed hyaluronic acid methacrylate/pancreatic extracellular matrix islet organoids. (B) Scale bar = 2 mm, (C) Scale bar = 2 mm, (D) Scale bar = 300 μm, (E) Scale bar = 50 μm, (G) Scale bar = 50 μm. 4A: (B) Scale bar = 2 mm, (C) Scale bar = 2 mm, (D) Scale bar = 300 μm, (E) Scale bar = 50 μm, (G) Scale bar = 50 μm. Adapted with permission from Wang et al.[94] Copyright© Elsevier 2023. (B) Low-cost and computer numerical control-driven 3D bioprinting for mammary epithelial organoid, including "sphere-like," "duct-like," and "star-like" cells. Scale bar = 200 μm. Adapted with permission from Reid et al.[96] Copyright© BMC 2018. (C) The embedded bioprinted breast tumor organoids in low-concentration collagen-based bioink recapitulated *in vivo* tumor morphology. Scale bar = 200 μm. Adapted with permission from Shi et al.[97] Copyright© Wiley 2022.

derived organoids has revolutionized precision medicine by facilitating the creation of personalized disease models and advancing therapeutic research.[98,103] By incorporating immune cells, stromal cells, and other pertinent cell types as well as vascular networks into organoid models, the ability to embed organoids within customizable bioprinted geometries allows researchers to simulate the spatial heterogeneity of inflammatory microenvironments, including immune cell infiltration and cytokine gradients, and delve into the exploration of disease mechanisms, such as the impact of specific genes and signaling pathways in inflammation, and facilitate the screening of potential therapeutic interventions.[106,107] Bioprinted organoids offer valuable insights into the pathogenesis of rheumatoid arthritis, characterized by persistent joint inflammation and cartilage degradation. By mimicking interactions between synovial tissue and immune cells, these models facilitate the investigation of inflammatory mediators and genetic determinants. Furthermore, they enhance the optimization of pharmacological interventions that target inflammatory pathways, holding promise for personalized medicine.[108]

Intestinal organoids incorporating patient-derived cells have been leveraged to study gut inflammation and epithelial barrier dysfunction, providing insights into inflammatory bowel disease pathogenesis.[109,110] 3D bioprinting is notably applied in generating gastrointestinal organoids, which demonstrate distinctive transcriptomic and secretomic signatures in patients with Crohn's disease, elucidating disease-specific mechanisms and responses.[111] The quest to replicate the small intestine's intricate architecture and physiology *in vitro* has driven innovations in 3D bioprinting. Conventional planar epithelial monolayers lack the 3D villi topography essential for mimicking native absorption and barrier dynamics. Stereolithographic 3D printing now addresses this gap through THE precision fabrication of villus-like micropillar arrays using tunable poly(ethylene glycol) diacrylate hydrogels.[112] These scaffolds sustain month-long Caco-2 cultures, inducing apicobasal polarization akin to *in vivo* epithelial organization, which serves as a critical advance for physiologically relevant transport and metabolic studies (Figure 5A). A key innovation lies in dual-material printing strategies that integrate diffusion-open villus microstructures with diffusion-closed hydrogel walls. This compartmentalization isolates epithelial transport processes, emulating the luminal-interstitial interface while enabling high-throughput analysis. Functionally, Caco-2 cells on these platforms form confluent barriers with tight junctions, validated by transepithelial electrical resistance and fluorescent tracer assays (Figure 5B). The compatibility with compound screening allows real-time evaluation of barrier integrity under pharmacological challenges, offering a scalable tool for drug permeability and toxicity studies. Similarly, Jonathan et al.[113] have developed the BATE technique to create centimeter-sized gastrointestinal tissues with self-organizing features, enhancing the scalability and applicability of bioprinted organoids in various research areas.[113] By merging microstructural fidelity with functional compartmentalization, these approaches transcend the limitations of traditional models.[114] Their scalability and adaptability pave the way for advanced intestinal niches incorporating dynamic cues or microbial interactions, promising transformative applications in drug discovery and gut pathophysiology research.

### 3.2. Genetic diseases

Genetic disorders such as cystic fibrosis and hereditary metabolic defects have been modeled using bioprinted organoids derived from iPSCs. Bioprinting enables the precise arrangement of wild-type and mutant cells within organoids, facilitating the analysis of cell-autonomous versus non-cell-autonomous disease mechanisms. Human iPSC-derived neural organoids with patterned differentiation have been used to study neurodevelopmental disorders caused by genetic mutations.[84] Organoids offer valuable insights into the cellular mechanisms involved in such disorders and the effectiveness of gene editing tools such as CRISPR/Cas9 for potential therapeutic applications.[115] The integration of CRISPR genome editing techniques (including knockout, base editing, and prime editing) with 3D organoid cultures has enabled the modeling of disease progression and the investigation of genetic disorders. This approach effectively bridges the gap between patient-derived cells and our understanding of diseases.[116] Schene et al.[117] demonstrated the utility of prime editing in repairing genetic defects within patient-derived organoids, showcasing the precision and therapeutic potential of this gene editing strategy.[117] Leigh syndrome, an untreatable mitochondrial disorder associated with a *SURF1* mutation, has been addressed using Cas9 genome editing to rectify the mutated *SURF1* gene in patient-derived organoids.[118] This approach has provided insights into the physiological correlation between *SURF1* and Leigh syndrome. Additionally, patient-specific iPSCs have been utilized to replicate macrocephaly/autism phenotypes, demonstrating the adaptability of patient-derived organoids in investigating intricate genetic conditions. Furthermore, the CRISPR-Cas9 system has been employed in brain organoid models to induce models of congenital nervous system malformation diseases, facilitating the exploration of these intricate disorders.[119,120] Additionally, bone organoids bioprinted with GelMA bioinks have replicated genetic bone disorders, like osteogenesis

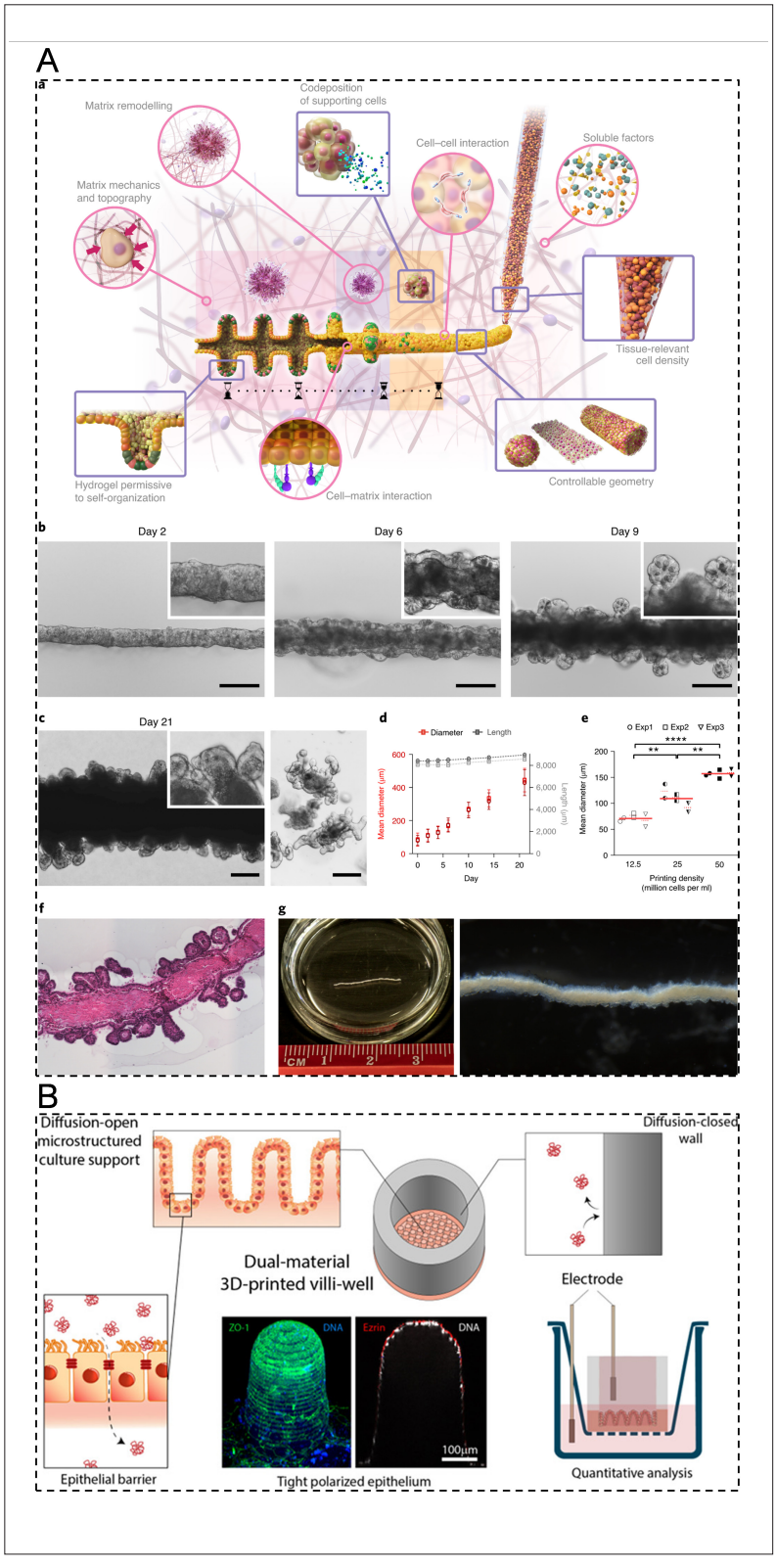



**Figure 5.** Bioprinted models for engineering functional intestinal structures and analysis platforms. (A) Macroscopic intestinal tube printing. Scale bar: 200 μm. Adapted with permission from Brassard et al.[113] Copyright© Nature 2021. (B) Engineering monolithic transport analysis devices with villi-like surface microstructures. Adapted with permission from Taebnia et al.[112] Copyright© American Chemical Society 2021.

imperfecta, characterized by brittle bones, enabling the study of mutations in collagen-producing genes and their impact on bone structure and strength.[121]

### 3.3. Metabolic diseases

Metabolic diseases, including diabetes and non-alcoholic fatty liver disease, have been modeled using bioprinted hepatic and pancreatic organoids. Bioprinted liver organoids with perfusable vascular networks mimic the zonated metabolism of hepatocytes, enabling the study of lipid accumulation and insulin resistance.[78] Recent advances in nozzle-free bioprinting have preserved organoid polarization and liver-specific enzyme activity, which are critical for replicating metabolic dysfunction.[25] Breakthroughs in bioprinted pancreatic organoids have demonstrated transformative potential for diabetes research and β-cell regeneration. A seminal study by Ahn et al.[122] has achieved functional restoration in diabetic murine models through extrusion-based bioprinting of human iPSC-derived β-cells co-encapsulated with endothelial progenitors in a tunable alginate matrix. These constructs exhibited glucose-responsive insulin secretion and sustained normoglycemia post-implantation, attributed to the integration of pre-vascular networks via co-printed endothelial cells. This enabled perfusable microvasculature formation within 7 days and an immunoprotective hydrogel design balancing crosslinking density to permit metabolic exchange while evading immune detection.[122] Furthermore, vascularized pancreatic organoids have been employed to investigate β-cell dysfunction and insulin secretion dynamics under hyperglycemic conditions.[123] Bone/cartilage organoids enable the study of metabolic pathways in cartilage development and pathologies. Analyzing the metabolomic profiles of these organoids reveals insights into metabolic changes linked to conditions such as OA. Understanding these alterations is vital for designing therapies targeting specific metabolic pathways to manage cartilage degradation. Integrating metabolomics with organoid technology offers new research opportunities in bone and cartilage biology, disease mechanisms, biomaterials, and pharmacology. This approach lays the groundwork for future research utilizing metabolomics for enhanced diagnostics, treatments, and regenerative approaches.[124]

### 3.4. Immune diseases

Bioprinted organoids are increasingly used to model autoimmune and immune-mediated disorders. The incorporation of endothelial and immune cells into bioprinted constructs allows the recapitulation of immune cell trafficking and vascular inflammation.[123,125] For example, vascularized skin organoids with hierarchical structures have been developed to study dermatological and lupus-related vasculopathy.[39,125] Parallel advancements in psoriatic modeling have been achieved through laser-assisted bioprinting of stratified skin organoids by Michael et al.[126] Their multilayered architecture, incorporating patient-derived keratinocytes, fibroblasts, and immune cells, recapitulated hallmarks of disease pathogenesis, including hyperproliferative epidermis, interleukin-23/interleukin-17 axis dysregulation, and T-cell infiltration dynamics visualized via integrated microfluidics.[127,128] The platform's dual utility in mechanistic interrogation and therapeutic screening was evidenced by the successful suppression of inflammatory markers using anti-tumor necrosis factor alpha biologics.[129] Nevertheless, the absence of neural components limits its capacity to model neuroimmune crosstalk, which is particularly relevant to pruritus mechanisms.[130] Future iterations incorporating sensory neurons and Schwann cells could bridge this gap, enabling a comprehensive study of itch pathways while preserving the strength in real-time immune monitoring and high-content drug evaluation.[131]

Additionally, lymph node organoids with spatially organized stromal and immune cell populations provide platforms to investigate antigen presentation and T-cell activation in autoimmune contexts.[132] The translational challenge for immune organoid research lies in modeling sensitivity and resistance to immunotherapies, encompassing checkpoint inhibition, novel pathways, and adoptive cell transfer strategies. The identification of underlying resistance pathways also holds considerable promise for human tumor immunotherapy.[133] Together, these studies exemplify how spatial precision in bioprinting accelerates both disease deconstruction and regenerative strategy development.[134] Furthermore, combining organoids with advanced imaging techniques and multi-omics approaches can enhance our understanding of immune responses and disease progression, paving the way for more effective therapeutic interventions.

While significant progress has been made in modeling metabolic and immune diseases using bioprinted organoids, further research is needed to overcome technical challenges and translate these models into clinical applications. The integration of organ-on-a-chip technology with metabolomics and advanced imaging techniques will undoubtedly continue to drive innovation in disease modeling and therapy development.

### 3.5. Neurodegenerative diseases

Neurodegenerative disorders such as Alzheimer's and Parkinson's diseases have been modeled using bioprinted neural organoids. Multi-material bioprinting of human iPSC-derived neural stem cells and endothelial progenitors has generated cortical organoids with layered regions and

perfusable microvasculature, mimicking the blood–brain barrier and neuronal degeneration.[84] These models enable the study of amyloid-β aggregation and tauopathy in a 3D microenvironment. Moreover, bioprinted midbrain organoids containing dopaminergic neurons have been used to evaluate mitochondrial dysfunction and oxidative stress in Parkinson's disease.[84,123] Wang et al.[135] aimed to create a Corti organoid using 3D bioprinting, integrating a "3D culture scaffold + multiple induction signals + inner ear stem cells." This approach addressed the limitation of regenerated hair cells in forming functional ciliary bundles and establishing synaptic connections with spiral ganglion cells in a 2D system, hindering the achievement of effective physiological repair of hearing. Their study demonstrated that the organoids facilitated the adhesion and proliferation of inner ear stem cells, leading to the generation of both hair cells and nerve cells. This work presents a promising avenue for investigating auditory cell regeneration and repairing hearing loss.[135]

3D bioprinting of organoids has significantly advanced disease modeling by combining stem cell self-organization with engineered architectures. While challenges remain in replicating the full complexity of human tissues, recent innovations in vascularization,[123,125] multicellular patterning,[84] and biomaterial design are bridging these gaps.[25,121] Future efforts should focus on integrating dynamic stimuli, such as mechanical forces and immune cues, to further enhance pathophysiological relevance.[106]

## 4. Bioprinting tumor organoids: simulacrum or throughput

Bioprinting technology can recreate the spatial topological relationships between different cellular components within tumor organoids, including immune cells, CAFs, and vascular and lymphatic structures, thus reproducing the heterogeneous structure of the primary tumor.[136] Emerging platforms such as organ-on-a-chip, organoids, and bioprinting within micro-physiological systems are increasingly employed to elucidate these interactions. These systems accurately recapitulate key features of tumor microenvironments (TMEs) and immune responses, offering physiologically relevant platforms for investigating cancer progression, immune evasion, and therapeutic interventions.[137] In addition, the application of bioprinting technology has been extended to high-throughput screening in tumor model systems.[138] Tumor organoids, which can be produced at scale and tested systematically through automated bioprinting processes, play a pivotal role in drug development and personalized treatment strategy optimization. The advancement of this technology has facilitated more rapid and precise evaluation of anticancer drug efficacy while also enabling the exploration of personalized cancer therapies.

### 4.1. Tumor biology-based bioprinting of tumor-like organoids

3D printing enables the reconstruction of quasi-native spatial topological relationships among different cell types within tumor tissue components. This primarily involves the first-level tumor cell components and specific structures, particularly those located on the periphery of the tumor cells, such as immune cells, blood vessels, lymphatic vessels, nerves, and paracancerous cells. Using 3D printing technology, a complex co-culture model with three or even multiple components can be established based on predefined spatial positioning and cell composition ratios. Additionally, the spatial arrangement of different clones between tumor cells and their biological interactions warrants further emphasis. Such intricate constructs of tumor-induced organs or organoids cannot be achieved by conventional methods and necessitate the use of 3D bioprinting. Bioprinting technologies have revolutionized the fabrication of tumor organoids by enabling precise spatial control over cellular and extracellular components, thereby recapitulating the heterogeneous architecture of native tumors. This approach addresses the critical limitations of conventional organoid models, which often fail to mimic the multicellular complexity and spatial organization of TMEs.[25,99]

#### *4.1.1. Spatial arrangement of tumor niche*

The spatial heterogeneity of tumor subclones and their dynamic crosstalk with stromal components, driven by genetic and epigenetic variations, constitute a hallmark of cancer progression and therapeutic resistance. Single-cell and spatial transcriptomic analyses reveal that intra-tumoral heterogeneity in gastric cancer arises not only from genetic diversity but also from spatially organized interactions between proliferative/invasive cancer cells and tumor-associated fibroblasts,[139,140] among which CAFs emerge as central orchestrators. CAFs secrete transforming growth factor-beta and matrix metalloproteinases to remodel the ECM, thereby creating biomechanical niches that promote immune evasion and chemoresistance.[141] Multi-material bioprinting strategies enable the integration of tumor cells, patient-derived CAFs, and immune cells into predefined geometries, mimicking the *in vivo* spatial hierarchy observed in solid tumors.[42,99,142,143] Studies have elucidated how CAF-secreted interleukin-6 and C-X-C motif chemokine ligand 12 gradients drive epithelial-mesenchymal transition in breast cancer organoids, while nanoparticle-mediated targeting of fibroblast activation protein enhances T-cell infiltration in murine models.[144,145] Such models have captured dynamic

feedback loops between transforming growth factor-beta-mediated matrix remodeling and tumor cell migration, offering unprecedented resolution of microenvironmental regulation.

Advanced 3D bioprinting platforms have achieved precise reconstruction of spatial topology to organize triple-negative breast cancer cells (MDA-MB-231) alongside stromal fibroblasts and endothelial cells by extrusion-based multi-nozzle bioprinting. This approach replicates the cellular and TME diversity observed in native tumors, preserving subclone viability and minimizing mechanical stress during printing.[25,84] Stratified deposition of *KRAS*-mutated and wild-type cells recapitulates *in vivo* spatial competition dynamics, mirroring the clonal hierarchy observed in native tumors.[146] Complementary approaches, such as magnetic levitation 3D culture systems, have successfully replicated the radial distribution of $CD133^+$ glioblastoma stem cells and non-stem counterparts, mimicking their *in situ* spatial organization.[147] Similarly, bioprinted pancreatic ductal adenocarcinoma models demonstrated the self-organization of cancer cells into heterogeneous aggregates, reflecting the clonal dynamics and spatial complexity of human tumors.[148] Microfluidic-integrated bioprinting systems permit the spatial embedding of vascular endothelial cells, CAFs, and immune cells within biomimetic matrices, thus establishing oxygen/nutrient gradients that emulate tumor ecological heterogeneity.[149] Notably, in GelMA hydrogels containing 10% Matrigel, CAFs self-organize into 50–100 μm fibrous bundles, guiding tumor cells along defined invasion trajectories. These engineered architectures provide a physiologically relevant platform to investigate paracrine signaling networks among sub-clonal niches.

Integration of fluorescent barcoding with 3D bioprinting allows real-time tracking of sub-clonal spatial dynamics, which enables the quantitative profiling of spatial heterogeneity. In hepatocellular carcinoma models, serial section imaging revealed epidermal growth factor receptor-high subclones preferentially colonizing nutrient-rich zones within 300 μm of artificial vasculature, exhibiting a 2.3-fold proliferation advantage over peripheral populations.[149–151] Spatial transcriptomics further identified elevated Wnt/β-catenin pathway activity in these regions, correlating with immunohistochemical patterns in clinical specimens.[136]

Bioprinted glioblastoma models with concentric architectures simulate temozolomide diffusion gradients, revealing that $CD133^+$ cells located 800 μm from drug release sites demonstrated 4.7-fold higher survival rates, attributable to hypoxia-induced adenosine triphosphate binding cassette subfamily G member 2 upregulation.[146] Intriguingly, macrophage incorporation exacerbated peripheral cell resistance via interleukin-6/Janus kinase/signal transducer and activator of transcription 3 activation, highlighting spatially compartmentalized stroma-tumor interactions in therapeutic evasion.[152]

These models have demonstrated clinical utility in predicting colorectal cancer liver metastasis patterns (82% accuracy), optimizing anti-angiogenic dosing regimens (37% survival extension in murine models, and deconvoluting transforming growth factor-beta/receptor activator of nuclear factor-κB ligand synergy in breast cancer bone niches.[149,153,154] Convergence with spatial omics and smart biomaterials promises to reconstruct tumor evolutionary landscapes at single-cell resolution, heralding a new era in precision oncology research.

#### *4.1.2. Intercellular communication within tumor organoids*

Bioprinting allows for the exact positioning of different cell types and biomaterials in a 3D space with the help of a mechanical and computer-assisted system to mimic the *in vivo* spatial architecture of a tissue or tumor and its microenvironment. This facilitates the study of bidirectional signaling between cancer cells and stromal components, such as CAFs, immune cells, and endothelial cells.[105] A key advantage of bioprinting-based preclinical cancer models is the standardization of cell deposition,[155] along with the ability to construct artificial 3D tumors incorporating multiple cell types, structural elements, and ECM. This capability enhances the potential for more accurate personalized medicine strategies.[156] Early research primarily utilized bioprinted cancer cell lines or single-cell suspensions to generate such models.[157] More recently, advancements in dispersing organoids and tumoroids alongside stromal cells have enabled the development of co-culture models that incorporate the TMEs.[158] It also facilitates the construction of vascularized tumor organoids by embedding perfusable channels lined with endothelial cells, thereby simulating nutrient exchange and immune cell trafficking.[39,42,123] Sequential printing of sacrificial inks enables the creation of hierarchical vascular networks that support long-term organoid survival and maturation.[42,159]

The mammary microenvironment has been shown to suppress tumor progression by redirecting cancer cells to adopt a normal mammary epithelial progenitor fate *in vivo*. However, the mechanism(s) by which this alteration occurs have yet to be defined. A 3D bioprinted fibroblast-mediated breast tumor organoid revealed that CAFs enhance ECM remodeling and paracrine communication, promoting tumor stiffness and conferring resistance to radiotherapy. Similarly, researchers evaluated mitochondrial transfer in 3D bioprinted chimeric organoids to test the hypothesis

that mitochondrial transfer from normal mammary epithelial cells to breast cancer cells plays a role in the redirection process. Their results demonstrate that mitochondrial transfer contributes to microenvironmental redirection of cancer cells through alteration of metabolic and molecular functions of the recipient cancer cells, which is the first description of a 3D bioprinter-assisted organoid system for studying mitochondrial transfer. Furthermore, these studies are also the first mechanistic insights into the process of mammary microenvironmental redirection of cancer, providing a framework for new therapeutic strategies to control cancer.[160] Khan et al.[161] introduced a human bone marrow organoid capable of sustaining the proliferation of primary cells derived from individuals with myeloid and lymphoid hematologic malignancies. This model facilitates in-depth investigations into the pathophysiology of blood cancers within their TMEs and offers a valuable *ex vivo* platform for evaluating novel therapeutic agents.[161] Chen et al.[162] generated colorectal cancer microtissues based on patient-specific colonoscopy images by printing photodynamic therapies enclosed by healthy organoids to mimic the tumor's interaction with adjacent normal tissue.[162] The *in vitro* response of these microtissues to standard 5-fluorouracil therapy mirrored patient responses, suggesting the model's potential as a physiologically relevant platform for drug screening. Furthermore, the model enabled the calculation of patient-specific risk for tumor invasion into neighboring tissues by assessing the quantity and proximity of invading tumor cells. This approach offers a real-time quantitative assessment for studying cancer advancement and metastasis.

In a recent study, microtissues comprising patient-derived lung tumoroids were co-cultured with corresponding CAFs and endothelial cells. The process involved printing vessel structures, seeding CAFs, and subsequently printing tumoroids suspended in a hydrogel sourced from porcine lung tissue into the same compartment. Notably, an active fusion between stromal cells and tumoroids occurred, leading to the formation of microvessels that directly engaged with other cell types. Upon administering the drug poziotinib through the vessel structures, it was observed that both endothelial cells and CAFs, along with the CAF-secreted matrix, shielded the lung tumoroids from the treatment. Consequently, this model holds promise for investigating the impact of cell–cell and cell–matrix interactions on the effectiveness of drug delivery to tumor tissues.[163]

In ovarian cancer models, bioprinted microtumors composed of leukemia (HL-60) and stromal cells exhibited dynamic cell–cell interactions, where stromal cells modulated cancer cell proliferation and invasion through cytokine signaling. Such models underscore the role of bioprinting in capturing the dynamic crosstalk within the TME, which drives drug resistance and tumor progression. Additionally, the incorporation of neural cells and lymphatic analogs into bioprinted models has revealed insights into tumor innervation and immune evasion mechanisms.[39,159] These models also capture cytokine-mediated crosstalk, such as CAF-driven ECM remodeling and immunosuppressive signaling, which are pivotal for drug resistance studies.[121]

### 4.2. High-throughput tumor organoid auto-printing for drug screening

Conventional organoid cultures face challenges in terms of batch-to-batch variability and labor-intensive protocols. Automated bioprinting systems, however, ensure consistent deposition of cells and bioinks, enabling mass production of uniform tumor organoids, which greatly avoids the bias caused by operators' manipulation.[78,159,164] Kim et al.[164] engineered a fully automated workstation that streamlines the entire workflow from tissue dissociation to drug screening, achieving a 20-fold increase in processing efficiency. Capable of handling 200 specimens per run, this system eliminates manual variability while enabling large-scale phenotypic and molecular profiling. Hou et al.[165] engineered a high-throughput screening-compatible approach that standardizes organoid generation in conventional flat-bottom 384- and 1536-well plates.[165] This method integrates magnet-assisted bioprinting technology with cell-repellent surface engineering to ensure precision and scalability. To demonstrate its applicability in automated drug discovery workflows, the researchers conducted a pilot cytotoxicity assessment of approximately 3300 clinically approved compounds. The results underscore the platform's breakthrough to facilitate large-scale drug screening through patient-derived 3D oncology models. By enabling robust analysis of clinically relevant tissues, this innovation represents a critical advancement toward personalized therapeutic development.

For personalized medicine, bioprinted constructs using patient-derived cells have been utilized to predict drug responses. In head and neck squamous cell carcinoma, bioprinted models maintained epithelial phenotypes and exhibited reduced cytotoxicity to radiochemotherapy compared to spheroids, better reflecting clinical resistance patterns.[166,167] Ovarian and colorectal tumor models bioprinted with nanocomposite hydrogels were screened for gemcitabine and oxaliplatin responses, revealing drug-specific resistance mechanisms.[168] These advances highlight the potential of automated bioprinting to generate patient-specific organoids at scale, bridging the gap between *in vitro* assays and clinical outcomes. Tebon et al.[169] introduced an integrated platform combining bioprinted tumor

organoids with label-free, time-resolved imaging using high-speed live cell interferometry and machine learning-driven analytical tools (Figure 6A).[169] Bioprinting generates 3D tumor structures that preserve native histology and transcriptional profiles. Coupled with high-speed live cell interferometry, this system enables non-invasive, parallelized mass quantification of thousands of organoids over time. Machine learning algorithms further enhance segmentation accuracy and phenotypic classification. They also demonstrated the platform's ability to distinguish organoids exhibiting transient or persistent sensitivity versus resistance to targeted therapies. This approach provides a scalable framework for resolving temporal and heterotypic adaptations in tumor populations, offering actionable insights to accelerate personalized therapeutic decision-making. Nonetheless, shortcomings exist in cost constraints, the availability of specific cell types, the time needed for model establishment and growth, and success rates. Parallel innovations in miniaturization have redefined screening economics. Phan et al.[170] pioneered a nanoscale microplate platform (200 nL/well) coupled with artificial intelligence-driven hyperspectral imaging, permitting simultaneous evaluation of 1536 drug combinations per assay.[170] This approach reduces reagent consumption and operational costs to 20% of conventional methods without compromising data resolution. Individual models vary in their representation of important features such as tumor heterogeneity, spatial interactions between tumor and stromal microenvironments, metabolic and nutritional gradients, and immunological responses. Consequently, a strategic integration of diverse models may be necessary to enhance the efficacy of clinical studies by bolstering the foundation of preclinical data.[171]

Long-term organoid modeling enables complex therapeutic screening. However, technical challenges include limited user-friendliness in long-term dynamic cell culture, incompatibility with rapid cell encapsulation in biomimetic hydrogels, and low throughput for compound screening. To address these issues, a micro-solenoid valve-driven bioprinting system was developed by Joshi et al.[171] This system fabricates the alginate-encapsulated Hep3B liver tumor spheroids in a 144-well plate, achieving rapid biomimetic tissue formation for large-scale compound screening.

Bioprinting offers applications beyond TME-mimicking models, including standardized cell dispensing for high-throughput studies. A key limitation, however, is the tendency of bioinks to spread within small wells, compromising the structural integrity required for 3D cell culture.[173] To address this issue, researchers have employed strategies such as mixing patient-derived glioblastoma or sarcoma cells with hyaluronic acid-collagen bioinks, printing them onto gelatin-coated wells, and subsequently replacing the gelatin with culture medium.[174] Alternatively, acoustic bioprinting has been used to deposit small droplets onto hydrophobic substrates, generating bladder cancer-derived tumoroids containing both cancer cells and CAFs.[175] This approach enables the scalable production of uniform tumoroids that recapitulate the TME while remaining compatible with high-throughput drug screening for personalized therapy. While 3D bioprinted models enhance reproducibility in drug testing and facilitate the study of multicellular interactions in a 3D context, they remain static systems. Consequently, they fail to incorporate dynamic mechanical forces (e.g., fluid flow) or chemical gradients, both of which critically influence tumor cell behavior *in vivo*.[176]

## 5. Beyond organoids: three-dimensional-printed biocompatible accessories

The convergence of 3D-printed biocompatible accessories, like artificial intelligence-driven design, organ-on-chip technologies, and organoid morphology recognition and deconvolution, enhances the functionality and realism of the models, enabling intelligence and automation in the construction of high-fidelity organoid models.[177–179] This section explores the role of functional biocompatible accessories beyond 3D-printed organoid culture, focusing on structural supports, functional interfaces, and integrated systems that augment organoid utility.[180] By leveraging advanced biomaterials, innovative printing techniques, and interdisciplinary engineering, these accessories bridge the gap between *in vitro* organoids and *in vivo* physiological complexity, opening new frontiers in precision medicine, tissue engineering, and regenerative therapy.[181,182]

### 5.1.icrofluidic devices for perfusable cultures

Microfluidic systems mimic the physiological flow of body fluids, allowing precise control of nutrient delivery, shear stress, and waste removal.[183,184] 3D bioprinting facilitates the fabrication of complex microchannels with integrated sensors or valves, creating organ-on-a-chip platforms that combine organoids with fluidic networks.[89,182,185,186] 3D-printed microfluidics can also create chemical gradients (e.g., oxygen, growth factors) to guide organoid morphogenesis.[187] Researchers have employed printed gradient chambers to induce regional differentiation in hepatic organoids, forming distinct zones of hepatocytes and cholangiocytes, similar to native liver lobules.[188,189] Such spatial control over microenvironments is crucial for modeling organ-level functional zonation.[82,190] Recent advancements in 3D printing technology have enabled the creation of more complex gradient-generating devices,

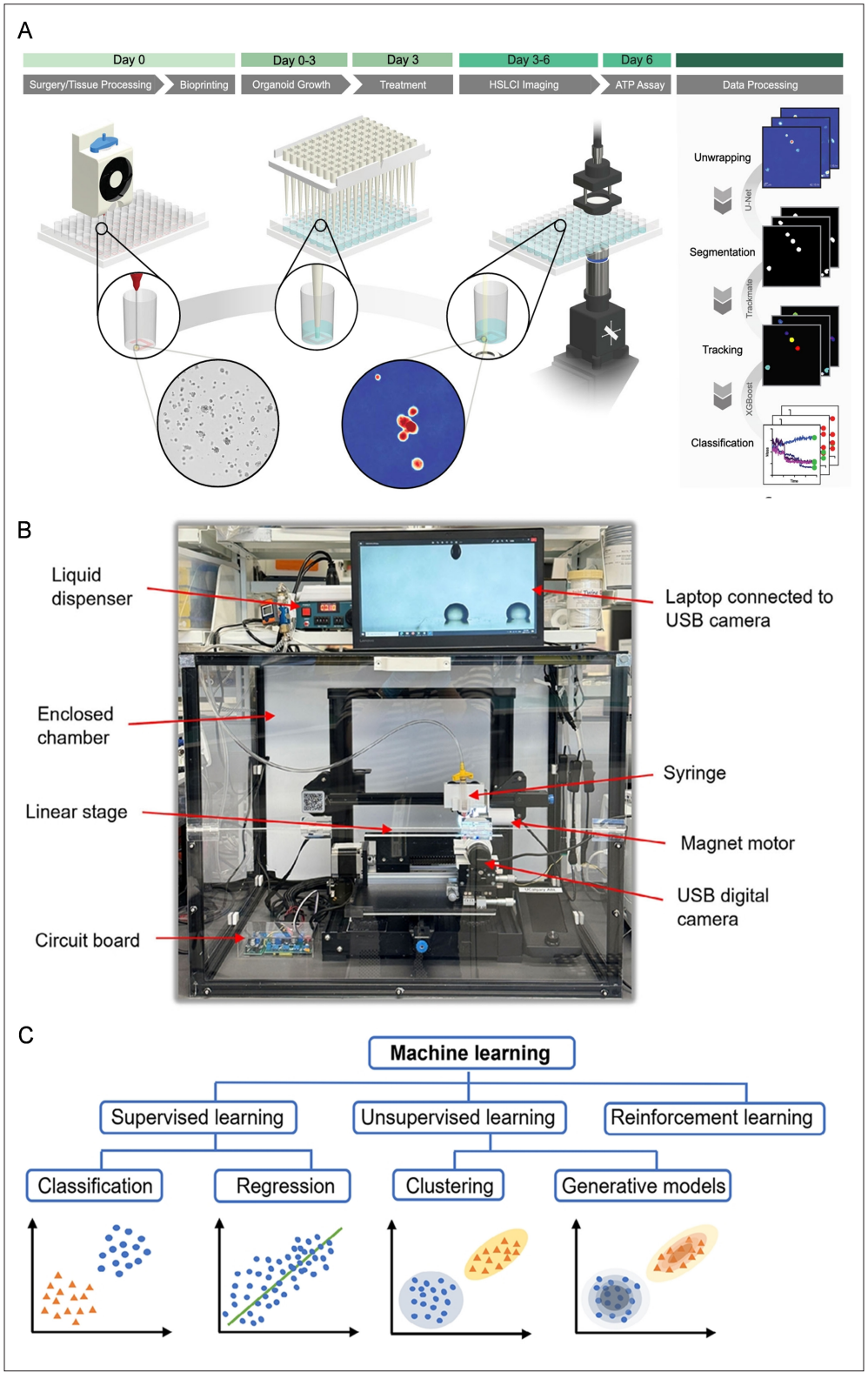



**Figure 6.** Emerging bioprinting-integrated platforms for high-throughput organoid analysis and fabrication. (A) Extrusion-based bioprinting enables single-organoid tracking with high-speed live cell interferometry (HSLCI). Adapted with permission from Tebon et al.[169] Copyright© Nature 2023. (B) Overview of machine learning setup. (C) Modified and developed cellular droplet three-dimensional bioprinting setup for high-throughput precision. Adapted with permission from Shin et al.[221] Copyright© Wiley 2025.

which can generate multiple gradients simultaneously and with higher precision, further improving the ability to control organoid development.[191–193]

### 5.2. Sensory accessories for real-time monitoring

Incorporating sensors into 3D printed accessories allows non-invasive monitoring of organoid physiology, including metabolic activity, electrical signals, and mechanical strain.[194]

#### *5.2.1. Biosensors for metabolite detection*

Printable conductive materials, like carbon nanotubes and graphene oxide, can be integrated into scaffolds to create electrochemical sensors.[195] For instance, a glucose-sensitive sensor printed within a pancreatic islet organoid scaffold continuously monitored insulin secretion in response to glucose challenges, providing real-time data for diabetes research.[196,197] Similarly, lactate sensors in printed brain organoid cultures correlated metabolic activity with neuronal network maturation, offering insights into neurodevelopmental disorders.[196,198] These biosensors have the potential to revolutionize the field of organoid research by providing real-time, non-invasive monitoring of organoid function, which can help to better understand the underlying mechanisms of disease and develop more effective treatments.

#### *5.2.2. Electrophysiological probes for neural organoids*

Traditional organoid research has been constrained by the limitation of surface-level signal acquisition, rendering the spatiotemporal dynamics of internal physiological activities inaccessible, which is a critical drawback, particularly in cardiac and neural organoid studies. To address this challenge, Park et al.[199] developed a soft bioelectrode platform based on liquid metal 3D printing.[199] This system enables customizable geometric parameters (height, diameter) to accommodate diverse organoid structures while maintaining mechanical compliance comparable to biological tissues. This compatibility prevents mechanical damage to cardiac organoids during contraction or fluidic movement, enabling stable, long-term monitoring. The electrode array further facilitates simultaneous electrophysiological monitoring of 32 organoids, successfully capturing drug-induced electrophysiological responses. This scalable capability establishes a robust platform for high-throughput drug screening and disease modeling.

Lee et al.[200] extended the potential of liquid metal microelectrodes through high-resolution 3D printing. By precisely embedding electrodes into specific inner layers of retinal organoids, the researchers achieved spatiotemporally controlled electrophysiological recordings of retinal ganglion cell activity. This approach not only validated developmental patterns of inner retinal signaling but also revealed critical insights into early-stage neural organoid modeling of retinal degeneration. The findings underscore the technology's utility in dissecting disease mechanisms through dynamic, cell-type-specific observations. Both studies leverage liquid metal's mechanical adaptability to overcome the limitations of rigid conventional electrodes, yet their designs diverge to address distinct challenges. Park's approach emphasizes scalability and dynamic stability for cardiac electrophysiology, whereas Lee's work prioritizes submillimeter precision for neural circuit interrogation. This complementary framework establishes a modular toolkit for organoid electrophysiology, enabling researchers to tailor electrode configurations to specific experimental demands. Integration with multimodal sensing technologies could further advance applications in organ development studies, disease progression modeling, and personalized medicine, paving the way for unprecedented insights into biological systems at the organoid scale. The elucidation of brain function in neural organoids necessitates precise electrophysiological monitoring of neuronal activity.[201] To this end, 3D-printed microelectrodes, coated with biocompatible polymers, have been engineered to penetrate cerebral organoids, enabling the detection of action potentials and synaptic connectivity.[202] A pioneering study by Acha et al.[202] employed printed multi-electrode arrays (MEAs) to systematically map electrical signals in cortical organoids. Their findings revealed spontaneous network oscillations resembling those observed in fetal brain development, underscoring the utility of these systems in modeling neurodevelopmental dynamics.[203-205] The research introduced shell-like MEA configurations analogous to electroencephalography caps, designed to interface with the surface of neural organoids while enabling 3D spatiotemporal recording. This design not only facilitated non-invasive longitudinal monitoring of neuronal firing but also demonstrated a statistically significant increase in action potential generation following external stimulation. Crucially, the biocompatible architecture preserved organoid viability, thereby supporting prolonged *in vitro* studies of neurodevelopmental processes.

A novel microfabrication technique has further advanced this field by addressing the mechanical constraints of traditional MEAs. By depositing material layers over a sacrificial template and strategically releasing internal stresses, researchers constructed vertically protruding cantilever microelectrodes. These slender beams extend over 200 μm from their base, allowing deep penetration into organoid interiors. This innovation enables the recording of local field potentials from deeply

embedded neurons, previously inaccessible with surface-based systems, thereby offering unprecedented insights into intracellular network dynamics.

Complementary advancements in 3D microfabrication further expanded the versatility of electrophysiological platforms. For instance, curved microfluidic systems and self-folding electrodes have been proposed to optimize spatial compatibility with diverse organoid morphologies. These customizable tools adapt to the unique structural demands of specific neural organoid models, enhancing data accuracy and experimental reproducibility. Such innovations collectively establish a modular framework for electrophysiological analysis, bridging the gap between *in vitro* organoid systems and complex *in vivo* neural activity.

### 5.3. Actuating accessories for mechanical stimulation

Mechanical stimuli, such as cyclic stretch and compression, are critical determinants of tissue maturation, particularly in the context of muscular and cardiovascular organoids. To replicate the dynamic mechanical microenvironments encountered *in vivo*, researchers have increasingly employed 3D-printed actuators, including pneumatic and hydraulic microsystems, as precise tools for delivering controlled mechanical signals.[205] This approach has been pivotal in advancing the functional maturation of engineered tissues.

In cardiac organoid models, silicone-based 3D-printed actuators have been instrumental in applying cyclic stretching, which facilitates myofibril alignment and enhances contractile performance.[205,206] Furthermore, studies utilizing 3D-printed piston-driven systems demonstrated that mechanical stimulation can significantly improve the electrophysiological properties of cardiac tissues, rendering them more physiologically relevant for disease modeling, such as arrhythmia studies.[207,208] Recent advancements have expanded this paradigm by incorporating additional mechanical modalities, such as shear stress and torsion, to further refine cardiac organoid maturation, thereby elevating their functional complexity and utility in both research and translational applications.[209,210]

Beyond cardiovascular systems, 3D printing has enabled the development of piezoelectric scaffolds capable of generating ultrasonic vibrations to simulate physiological mechanical loading. Such scaffolds have been shown to promote osteoblast differentiation and mineralization by mimicking *in vivo* mechanical cues, with studies reporting a 40% increase in calcium deposition in bone organoids compared to static culture conditions.[121] The customizable nature of 3D-printed piezoelectric structures further allows tailoring their mechanical properties to specific bone organoid models, thereby optimizing stimulation efficacy and enhancing the structural quality of engineered bone tissues.[211]

### 5.4. Multicomponent bio-assembly for complex organoid models

#### *5.4.1. Heterotypic co-culture platforms*

3D bioprinting enables the precise co-deposition of multiple cell types and biomaterial components, creating heterotypic co-culture systems that replicate complex tissue–tissue interfaces and physiological interactions.[212]

A compelling application is the integration of brain organoids with endothelial cells and astrocytes within perfusable scaffolds, which generates a functional blood–brain barrier model.[213] These platforms exhibit robust tight junction formation and selective permeability, offering a physiologically accurate platform to study neurotoxicity, drug permeability, and blood–brain barrier dysfunction.[214,215] Recent advances have refined these systems by incorporating species-specific cell lines, bioactive ECM components, and dynamic perfusion protocols to better mimic *in vivo* microenvironments, thereby enhancing model fidelity.[216,217]

Similarly, cancer organoids co-cultured with stromal cells or immune cells within 3D-printed matrices recapitulate critical features of TMEs. For instance, colorectal cancer organoids embedded with immune cells in printed matrices enabled the evaluation of immunotherapy efficacy in a context that mirrors clinical tumor-immune interactions.[213] Such systems hold transformative potential for cancer research, offering insights into tumor progression, metastasis, and therapeutic resistance.

Despite these advancements, challenges remain. Heterotypic co-cultures require meticulous regulation of cell–cell interactions, as uncontrolled cross-talk may lead to signal interference or cross-contamination over time. Additionally, maintaining physiological relevance across diverse cell populations demands precise spatiotemporal control of growth factors, mechanical cues, and metabolic gradients, which is a complex interplay that necessitates further optimization.

#### *5.4.2. Miniature bioreactors for scalable culture*

The integration of 3D-printed scaffolds with microfluidic bioreactors has enabled high-throughput organoid culture systems capable of parallel production, monitoring, and analysis.[218] These platforms, often equipped with automated sampling and imaging capabilities, are indispensable for accelerating drug discovery and toxicity testing.[201,219]

A notable example is the 3D-printed polycarbonate perfusion bioreactor, which supports the simultaneous culture of hundreds of hepatic organoids under controlled perfusion. By maintaining consistent metabolic activity for up to 4 weeks, these systems address a critical limitation of traditional static cultures and advance their utility in drug metabolism studies.[220] Recent design improvements prioritize uniform nutrient delivery, efficient waste removal, and minimized shear stress, thereby enhancing organoid viability and experimental reproducibility.[175]

To standardize output, another 3D bioprinting platform presents a promising alternative. Shin et al.[221] addressed the limitations of optimization of critical process variables (bioink viscosity, nozzle size, printing duration, pressure, and cell density) by leveraging machine learning to streamline parameter optimization and enable real-time prediction of cellular droplet dimensions.[221] This high-throughput bioprinting system was engineered to generate over 50 cellular droplets per cycle, facilitating the rapid compilation of datasets essential for robust algorithmic training. Five machine learning models were evaluated for performance, with the multilayer perceptron exhibiting superior predictive accuracy and the decision tree achieving the shortest computational latency. To enhance practical utility, these optimized algorithms were embedded into an intuitive interface designed for seamless integration into experimental workflows, which bridges bioprinting automation with data-driven parameter refinement, holding significant potential to advance scalable organoid manufacturing (Figure 6B & C). By harmonizing precision and efficiency, this framework is poised to synergize with diverse biomanufacturing technologies, accelerating applications in drug development, disease modeling, and regenerative medicine.

Complementary to perfusion systems, inkjet bioprinting enables the precise deposition of organoids onto pre-patterned substrates, forming high-density arrays ideal for large-scale screening. A 96-well plate format with printed ECM microdots, for instance, facilitates uniform culture of intestinal organoids and accelerates high-throughput toxicity testing of anticancer agents.[222,223] Such array-based systems significantly streamline drug discovery workflows, reducing time and resource demands while improving data consistency.

Despite these strides, scaling organoid cultures requires balancing system complexity with cost-effectiveness. Future innovations in bioreactor design must prioritize scalability, automation, low-cost accessibility, computer numerical control-driven platform, and integration with artificial intelligence-driven analytics to fully unlock the potential of organoid-based platforms in translational research.[96]

### 5.5. Challenges and future directions

Despite advancements in printing vascular networks, seamless anatomical and functional integration between engineered vessels and organoid-derived microvasculature remains elusive. Overcoming this hurdle requires innovations in two key areas. First, the co-printing of endothelial cells with angiogenic factors to promote vasculogenesis, and second, the development of advanced imaging or sensor-based tools to evaluate vascular functionality and optimize tissue–tissue interfaces. Meanwhile, short-term biocompatibility does not guarantee safety over extended periods, as degradation byproducts may trigger inflammatory responses or compromise organoid viability. Future efforts must prioritize the development of fully biodegradable materials that mimic the dynamic turnover of native ECM while exerting immunomodulatory effects. Concurrently, systematic investigations into the long-term biological impacts of material degradation are essential to refine strategies for mitigating adverse outcomes.

Current 3D-printed systems excel at providing structural frameworks but often fail to induce functional maturation, such as the formation of innervated muscle or vascularized glomeruli. To bridge this gap, researchers must integrate multifaceted stimuli into engineered environments, including mechanical forces, electrical signaling, and spatiotemporal biochemical gradients. Furthermore, elucidating the molecular and cellular mechanisms governing maturation will inform the design of targeted interventions.

Innovations in artificial intelligence present a transformative opportunity for clinical translation. Artificial intelligence-driven platforms can streamline organoid design through rapid screening, enable high-resolution analysis of multiscale imaging and multi-omics datasets, and facilitate precise preclinical drug testing and disease modeling.[224] Achieving clinical relevance also demands rigorous standardization. Scalable production protocols must ensure precision and reproducibility, while standardized sterilization, cell seeding, and functional validation procedures are indispensable for safe patient applications.

## 6. Conclusion

Advances in bioprinting have profoundly advanced our comprehension of organoid and tumoroid development, regeneration, pathophysiological mechanisms, drug sensitivity assessment, and clinical translation.[225]

Innovations in culture optimization and the discovery of novel ECM-mimetic scaffold materials have partially mitigated longstanding challenges related to poor standardization and suboptimal cell yield.[226] However, persistent technical barriers impede progress, particularly in balancing geometric precision with the preservation of cellular viability and functional integrity. Current systems often struggle to achieve high-fidelity without compromising the metabolic and physiological activity of encapsulated cells.

To unlock the full potential of 3D bioprinted organoids, future research must prioritize three interrelated avenues: (i) the engineering of advanced bioinks with tunable mechanical, biochemical, and degradation properties to better emulate native tissues, (ii) the refinement of printing methodologies to enhance spatial resolution while minimizing mechanical stress on delicate cellular components, and (iii) the synergistic integration of organoid systems with complementary biofabrication technologies (e.g., microfluidics, organ-on-a-chip platforms) to construct physiologically relevant, multi-tissue models. Such advancements will not only address existing technical limitations but also expand the scope of organoid applications in precision medicine, disease modeling, and high-throughput drug screening.

## Acknowledgment

The author team would like to extend their sincere gratitude to the research teams whose works have been cited in this review. Special thanks are given to those who generously granted permission for the reuse of their data. Their valuable contributions have laid a solid foundation for the advancement of 3D-printed organoid research and have provided important insights that shaped the perspectives discussed in this manuscript.

## Funding

This work was supported by the National Key Research and Development Program of China (Grant No. 2022YFC2504200) and the National Natural Science Foundation of China (Grant Nos. 82373434 and 82002878).

## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

*Conceptualization:* All authors
*Funding acquisition:* Zhigang Cai, Shang Xie
*Resources:* Zhigang Cai, Shang Xie, Xiaofeng Shan
*Supervision:* Zhigang Cai, Shang Xie, Xiaofeng Shan
*Validation:* Zhigang Cai, Shang Xie, Xiaofeng Shan
*Writing–original draft:* Lingzi Liao
*Writing–review & editing:* Shang Xie, Zhigang Cai, Qiushi Feng

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data

Not applicable.

## References

1. Zhao Z, Chen X, Dowbaj AM, et al. Organoids. *Nat Rev Methods Primers*. 2022;2(1):94. doi: 10.1038/s43586-022-00174-y
2. Geng Y, Chen Z, Luo T, et al. Innovative construction and application of bile duct organoids: unraveling the complexity of bile duct diseases and potential therapeutic strategies. *Cancer Lett*. 2025;618:217619. doi: 10.1016/j.canlet.2025.217619
3. Hermans F, Hasevoets S, Vankelecom H, Bronckaers A, Lambrichts I. From pluripotent stem cells to organoids and bioprinting: recent advances in dental epithelium and ameloblast models to study tooth biology and regeneration. *Stem Cell Rev Rep*. 2024;20(5):1184-1199. doi: 10.1007/s12015-024-10702-w
4. Mallya D, Gadre MA, Varadharajan S, Vasanthan KS. 3D bioprinting for the construction of drug testing models-development strategies and regulatory concerns. *Front Bioeng Biotechnol*. 2025;13:1457872. doi: 10.3389/fbioe.2025.1457872
5. Nejati B, Shahhosseini R, Hajiabbasi M, et al. Cancer-on-chip: a breakthrough organ-on-a-chip technology in cancer cell modeling. *Med Biol Eng Comput*. 2025;63(2):321-337. doi: 10.1007/s11517-024-03199-5
6. Hu Y, Zhu T, Cui H, Cui H. Integrating 3D bioprinting and organoids to better recapitulate the complexity of cellular microenvironments for tissue engineering. *Adv Healthc Mater*. 2025;14(3):e2403762. doi: 10.1002/adhm.202403762
7. Huang MS, Christakopoulos F, Roth JG, Heilshorn SC. Organoid bioprinting: from cells to functional tissues. Review. *Nat Rev Bioeng*. 2025;3(2):126-142. doi: 10.1038/s44222-024-00268-0
8. Ju M, Jin Z, Yu X, et al. Gastric cancer models developed via GelMA 3D bioprinting accurately mimic cancer hallmarks,

tumor microenvironment features, and drug responses. *Small*. 2025;21(8):e2409321.
doi: 10.1002/smll.202409321

9. Ding Z, Huang J, Ren Y, et al. 3D bioprinted advanced cartilage organoids with engineered magnetic nanoparticles polarized-BMSCs/alginate/gelatin for cartilage tissue regeneration. Article. *Nano Res*. 2025;18(2)94907084.
doi: 10.26599/nr.2025.94907084

10. Shyu J-F, Chu T-H, Lo Y-C, et al. Fabrication of 3D bioprinting vascularized bone organoid under compressive stimulation for study of osteogenesis and angiogenesis. Meeting Abstract. *J Bone Miner Res*. 2023;38:304-304.

11. Abaci A, Camci-Unal G, Guvendiren M. Three-dimensional bioprinting for medical applications. Article. *MRS Bulletin*. 2023;48(6):624-631.
doi: 10.1557/s43577-023-00546-z

12. Shen N, Li Z, Yang P, et al. Designing methacrylic anhydride-based hydrogels for 3D bioprinting. *IJB*. 2024;11(1):84-138.
doi: 10.36922/ijb.4650

13. Chen Z, Zhang H, Huang J, et al. DNA-encoded dynamic hydrogels for 3D bioprinted cartilage organoids. *Mater Today Bio*. 2025;31:101509.
doi: 10.1016/j.mtbio.2025.101509

14. Shen C, Wang J, Li G, et al. Boosting cartilage repair with silk fibroin-DNA hydrogel-based cartilage organoid precursor. Article. *Bioact Mater*. 2024;35:429-444.
doi: 10.1016/j.bioactmat.2024.02.016

15. Ali EAM, Smaida R, Meyer M, et al. iPSCs chondrogenic differentiation for personalized regenerative medicine: a literature review. *Stem Cell Res Ther*. 2024;15(1):185.
doi: 10.1186/s13287-024-03794-1

16. Lawlor KT, Vanslambrouck JM, Higgins JW, et al. Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation. *Nat Mater*. 2021;20(2):260-271.
doi: 10.1038/s41563-020-00853-9

17. Long B, Mengmeng L, Jiacan S. A perspective on light-based bioprinting of DNA hydrogels for advanced bone regeneration: implication for bone organoids. *Int J Bioprint*. 2023;9(2):688.
doi: 10.18063/ijb.688

18. Wang Z, Wang X, Huang Y, et al. $Ca_v3.3$-mediated endochondral ossification in a three-dimensional bioprinted GelMA hydrogel. Article. *Bio-Des Manuf*. 2024;7(6):983-999.
doi: 10.1007/s42242-024-00287-1

19. Wang J. Engineering large-scale self-mineralizing bone organoids with bone matrix-inspired hydroxyapatite hybrid bioinks. *Adv Mater (Weinheim)*. 2024;36(30):e2309875.
doi: 10.1002/adma.202309875

20. Ren X, Wang J, Wu Y, et al. One-pot synthesis of hydroxyapatite hybrid bioinks for digital light processing 3D printing in bone regeneration. *J Mater Sci Technol*. 2024;188:84-97.
doi: 10.1016/j.jmst.2024.01.001

21. Wang J, Zhou D, Li R, et al. Protocol for engineering bone organoids from mesenchymal stem cells. *Bioact Mater*. 2025;45:388-400.
doi: 10.1016/j.bioactmat.2024.11.017

22. Li H, Chen H, Du C, et al. Effect of hydroxyapatite nanowires on formation and bioactivity of osteoblastic cell spheroid. *ACS Biomater Sci Eng*. 2024;10(12):7413-7428.
doi: 10.1021/acsbiomaterials.4c01159

23. Fang Y, Ji M, Wu B, et al. Engineering highly vascularized bone tissues by 3d bioprinting of granular prevascularized spheroids. *ACS Appl Mater Interfaces*. 2023;15(37):43492-43502.
doi: 10.1021/acsami.3c08550

24. Loukelis K, Koutsomarkos N, Mikos AG, Chatzinikolaidou M. Advances in 3D bioprinting for regenerative medicine applications. *Regen Biomater*. 2024;11:rbae033.
doi: 10.1093/rb/rbae033

25. Bernal PN, Bouwmeester M, Madrid-Wolff J, et al. Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories. *Adv Mater (Deerfield Beach, Fla)*. 2022;34(15):e2110054.
doi: 10.1002/adma.202110054

26. Luo Y, Xu R, Hu Z, et al. Gel-based suspension medium used in 3D bioprinting for constructing tissue/organ analogs. *Gels*. 2024;10(10):644.
doi: 10.3390/gels10100644

27. De Leeuw A, Graf R, Zhang J, et al. Increased cell density increases mineral formation rates and stiffness in 3D bioprinted patient-derived bone organoids using dynamic loading. Meeting Abstract. *Tissue Eng Part A*. 2023;29(11-12):582-583.

28. Wang J, Chen X, Li R, et al. Standardization and consensus in the development and application of bone organoids. *Theranostics*. 2025;15(2):682-706.
doi: 10.7150/thno.105840

29. Park S, Cho SW. Bioengineering toolkits for potentiating organoid therapeutics. *Adv Drug Deliv Rev*. 2024;208:115238.
doi: 10.1016/j.addr.2024.115238

30. Frenz-Wiessner S, Fairley SD, Buser M, et al. Generation of complex bone marrow organoids from human induced pluripotent stem cells. Article. *Nat Methods*. 2024;21(5).
doi: 10.1038/s41592-024-02172-2

31. De Leeuw A, Schadli GN, Steffi C, et al. A novel 3D-bioprinted patient-specific biomimetic bone organoid to model osteogenesis imperfecta. Meeting Abstract. *Tissue Eng Part A*. 2023;29(13-14):582-583.

32. Abbott A. Cell culture: biology's new dimension. *Nature*. 2003;424(6951):870-872.
doi: 10.1038/424870a

33. Li J, Han S, Yu F, Li T, Liao B, Liu F. Mapping the landscape of PSC-CM research through bibliometric analysis. *Front Cardiovasc Med*. 2024;11:1435874. doi: 10.3389/fcvm.2024.1435874

34. Wang Y, Hou Y, Hao T, et al. Model construction and clinical therapeutic potential of engineered cardiac organoids for cardiovascular diseases. *Biomater Transl*. 2024;5(4):337-354. doi: 10.12336/biomatertransl.2024.04.002

35. Nwokoye PN, Abilez OJ. Blood vessels in a dish: the evolution, challenges, and potential of vascularized tissues and organoids. *Front Cardiovasc Med*. 2024;11:1336910. doi: 10.3389/fcvm.2024.1336910

36. Khoury RE, Nagiah N, Mudloff JA, Thakur V, Chattopadhyay M, Joddar B. 3D bioprinted spheroidal droplets for engineering the heterocellular coupling between cardiomyocytes and cardiac fibroblasts. *Cyborg Bionic Syst*. 2021;2021:9864212. doi: 10.34133/2021/9864212

37. Mohr E, Thum T, Bär C. Accelerating cardiovascular research: recent advances in translational 2D and 3D heart models. *Eur J Heart Fail*. Oct 2022;24(10):1778-1791. doi: 10.1002/ejhf.2631

38. Zhang W, Chen Y, Li M, et al. A PDA-functionalized 3d lung scaffold bioplatform to construct complicated breast tumor microenvironment for anticancer drug screening and immunotherapy. *Adv Sci (Weinh)*. 2023;10(26):e2302855. doi: 10.1002/advs.202302855

39. Li S, Li J, Xu J, et al. Removal-free and multicellular suspension bath-based 3D bioprinting. *Adv Mater (Deerfield Beach, Fla)*. 2024;36(48):e2406891. doi: 10.1002/adma.202406891

40. Hoang P, Sun S, Tarris BA, Ma Z. Controlling morphology and functions of cardiac organoids by two-dimensional geometrical templates. *Cells Tissues Organs*. 2023;212(1):64-73. doi: 10.1159/000521787

41. Noël ES. Cardiac construction—recent advances in morphological and transcriptional modeling of early heart development. *Curr Top Dev Biol*. 2024;156:121-156. doi: 10.1016/bs.ctdb.2024.02.005

42. Fang Y, Guo Y, Wu B, et al. Expanding embedded 3D bioprinting capability for engineering complex organs with freeform vascular networks. *Adv Mater (Deerfield Beach, Fla)*. 2023;35(22):e2205082. doi: 10.1002/adma.202205082

43. Cui H, Liu C, Esworthy T, et al. 4D physiologically adaptable cardiac patch: A 4-month in vivo study for the treatment of myocardial infarction. *Sci Adv*. 2020;6(26):eabb5067. doi: 10.1126/sciadv.abb5067

44. Zhang Z, Wu C, Dai C, et al. A multi-axis robot-based bioprinting system supporting natural cell function preservation and cardiac tissue fabrication. *Bioact Mater*. 2022;18:138-150. doi: 10.1016/j.bioactmat.2022.02.009

45. Fareez UNM, Naqvi SAA, Mahmud M, Temirel M. Computational fluid dynamics (CFD) analysis of bioprinting. *Adv Healthc Mater*. 2024;13(20):e2400643. doi: 10.1002/adhm.202400643

46. Abolhassani S, Fattahi R, Safshekan F, Saremi J, Hasanzadeh E. Advances in 4D bioprinting: the next frontier in regenerative medicine and tissue engineering applications. *Adv Healthc Mater*. 2025;14(4):e2403065. doi: 10.1002/adhm.202403065

47. Wu J, Fu J. Toward developing human organs via embryo models and chimeras. *Cell*. 2024;187(13):3194-3219. doi: 10.1016/j.cell.2024.05.027

48. Wu Y, Qin M, Yang X. Organ bioprinting: progress, challenges and outlook. *J Mater Chem B*. 2023;11(43):10263-10287. doi: 10.1039/d3tb01630g

49. Roza Vaez G, Ileana LC, Matthew CM, Vikramaditya GY. Brain organoids: a new, transformative investigational tool for neuroscience research. *Adv Biosyst*. 2018;3(1):174. doi: 10.1002/adbi.201800174

50. Renjitha G, Rakhi P. Bioengineering of brain organoids: advancements and challenges. *Tissue Eng*. 2022:399-414. doi: 10.1016/b978-0-12-824064-9.00002-2

51. Madeline AL, Magdalena R, Carol-Anne M, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501(7467):373-379. doi: 10.1038/nature12517

52. Jeong E, Choi S, Cho SW. Recent advances in brain organoid technology for human brain research. *ACS Appl Mater Interfaces*. 2023;15(1):200-219. doi: 10.1021/acsami.2c17467

53. Cadena MA, Sing A, Taylor K, et al. A 3D bioprinted cortical organoid platform for modeling human brain development. *Adv Healthc Mater*. 2024;13(27):e2401603. doi: 10.1002/adhm.202401603

54. Jihoon K, Sujin H, Sunghun C, Yoojin C, Noo Li J. Revealing the clinical potential of high-resolution organoids. *Adv Drug Deliv Rev*. 2024;207:115202. doi: 10.1016/j.addr.2024.115202

55. Natan Roberto de B, Canran W, Surjendu M, et al. Engineered organoids for biomedical applications. *Adv Drug Deliv Rev*. 2023;203:115142. doi: 10.1016/j.addr.2023.115142

56. Zhe L, Weizi G, Fukang L, et al. Vat photopolymerization based digital light processing 3D printing hydrogels in biomedical fields: key parameters and perspective. *Addit Manuf*. 2024;94:104443. doi: 10.1016/j.addma.2024.104443

57. Jennifer Sally S, Anuradha R, Venkatachalam Deepa P. Development of midbrain dopaminergic neurons and the advantage of using hiPSCs as a model system to study Parkinson's disease. *Neuroscience*. 2024;546:1-19. doi: 10.1016/j.neuroscience.2024.03.025

58. Yan Y, Li X, Gao Y, et al. 3D bioprinting of human neural tissues with functional connectivity. *Cell Stem Cell*. 2024;31(2):260-274.e7. doi: 10.1016/j.stem.2023.12.009

59. Layrolle P, Payoux P, Chavanas S. Message in a scaffold: natural biomaterials for three-dimensional (3D) bioprinting of human brain organoids. *Biomolecules*. 2022;13(1):25. doi: 10.3390/biom13010025

60. Gabriel E, Albanna W, Pasquini G, et al. Human brain organoids assemble functionally integrated bilateral optic vesicles. *Cell Stem Cell*. 2021;28(10):1740-1757.e8. doi: 10.1016/j.stem.2021.07.010

61. Jing G, Jiahui K, Minghui L, Xiao L, Jun Y, Haiwei X. Applications of neural organoids in neurodevelopment and regenerative medicine. *Biomed Eng*. 2022. doi: 10.5772/intechopen.104044

62. Tariku Sinshaw T, Frehiwot Bayelign T, Xijin H, et al. A review of advances in 3D and 4D bioprinting: toward mass individualization paradigm. *J Intell Manuf*. 2024. doi: 10.1007/s10845-024-02529-6

63. Wang X, Yang X, Liu Z, et al. 3D bioprinting of an in vitro hepatoma microenvironment model: establishment, evaluation, and anticancer drug testing. *Acta Biomater*. 2024;185:173-189. doi: 10.1016/j.actbio.2024.07.019

64. Zhuang X, Deng G, Wu X, et al. Recent advances of three-dimensional bioprinting technology in hepato-pancreato-biliary cancer models. *Front Oncol*. 2023;13:1143600. doi: 10.3389/fonc.2023.1143600

65. Kim MH, Singh YP, Celik N, et al. High-throughput bioprinting of spheroids for scalable tissue fabrication. *Nat Commun*. 2024;15(1):10083. doi: 10.1038/s41467-024-54504-7

66. Lekkala VKR, Shrestha S, Al Qaryoute A, et al. Enhanced maturity and functionality of vascular human liver organoids through 3D bioprinting and pillar plate culture. *ACS Biomater Sci Eng*. 2025;11(1):506-517. doi: 10.1021/acsbiomaterials.4c01658

67. Kang SY, Kimura M, Shrestha S, et al. A pillar and perfusion plate platform for robust human organoid culture and analysis. *Adv Healthc Mater*. 2024;13(21):e2302502. doi: 10.1002/adhm.202302502

68. Shrestha S, Lekkala VKR, Acharya P, Kang SY, Vanga MG, Lee MY. Reproducible generation of human liver organoids (HLOs) on a pillar plate platform via microarray 3D bioprinting. *Lab Chip*. 2024;24(10):2747-2761. doi: 10.1039/d4lc00149d

69. Gao Z, Liu X, Zhao H, et al. Synthesis of easily-processable collagen bio-inks using ionic liquid for 3D bioprinted liver tissue models with branched vascular networks. *Sci China Chem*. 2023;66(5):1489-1499. doi: 10.1007/s11426-022-1472-6

70. Yan J, Ye Z, Lu Y, et al. 3D bioprinting lobule-like hepatorganoids with induced vascularization for orthotopic implantation. *Mater Today Bio*. 2025;31:101515. doi: 10.1016/j.mtbio.2025.101515

71. Brumberg VA, Bikmulina PY, Pozdnyakov AA, et al. Scaling liver bioprinting: a guide for usage of the hepatic extracellular matrix as a bioink. Review. *Int J Bioprint*. 2025;11(1):57-83. doi: 10.36922/ijb.4343

72. Zhang Y, Li L, Dong L, et al. Hydrogel-based strategies for liver tissue engineering. *Chem Bio Eng*. 2024;1(11):887-915. doi: 10.1021/cbe.4c00079

73. Cross-Najafi AA, Farag K, Chen AM, et al. The long road to develop custom-built livers: current status of 3D liver bioprinting. *Transplantation*. 2024;108(2):357-368. doi: 10.1097/tp.0000000000004668

74. Li W, Liu Z, Tang F, et al. Application of 3D bioprinting in liver diseases. *Micromachines (Basel)*. 2023;14(8):1648. doi: 10.3390/mi14081648

75. Willemse J, van der Laan LJW, de Jonge J, Verstegen MMA. Design by nature: emerging applications of native liver extracellular matrix for cholangiocyte organoid-based regenerative medicine. *Bioengineering (Basel)*. 2022;9(3):110. doi: 10.3390/bioengineering9030110

76. Shi W, Zhang Z, Wang X. The prospect of hepatic decellularized extracellular matrix as a bioink for liver 3D bioprinting. *Biomolecules*. 2024;14(8):1019. doi: 10.3390/biom14081019

77. Kim Y, Kang M, Mamo MG, Adisasmita M, Huch M, Choi D. Liver organoids: current advances and future applications for hepatology. *Clin Mol Hepatol*. 2025;31(Suppl):S327-S348. doi: 10.3350/cmh.2024.1040

78. Li G, He J, Shi J, et al. Bioprinting functional hepatocyte organoids derived from human chemically induced pluripotent stem cells to treat liver failure. *Gut*. 2025; 74(7):1150-1164. doi: 10.1136/gutjnl-2024-333885

79. Sun H, Sun L, Ke X, et al. Prediction of clinical precision chemotherapy by patient-derived 3D bioprinting models of colorectal cancer and its liver metastases. *Adv Sci (Weinh)*. 2024;11(2):e2304460. doi: 10.1002/advs.202304460

80. Chen F, Wei X, Chen K, Wang L, Xu M. Massive fabrication of functional hepatic cancer spheroids by micropatterned GelMA hydrogel chip for drug screening. *Colloids Surf B Biointerfaces*. 2024;244:114171. doi: 10.1016/j.colsurfb.2024.114171

81. Yeo M, Sarkar A, Singh YP, Derman ID, Datta P, Ozbolat IT. Synergistic coupling between 3D bioprinting and vascularization strategies. *Biofabrication*. 2023;16(1):e2302506. doi: 10.1088/1758-5090/ad0b3f

82. Reza HA, Santangelo C, Iwasawa K, et al. Multi-zonal liver organoids from human pluripotent stem cells. *Nature*. 2025; 641(8065):1258-1267.
doi: 10.1038/s41586-025-08850-1

83. Falandt M, Bernal PN, Longoni A, et al. Hybrid supramolecular-covalent bioresin promotes cell migration and self-assembly in light-based volumetric bioprinted constructs. preprint. *bioRxiv*. 2025.
doi: 10.1101/2025.01.06.631505

84. Skylar-Scott MA, Huang JY, Lu A, et al. Orthogonally induced differentiation of stem cells for the programmatic patterning of vascularized organoids and bioprinted tissues. *Nature Biomed Eng*. 2022;6(4):449-462.
doi: 10.1038/s41551-022-00856-8

85. Urkasemsin G, Rungarunlert S, Ferreira JN. Bioprinting strategies for secretory epithelial organoids. *Methods Mol Biol*. 2020;2140:243-249.
doi: 10.1007/978-1-0716-0520-2_16

86. Klangprapan J, Souza GR, Ferreira JN. Bioprinting salivary gland models and their regenerative applications. *BDJ Open*. 2024;10(1):39.
doi: 10.1038/s41405-024-00219-2

87. Liu N, Huang S, Yao B, Xie J, Wu X, Fu X. 3D bioprinting matrices with controlled pore structure and release function guide in vitro self-organization of sweat gland. *Sci Rep*. 2016;6:34410.
doi: 10.1038/srep34410

88. Dai R, Chen W, Chen Y, et al. 3D bioprinting platform development for high-throughput cancer organoid models construction and drug evaluation. *Biofabrication*. 2024;16(3):34410.
doi: 10.1088/1758-5090/ad51a6

89. Shiwarski DJ, Hudson AR, Tashman JW, et al. 3D bioprinting of collagen-based microfluidics for engineering fully-biologic tissue systems. *bioRxiv*. 2024.
doi: 10.1101/2024.01.26.577422

90. Maciel BR, Grimm A, Oelschlaeger C, Schepers U, Willenbacher N. Targeted micro-heterogeneity in bioinks allows for 3D printing of complex constructs with improved resolution and cell viability. *Biofabrication*. 2023;15(4):042004.
doi: 10.1088/1758-5090/acee22

91. Markstedt K, Mantas A, Tournier I, Martínez Ávila H, Hägg D, Gatenholm P. 3D bioprinting human chondrocytes with nanocellulose-alginate bioink for cartilage tissue engineering applications. *Biomacromolecules*. 2015;16(5):1489-96.
doi: 10.1021/acs.biomac.5b00188

92. Maharjan S, Ma C, Singh B, et al. Advanced 3D imaging and organoid bioprinting for biomedical research and therapeutic applications. *Adv Drug Deliv Rev*. 2024;208:115237.
doi: 10.1016/j.addr.2024.115237

93. Gugulothu SB, Chatterjee K. Visible light-based 4D-bioprinted tissue scaffold. *ACS Macro Lett*. 2023;12(4):494-502.
doi: 10.1021/acsmacrolett.3c00036

94. Wang D, Guo Y, Zhu J, et al. Hyaluronic acid methacrylate/pancreatic extracellular matrix as a potential 3D printing bioink for constructing islet organoids. *Acta Biomater*. 2023;165:86-101.
doi: 10.1016/j.actbio.2022.06.036

95. Kim M, Cho S, Hwang DG, et al. Bioprinting of bespoke islet-specific niches to promote maturation of stem cell-derived islets. *Nat Commun*. 2025;16(1):1430.
doi: 10.1038/s41467-025-56665-5

96. Reid JA, Mollica PA, Bruno RD, Sachs PC. Consistent and reproducible cultures of large-scale 3D mammary epithelial structures using an accessible bioprinting platform. *Breast Cancer Res*. 2018;20(1):122.
doi: 10.1186/s13058-018-1045-4

97. Shi W, Mirza S, Kuss M, et al. Embedded bioprinting of breast tumor cells and organoids using low-concentration collagen-based bioinks. *Adv Healthc Mater*. 2023;12(26):e2300905.
doi: 10.1002/adhm.202300905

98. Zhang Y, Li G, Wang J, Zhou F, Ren X, Su J. Small joint organoids 3D bioprinting: construction strategy and application. *Small*. 2024;20(8):e2302506.
doi: 10.1002/smll.202302506

99. Bertassoni LE. Bioprinting of complex multicellular organs with advanced functionality-recent progress and challenges ahead. *Adv Mater (Deerfield Beach, Fla)*. 2022;34(3):e2101321.
doi: 10.1002/adma.202101321

100. Freedman BS, Brooks CR, Lam AQ, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun*. 2015;6:8715.
doi: 10.1038/ncomms9715

101. Votanopoulos KI, Forsythe S, Sivakumar H, et al. Model of patient-specific immune-enhanced organoids for immunotherapy screening: feasibility study. *Ann Surg Oncol*. 2020;27(6):1956-1967.
doi: 10.1245/s10434-019-08143-8

102. Childs CJ, Poling HM, Chen K, et al. Coordinated differentiation of human intestinal organoids with functional enteric neurons and vasculature. *Cell Stem Cell*. 2025;32(4):640-651.e9.
doi: 10.1016/j.stem.2025.02.007

103. Park HS, Park JH, Oh M-K, Yu K-R. Advancements in 3D bioprinting for precision medicine: enhancing patient-derived organoids and extracellular vesicle applications in inflammatory diseases. Article. *Int J Bioprint*. 2024;10(5)4054.
doi: 10.36922/ijb.4054

104. Datta P, Dey M, Ataie Z, Unutmaz D, Ozbolat IT. 3D bioprinting for reconstituting the cancer microenvironment. *NPJ Precis Oncol*. 2020;4:18.
doi: 10.1038/s41698-020-0121-2

105. Augustine R, Kalva SN, Ahmad R, et al. 3D bioprinted cancer models: revolutionizing personalized cancer therapy. *Transl Oncol*. 2021;14(4):101015.
doi: 10.1016/j.tranon.2021.101015

106. Qazi TH, Blatchley MR, Davidson MD, et al. Programming hydrogels to probe spatiotemporal cell biology. *Cell Stem Cell*. 2022;29(5):678-691.
doi: 10.1016/j.stem.2022.03.013

107. Gopalakrishnan S, Bakke I, Hansen MD, et al. Comprehensive protocols for culturing and molecular biological analysis of IBD patient-derived colon epithelial organoids. *Front Immunol*. 2023;14:1097383.
doi: 10.3389/fimmu.2023.1097383

108. Caire R, Audoux E, Courbon G, et al. YAP/TAZ: key players for rheumatoid arthritis severity by driving fibroblast like synoviocytes phenotype and fibro-inflammatory response. *Front Immunol*. 2021;12:791907.
doi: 10.3389/fimmu.2021.791907

109. Günther C, Winner B, Neurath MF, Stappenbeck TS. Organoids in gastrointestinal diseases: from experimental models to clinical translation. *Gut*. 2022;71(9): 1892-1908.
doi: 10.1136/gutjnl-2021-326560

110. Sachs N, Tsukamoto Y, Kujala P, Peters PJ, Clevers H. Intestinal epithelial organoids fuse to form self-organizing tubes in floating collagen gels. *Development*. 2017;144(6):1107-1112.
doi: 10.1242/dev.143933

111. Niklinska-Schirtz BJ, Venkateswaran S, Anbazhagan M, et al. Ileal derived organoids from Crohn's disease patients show unique transcriptomic and secretomic signatures. *Cell Mol Gastroenterol Hepatol*. 2021;12(4):1267-1280.
doi: 10.1016/j.jcmgh.2021.06.018

112. Taebnia N, Zhang R, Kromann EB, Dolatshahi-Pirouz A, Andresen TL, Larsen NB. Dual-material 3D-printed intestinal model devices with integrated villi-like scaffolds. *ACS Appl Mater Interfaces*. 2021;13(49): 8434-58446.
doi: 10.1021/acsami.1c22185

113. Brassard JA, Nikolaev M, Hübscher T, Hofer M, Lutolf MP. Recapitulating macro-scale tissue self-organization through organoid bioprinting. *Nat Mater*. 2021;20(1):22-29.
doi: 10.1038/s41563-020-00803-5

114. Carvalho MR, Yan L-P, Li B, et al. Gastrointestinal organs and organoids-on-a-chip: advances and translation into the clinics. Review. *Biofabrication*. 2023;15(4):042004.
doi: 10.1088/1758-5090/acf8fb

115. Xiaoshuai L, Qiushi W, Rui W. Advantages of CRISPR-Cas9 combined organoid model in the study of congenital nervous system malformations. *Front Bioeng Biotechnol*. 2022;10:932936.
doi: 10.3389/fbioe.2022.932936

116. Gopal S, Rodrigues AL, Dordick JS. Exploiting CRISPR Cas9 in three-dimensional stem cell cultures to model disease. *Front Bioeng Biotechnol*. 2020;8:692.
doi: 10.3389/fbioe.2020.00692

117. Schene IF, Joore IP, Oka R, et al. Prime editing for functional repair in patient-derived disease models. *Nat Commun*. 2020;11(1):5352.
doi: 10.1038/s41467-020-19136-7

118. Inak G, Rybak-Wolf A, Lisowski P, et al. Defective metabolic programming impairs early neuronal morphogenesis in neural cultures and an organoid model of Leigh syndrome. *Nat Commun*. 2021;12(1):1929.
doi: 10.1038/s41467-021-22117-z

119. Zhang W, Ma L, Yang M, et al. Cerebral organoid and mouse models reveal a RAB39b-PI3K-mTOR pathway-dependent dysregulation of cortical development leading to macrocephaly/autism phenotypes. *Genes Dev*. 2020;34(7-8):580-597.
doi: 10.1101/gad.332494.119

120. An HL, Kuo HC, Tang TK. Modeling human primary microcephaly with hiPSC-derived brain organoids carrying CPAP-E1235V disease-associated mutant protein. *Front Cell Dev Biol*. 2022;10:830432.
doi: 10.3389/fcell.2022.830432

121. Cidonio G, Glinka M, Dawson JI, Oreffo ROC. The cell in the ink: Improving biofabrication by printing stem cells for skeletal regenerative medicine. *Biomater*. 2019;209:10-24.
https://doi.org/10.1016/j.biomaterials.2019.04.009

122. Ahn CB, Lee J-H, Kim JH, et al. Development of a 3D subcutaneous construct containing insulin-producing beta cells using bioprinting. *Bio-Des Manuf*. 2022;5(2): 265-276.
doi: 10.1007/s42242-021-00178-9

123. Enrico A, Voulgaris D, Ostmans R, et al. 3D microvascularized tissue models by laser-based cavitation molding of collagen. *Adv Mater (Deerfield Beach, Fla)*. 2022;34(11):e2109823.
doi: 10.1002/adma.202109823

124. Bai L, Zhou D, Li G, Liu J, Chen X, Su J. Engineering bone/cartilage organoids: strategy, progress, and application. *Bone Res*. 2024;12(1):66.
doi: 10.1038/s41413-024-00376-y

125. O'Connor C, Brady E, Zheng Y, Moore E, Stevens KR. Engineering the multiscale complexity of vascular networks. *Nat Rev Mater*. 2022;7(9):702-716.
doi: 10.1038/s41578-022-00447-8

126. Michael S, Sorg H, Peck CT, et al. Tissue engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skin fold chamber in mice. *PLoS One*. 2013;8(3):e57741.
doi: 10.1371/journal.pone.0057741

127. Rioux G, Simard M, Morin S, Lorthois I, Guérin SL, Pouliot R. Development of a 3D psoriatic skin model optimized

for infiltration of IL-17A producing T cells: focus on the crosstalk between T cells and psoriatic keratinocytes. *Acta Biomater*. 2021;136:210-222.
doi: 10.1016/j.actbio.2021.09.018

128. Shin JU, Abaci HE, Herron L, et al. Recapitulating T cell infiltration in 3D psoriatic skin models for patient-specific drug testing. *Sci Rep*. 2020;10(1):4123.
doi: 10.1038/s41598-020-60275-0

129. Lorthois I, Simard M, Morin S, Pouliot R. Infiltration of T cells into a three-dimensional psoriatic skin model mimics pathological key features. *Int J Mol Sci*. 2019;20(7):1670.
doi: 10.3390/ijms20071670

130. Gong L, Li J, Zhang J, et al. An interleukin-4-loaded bi-layer 3D printed scaffold promotes osteochondral regeneration. *Acta Biomater*. 2020;117:246-260.
doi: 10.1016/j.actbio.2020.09.039

131. Derman ID, Rivera T, Garriga Cerda L, et al. Advancements in 3D skin bioprinting: processes, bioinks, applications and sensor integration. *Int J Extrem Manuf*. 2025;7(1):012009.
doi: 10.1088/2631-7990/ad878c

132. Zhou Z, Pang Y, Ji J, et al. Harnessing 3D in vitro systems to model immune responses to solid tumours: a step towards improving and creating personalized immunotherapies. *Nat Rev Immunol*. 2024;24(1):18-32.
doi: 10.1038/s41577-023-00896-4

133. Zhao K-y, Du Y-x, Cao H-m, Su L-y, Su X-l, Li X. The biological macromolecules constructed Matrigel for cultured organoids in biomedical and tissue engineering. Article. *Colloids Surf B Biointerfaces*. 2025;247:114435.
doi: 10.1016/j.colsurfb.2024.114435

134. Di Piazza E, Pandolfi E, Cacciotti I, et al. Bioprinting technology in skin, heart, pancreas and cartilage tissues: progress and challenges in clinical practice. *Int J Environ Res Public Health*. 2021;18(20):10806.
doi: 10.3390/ijerph182010806

135. Wang Y, Li H, Zhang J, Chen M, Pan Y, Lou X. 3D bioprinting inner ear organ of corti organoids induce hair cell regeneration. *J Biomed Mater Res A*. 2025;113(3):e37892.
doi: 10.1002/jbm.a.37892

136. Shukla P, Yeleswarapu S, Heinrich MA, Prakash J, Pati F. Mimicking tumor microenvironment by 3D bioprinting: 3D cancer modeling. *Biofabrication*. 2022;14(3):6d11.
doi: 10.1088/1758-5090/ac6d11

137. Lee Y, Min J, Kim S, Park W, Ko J, Jeon NL. Recapitulating the cancer-immunity cycle on a chip. *Adv Healthc Mater*. 2025;14(1):e2401927.
doi: 10.1002/adhm.202401927

138. Fan H, Demirci U, Chen P. Emerging organoid models: leaping forward in cancer research. *J Hematol Oncol*. 2019;12(1):142.
doi: 10.1186/s13045-019-0832-4

139. Mohamed E-T, Syed Arman R, Rasha B, et al. Unraveling the tumor microenvironment: insights into cancer metastasis and therapeutic strategies. *Cancer Lett*. 2024;591:216894.
doi: 10.1016/j.canlet.2024.216894

140. Wu X, Jin Z, Li B, et al. Deciphering of intra-tumoural heterogeneity and the interplay between metastasis-associated meta-program and myofibroblasts in gastric cancer. *Clin Transl Med*. 2025;15(5):e70319.
doi: 10.1002/ctm2.70319

141. Julia AL, Lance LM, Rakesh KJ. Compressive stresses in cancer: characterization and implications for tumour progression and treatment. *Nat Rev Cancer*. 2024;24(11):768-791.
doi: 10.1038/s41568-024-00745-z

142. Francisco B, Joana C, Maria M, João JS, Carla V. 3D bioprinting models for glioblastoma: from scaffold design to therapeutic application. *Adv Mater*. 2025;37(18):e2501994.
doi: 10.1002/adma.202501994

143. Yan L, Haijun C, Haitao C. Precision spatial control of tumor-stroma interactions in cancer models via 3D bioprinting for advanced research and therapy. *Adv Funct Mater*. 2025;2503391.
doi: 10.1002/adfm.202503391

144. Rong J, Xia L, Qian Z, et al. Anti-tumor immune potentiation targets-engineered nanobiotechnologies: design principles and applications. *Prog Mater Sci*. 2024;142:101230.
doi: 10.1016/j.pmatsci.2023.101230

145. Pengcheng Z, Xuanlong D, Weilu J, Kun F, Yewei Z. Engineered extracellular vesicles for targeted reprogramming of cancer-associated fibroblasts to potentiate therapy of pancreatic cancer. *Signal Transduct Target Ther*. 2024;9(1):1.
doi: 10.1038/s41392-024-01872-7

146. Hermida MA, Kumar JD, Schwarz D, et al. Three dimensional in vitro models of cancer: bioprinting multilineage glioblastoma models. *Adv Biol Regul*. 2020;75:100658.
doi: 10.1016/j.jbior.2019.100658

147. Sun Q, Tan SH, Chen Q, et al. Microfluidic formation of coculture tumor spheroids with stromal cells as a novel 3D tumor model for drug testing. *ACS Biomater Sci Eng*. 2018;4(12):4425-4433.
doi: 10.1021/acsbiomaterials.8b00904

148. Godier C, Baka Z, Lamy L, et al. A 3D bio-printed-based model for pancreatic ductal adenocarcinoma. *Diseases*. 2024;12(9):206.
doi: 10.3390/diseases12090206

149. Meng F, Meyer CM, Joung D, Vallera DA, McAlpine MC, Panoskaltsis-Mortari A. 3D bioprinted in vitro metastatic models via reconstruction of tumor microenvironments. *Adv Mater (Deerfield Beach, Fla)*. 2019;31(10):e1806899.
doi: 10.1002/adma.201806899

150. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer*. 2018;18(7):407-418.
doi: 10.1038/s41568-018-0007-6

151. Li Y, Liu J, Xu S, Wang J. 3D bioprinting: an important tool for tumor microenvironment research. *Int J Nanomed*. 2023;18:8039-8057. doi: 10.2147/ijn.S435845

152. Heinrich MA, Bansal R, Lammers T, Zhang YS, Michel Schiffelers R, Prakash J. 3D-bioprinted mini-brain: a glioblastoma model to study cellular interactions and therapeutics. *Adv Mater (Deerfield Beach, Fla)*. 2019;31(14):e1806590. doi: 10.1002/adma.201806590

153. Zhou X, Zhu W, Nowicki M, et al. 3D bioprinting a cell-laden bone matrix for breast cancer metastasis study. *ACS Appl Mater Interfaces*. 2016;8(44):30017-30026. doi: 10.1021/acsami.6b10673

154. Hughes AM, Kolb AD, Shupp AB, Shine KM, Bussard KM. Printing the pathway forward in bone metastatic cancer research: applications of 3D engineered models and bioprinted scaffolds to recapitulate the bone-tumor niche. *Cancers (Basel)*. 2021;13(3):507. doi: 10.3390/cancers13030507

155. Mazzocchi A, Soker S, Skardal A. 3D bioprinting for high-throughput screening: drug screening, disease modeling, and precision medicine applications. *Appl Phys Rev*. 2019;6(1):011302. doi: 10.1063/1.5056188

156. Kim J, Jang J, Cho D-W. Recapitulating the cancer microenvironment using bioprinting technology for precision medicine. *Micromachines*. 2021;12(9):1122. doi: 10.3390/mi12091122

157. Langer EM, Allen-Petersen BL, King SM, et al. Modeling tumor phenotypes in vitro with three-dimensional bioprinting. *Cell Rep*. 2019;26(3):608-623.e6. doi: 10.1016/j.celrep.2018.12.090

158. Calandrini C, Drost J. Normal and tumor-derived organoids as a drug screening platform for tumor-specific drug vulnerabilities. *STAR Protoc*. 2022;3(1):101079. doi: 10.1016/j.xpro.2021.101079

159. Wu P, Asada H, Hakamada M, Mabuchi M. Bioengineering of high cell density tissues with hierarchical vascular networks for ex vivo whole organs. *Adv Mater (Deerfield Beach, Fla)*. 2023;35(9):e2209149. doi: 10.1002/adma.202209149

160. Bjerring JS, Khodour Y, Peterson EA, Sachs PC, Bruno RD. Intercellular mitochondrial transfer contributes to microenvironmental redirection of cancer cell fate. *FEBS J*. 2025;292(9):2306-2322. doi: 10.1111/febs.70002

161. Khan AO, Rodriguez-Romera A, Reyat JS, et al. Human bone marrow organoids for disease modeling, discovery, and validation of therapeutic targets in hematologic malignancies. Article. *Cancer Discov*. 2023;13(2):364-385. doi: 10.1158/2159-8290.Cd-22-0199

162. Chen H, Wu Z, Gong Z, et al. Acoustic bioprinting of patient-derived organoids for predicting cancer therapy responses. *Adv Healthc Mater*. 2022;11(13):2102784. doi: 10.1002/adhm.202102784

163. Choi Y-m, Lee H, Ann M, Song M, Rheey J, Jang J. 3D bioprinted vascularized lung cancer organoid models with underlying disease capable of more precise drug evaluation. *Biofabrication*. 2023;15(3):034104. doi: 10.1088/1758-5090/acd95f

164. Jungeun K, Hoe Suk K, Ga Yeon K, et al. Abstract P5-02-02: development of automated 3d high-throughput drug screening platform for patient-derived breast cancer organoids. *Cancer Res*. 2022;82:2. doi: 10.1158/1538-7445.sabcs21-p5-02-02

165. Hou S, Tiriac H, Sridharan BP, et al. Advanced development of primary pancreatic organoid tumor models for high-throughput phenotypic drug screening. *SLAS Discov*. 2018;23(6):574-584. doi: 10.1177/2472555218766842

166. Arutyunyan I, Jumaniyazova E, Makarov A, Fatkhudinov T. In vitro models of head and neck cancer: from primitive to most advanced. *J Pers Med*. 2023;13(11):1575. doi: 10.3390/jpm13111575

167. Azhakesan A, Kern J, Mishra A, et al. 3D bioprinted head and neck squamous cell carcinoma (HNSCC) model using tunicate derived nanocellulose (NC) bioink. *Adv Healthc Mater*. 2025;14(7):e2403114. doi: 10.1002/adhm.202403114

168. Baka Z, Godier C, Lamy L, et al. A coculture based, 3D bioprinted ovarian tumor model combining cancer cells and cancer associated fibroblasts. *Macromol Biosci*. 2023;23(3):e2200434. doi: 10.1002/mabi.202200434

169. Tebon PJ, Wang B, Markowitz AL, et al. Drug screening at single-organoid resolution via bioprinting and interferometry. *Nat Commun*. 2023;14(1):3168. doi: 10.1038/s41467-023-38832-8

170. Nhan P, Jenny JH, Bobby T, et al. A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids. *Commun Biol*. 2019;2(1):1. doi: 10.1038/s42003-019-0305-x

171. Krendl FJ, Primavesi F, Oberhuber R, et al. The importance of preclinical models for cholangiocarcinoma drug discovery. *Expert Opin Drug Discov*. 2025;20(2):205-216. doi: 10.1080/17460441.2025.2457637

172. Joshi P, Nascimento HSD, Kang SY, et al. Dynamic culture of bioprinted liver tumor spheroids in a pillar/perfusion plate for predictive screening of anticancer drugs. *Biotechnol Bioeng*. 2025;122(4):995-1009. doi: 10.1002/bit.28924

173. Kalla J, Pfneissl J, Mair T, Tran L, Egger G. A systematic review on the culture methods and applications of 3D

tumoroids for cancer research and personalized medicine. *Cell Oncol*. 2025;48(1):1-26.
doi: 10.1007/s13402-024-00960-8

174. Maloney E, Clark C, Sivakumar H, et al. Immersion bioprinting of tumor organoids in multi-well plates for increasing chemotherapy screening throughput. *Micromachines*. 2020;11(2):208.
doi: 10.3390/mi11020208

175. Gong Z, Mao Y, Huang L, et al. Acoustic printing of patient-derived organoids that preserve tumor microenvironment for personalized drug screening. *Adv Mater Technol*. 2023;8(11):2201942.
doi: 10.1002/admt.202201942

176. Nieto D, Jiménez G, Moroni L, López-Ruiz E, Gálvez-Martín P, Marchal JA. Biofabrication approaches and regulatory framework of metastatic tumor-on-a-chip models for precision oncology. *Med Res Rev*. 2022;42(5):1978-2001.
doi: 10.1002/med.21914

177. Wang F, Song P, Wang J, et al. Organoid bioinks: construction and application. *Biofabrication*. 2024;16(3):3467c.
doi: 10.1088/1758-5090/ad467c

178. Wu Z, Liu R, Shao N, Zhao Y. Developing 3D bioprinting for organs-on-chips. *Lab Chip*. 2025;25(5):1081-1096.
doi: 10.1039/d4lc00769g

179. O'Connor CE, Zhang F, Neufeld A, et al. Bioprinted platform for parallelized screening of engineered microtissues in vivo. *Cell Stem Cell*. 2025;1;32(5):838-853.e6.
doi: 10.1016/j.stem.2025.03.002

180. Capeling MM, Czerwinski M, Huang S, et al. Nonadhesive alginate hydrogels support growth of pluripotent stem cell-derived intestinal organoids. *Stem Cell Rep*. 2019;12(2):381-394.
doi: 10.1016/j.stemcr.2018.12.001

181. Baptista LS, Porrini C, Kronemberger GS, Kelly DJ, Perrault CM. 3D organ-on-a-chip: the convergence of microphysiological systems and organoids. *Front Cell Dev Biol*. 2022;10:1043117.
doi: 10.3389/fcell.2022.1043117

182. Bengtsson A, Andersson R, Rahm J, Ganganna K, Andersson B, Ansari D. Organoid technology for personalized pancreatic cancer therapy. *Cell Oncol (Dordr)*. 2021;44(2):251-260.
doi: 10.1007/s13402-021-00585-1

183. Mahdavi R, Hashemi-Najafabadi S, Ghiass MA, et al. Design, fabrication, and characterization of a user-friendly microfluidic device for studying liver zonation-on-chip (ZoC). *Biomed Microdevices*. 2025;27(1):8.
doi: 10.1007/s10544-025-00738-1

184. Myszczyszyn A, Muench A, Lehmann V, et al. A hollow fiber membrane-based liver organoid-on-a-chip model for examining drug metabolism and transport. *Biofabrication*. 2025;17(2):206.
doi: 10.1088/1758-5090/adc3ce

185. Zheng F, Xiao Y, Liu H, Fan Y, Dao M. Patient-specific organoid and organ-on-a-chip: 3D cell-culture meets 3D printing and numerical simulation. *Adv Biol (Weinh)*. 2021;5(6):e2000024.
doi: 10.1002/adbi.202000024

186. Park B, Park J, Han S, et al. Advances in organoid-on-a-chip for recapitulation of human physiological events. *Mater Today*. 2025;84:75-94.
doi: 10.1016/j.mattod.2025.02.002

187. Tonon F, Giobbe GG, Zambon A, et al. In vitro metabolic zonation through oxygen gradient on a chip. *Sci Rep*. 2019;9(1):13557.
doi: 10.1038/s41598-019-49412-6

188. McCarty WJ, Usta OB, Yarmush ML. A microfabricated platform for generating physiologically-relevant hepatocyte zonation. *Sci Rep*. 2016;6(1):26868.
doi: 10.1038/srep26868

189. Mitani S, Takayama K, Nagamoto Y, et al. Human ESC/iPSC-derived hepatocyte-like cells achieve zone-specific hepatic properties by modulation of WNT signaling. *Mol Ther*. 2017;25(6):1420-1433.
doi: 10.1016/j.ymthe.2017.04.006

190. Wang Q, Liu J, Yin W, et al. Microscale tissue engineering of liver lobule models: advancements and applications. *Front Bioeng Biotechnol*. 2023;11:1303053.
doi: 10.3389/fbioe.2023.1303053

191. Saw TB, Doostmohammadi A, Nier V, et al. Topological defects in epithelia govern cell death and extrusion. *Nature*. 2017;544(7649):212-216.
doi: 10.1038/nature21718

192. Gupta K, Ng IC, Balachander GM, et al. Bile canaliculi contract autonomously by releasing calcium into hepatocytes via mechanosensitive calcium channel. *Biomaterials*. 2020;259:120283.
doi: 10.1016/j.biomaterials.2020.120283

193. Warmflash A, Sorre B, Etoc F, Siggia ED, Brivanlou AH. A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. *Nat Methods*. 2014;11(8):847-54.
doi: 10.1038/nmeth.3016

194. Kang R, Park S, Shin S, Bak G, Park JC. Electrophysiological insights with brain organoid models: a brief review. *BMB Rep*. 2024;57(7):311-317.
doi: 10.5483/BMBRep.2024-0077

195. Zhou J, Vijayavenkataraman S. 3D-printable conductive materials for tissue engineering and biomedical applications. *Bioprinting*. 2021;24:e00166.
doi: 10.1016/j.bprint.2021.e00166

196. Liu P. *3D da yin wei liu kong xin pian xi bao fen xi ping tai de gou jian ji qi ying yong [Construction and Application of a 3D-Printed Microfluidic Chip-Based Cell Analysis Platform]* [dissertation]. Shandong Normal University; 2023. Accessed May 20, 2025

https://d.wanfangdata.com.cn/thesis/vChhUaGVzaXNOZXdTMjAyNDA5MjAxNTE3MjUSCUQwMzAyNDMyOBoIeXRhZmhk ZXM%3D.Accessed XXX.

197. Mai S, Inkielewicz-Stepniak I. Graphene oxide nanoparticles and organoids: a prospective advanced model for pancreatic cancer research. *Int J Mol Sci*. 2024;25(2):1066. doi: 10.3390/ijms25021066

198. Salmon I, Grebenyuk S, Abdel Fattah AR, et al. Engineering neurovascular organoids with 3D printed microfluidic chips. *Lab Chip*. 2022;22(8):1615-1629. doi: 10.1039/d1lc00535a

199. Park YG, Kim S, Min S, et al. Soft 3D bioelectrodes for intraorganoid signal monitoring in cardiac models. *Nano Lett*. 2025;25(16):6481-6490. doi: 10.1021/acs.nanolett.5c00069

200. Lee S, Chung WG, Jeong H, et al. Electrophysiological analysis of retinal organoid development using 3D microelectrodes of liquid metals. *Adv Mater (Deerfield Beach, Fla)*. 2024;36(35):e2404428. doi: 10.1002/adma.202404428

201. Dong K, Liu WC, Su Y, et al. Scalable electrophysiology of millimeter-scale animals with electrode devices. *BME Front*. 2023;4:0034. doi: 10.34133/bmef.0034

202. Acha C, George D, Diaz LC, et al. Neuromodulation in neural organoids with shell MEAs. *bioRxiv*. 2025. doi: 10.1101/2025.02.18.637712

203. Saleh MS, Ritchie SM, Nicholas MA, et al. CMU array: a 3D nanoprinted, fully customizable high-density microelectrode array platform. *Sci Adv*. 2022;8(40):eabj4853. doi: 10.1126/sciadv.abj4853

204. Patel D, Shetty S, Acha C, et al. Microinstrumentation for brain organoids. *Adv Healthc Mater*. 2024;13(21):e2302456. doi: 10.1002/adhm.202302456

205. Li TL, Liu Y, Forro C, et al. Stretchable mesh microelectronics for the biointegration and stimulation of human neural organoids. *Biomaterials*. 2022;290:121825. doi: 10.1016/j.biomaterials.2022.121825

206. Mao M, Han K, Gao J, et al. Engineering highly aligned and densely populated cardiac muscle bundles via fibrin remodeling in 3D-printed anisotropic microfibrous lattices. *Adv Mater (Deerfield Beach, Fla)*. 2025;37(9):e2419380. doi: 10.1002/adma.202419380

207. Zilinskaite N, Shukla RP, Baradoke A. Use of 3D printing techniques to fabricate implantable microelectrodes for electrochemical detection of biomarkers in the early diagnosis of cardiovascular and neurodegenerative diseases. *ACS Meas Sci Au*. 2023;3(5):315-336. doi: 10.1021/acsmeasuresciau.3c00028

208. Kalmykov A, Huang C, Bliley J, et al. Organ-on-e-chip: three-dimensional self-rolled biosensor array for electrical interrogations of human electrogenic spheroids. *Sci Adv*. 2019;5(8):eaax0729. doi: 10.1126/sciadv.aax0729

209. Spedicati M, Tivano F, Zoso A, et al. 3D bioartificial stretchable scaffolds mimicking the mechanical hallmarks of human cardiac fibrotic tissue. *Int J Bioprint*. 2024;10(3):2247. doi: 10.36922/ijb.2247

210. Vashistha R, Kumar P, Dangi AK, Sharma N, Chhabra D, Shukla P. Quest for cardiovascular interventions: precise modeling and 3D printing of heart valves. *J Biol Eng*. 2019;13:12. doi: 10.1186/s13036-018-0132-5

211. Chen A, Su J, Li Y, et al. 3D/4D printed bio-piezoelectric smart scaffolds for next-generation bone tissue engineering. *Int J Extreme Manuf*. 2023;5(3):8. doi: 10.1088/2631-7990/acd88f

212. Simonneau C, Duschmalé M, Gavrilov A, et al. Investigating receptor-mediated antibody transcytosis using blood-brain barrier organoid arrays. *Fluids Barriers CNS*. 2021;18(1):43. doi: 10.1186/s12987-021-00276-x

213. Shen C, Zhang ZJ, Li XX, et al. Intersection of nanomaterials and organoids technology in biomedicine. *Front Immunol*. 2023;14:1172262. doi: 10.3389/fimmu.2023.1172262

214. Paone LS, Benmassaoud MM, Curran A, Vega SL, Galie PA. A 3D-printed blood-brain barrier model with tunable topology and cell-matrix interactions. *Biofabrication*. 2023;16(1):260. doi: 10.1088/1758-5090/ad0260

215. Marino A, Tricinci O, Battaglini M, et al. A 3D real-scale, biomimetic, and biohybrid model of the blood-brain barrier fabricated through two-photon lithography. *Small*. 2018;14(6):2959. doi: 10.1002/smll.201702959

216. Carton F, Malatesta M. In vitro models of biological barriers for nanomedical research. *Int J Mol Sci*. 2022;23(16):8910. doi: 10.3390/ijms23168910

217. Sharma A, Fernandes DC, Reis RL, et al. Cutting-edge advances in modeling the blood-brain barrier and tools for its reversible permeabilization for enhanced drug delivery into the brain. *Cell Biosci*. 2023;13(1):137. doi: 10.1186/s13578-023-01079-3

218. Reina-Mahecha A, Beers MJ, van der Veen HC, Zuhorn IS, van Kooten TG, Sharma PK. A review of the role of bioreactors for iPSCs-based tissue-engineered articular cartilage. *Tissue Eng Regen Med*. 2023;20(7):1041-1052. doi: 10.1007/s13770-023-00573-6

219. Xiu Z, Yang Q, Xie F, Han F, He W, Liao W. Revolutionizing digestive system tumor organoids research: exploring

the potential of tumor organoids. *J Tissue Eng*. 2024;15:20417314241255470.
doi: 10.1177/20417314241255470

220. Labour MN, Le Guilcher C, Aid-Launais R, et al. Development of 3D hepatic constructs within polysaccharide-based scaffolds with tunable properties. *Int J Mol Sci*. 2020;21(10):3644.
doi: 10.3390/ijms21103644

221. Shin J, Kang R, Hyun K, et al. Machine learning-enhanced optimization for high-throughput precision in cellular droplet bioprinting. Adv Sci (Weinh). 2025:12(20):e2412831.
doi: 10.1002/advs.202412831

222. Hwang HH, You S, Ma X, et al. High throughput direct 3D bioprinting in multiwell plates. *Biofabrication*. 2021;13(2):2200434.
doi: 10.1088/1758-5090/ab89ca

223. Hu W, Cao M, Liao L, et al. An automated digital microfluidic system based on inkjet printing. *Micromachines (Basel)*. 2024;15(11):2247.
doi: 10.3390/mi15111285

224. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: Construction, analysis, and application. *Bioact Mater*. 2024;31:525-548.
doi: 10.1016/j.bioactmat.2023.09.005

225. Ear PH, Marinoni I, Dayton T, et al. NET models meeting 2024 white paper: the current state of neuroendocrine tumour research models and our future aspirations. *Endocr Oncol*. 2024;4(1):e240055.
doi: 10.1530/eo-24-0055

226. Ma W, Lu H, Xiao Y, Wu C. Advancing organoid development with 3D bioprinting. *OR*. 2025;1(1):40004.
doi: 10.36922/or025040004