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3D-bioprinted tumor organoids enable programmable tumor microenvironment reconstruction for precision oncology

Running title: 3D-bioprinted tumor organoids

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Abstract

The high heterogeneity and complex cellular interactions within the tumor microenvironment (TME) pose a fundamental challenge to both cancer research and drug development. Conventional in vitro models fail to faithfully recapitulate the pathological features of tumors, thereby limiting the translation of basic research findings into clinical practice. Although patient-derived tumor organoids preserve the genetic and structural hallmarks of primary tumors, they are inherently constrained by limited control over spatial cellular organization, insufficient recapitulation of the biomimetic microenvironment, and a lack of model standardization. Three-dimensional (3D) bioprinting, characterized by precise spatial manipulation and multi-material deposition, offers a transformative strategy to address these limitations by converting key TME variables into programmable design parameters. Through the rational design of bioinks and optimization of printing parameters, bioprinting enables the programmable patterning of cells, extracellular matrix components, and bioactive factors, substantially enhancing the structural reproducibility, physiological relevance, and functional integrity of tumor organoids. This review systematically summarizes recent advances in bioprinted tumor organoids, with a focus on mainstream bioprinting technologies, the regulation of key process parameters, bioink design strategies for TME reconstruction, and the complete pipeline of organoid fabrication. We further delineate the translational impact of these models across personalized medicine, high-throughput drug discovery, immunotherapy evaluation, and the mechanistic dissection of drug resistance. We also discuss the current challenges in technical reproducibility, scalable manufacturing, ethical governance, and regulatory compliance. Finally, we outline future directions, including multimodal technological integration, four-dimensional (4D) bioprinting, tumor-on-a-chip platforms, and interdisciplinary innovation. Collectively, the convergence of bioprinting and tumor organoid technologies offers a robust platform for constructing highly biomimetic tumor models, dissecting tumor biology, and advancing precision oncology. This programmable reconstruction paradigm may help bridge the gap between basic cancer research, preclinical drug testing, and clinically actionable decision-making.

Keywords: Tumor organoids; Tumor microenvironment; Precision medicine; Drug screening; Immunotherapy

1. Introduction

A major challenge in modern oncology is the high heterogeneity of tumors and their dynamic interaction with the microenvironment. Traditional two-dimensional (2D) cell culture, while easy to establish and low in cost, fails to recapitulate *in vivo* conditions. Specifically, 2D culture alters cell morphology, differentiation status, and signaling pathway activity. These models fail to replicate the three-dimensional (3D) structure of tumor tissues, cellular polarity, and spatial interactions between cells and the extracellular matrix (ECM). Consequently, they remain limited in simulating tumor behavior and predicting drug responses, which restricts their ability to reflect tumor progression and clinical treatment outcomes¹⁻⁵.

To overcome these limitations, 3D tumor organoid technology has advanced rapidly, offering a more physiologically relevant platform for cancer research. Patient-derived tumor organoids (PDOs) are self-organized 3D cultures established from patient tumor tissues or cells. They largely preserve the genetic features and histological architecture of parental tumors while retaining some tumor microenvironment-related characteristics³. These models can be used to monitor tumor cell growth, invasion, and drug responses *in vitro*, providing physiologically relevant platforms for studying tumor initiation, progression, and therapy resistance^{4,6}. Furthermore, PDOs serve as an efficient, reproducible platform for personalized drug screening^{7,8} (**Figure 1**).

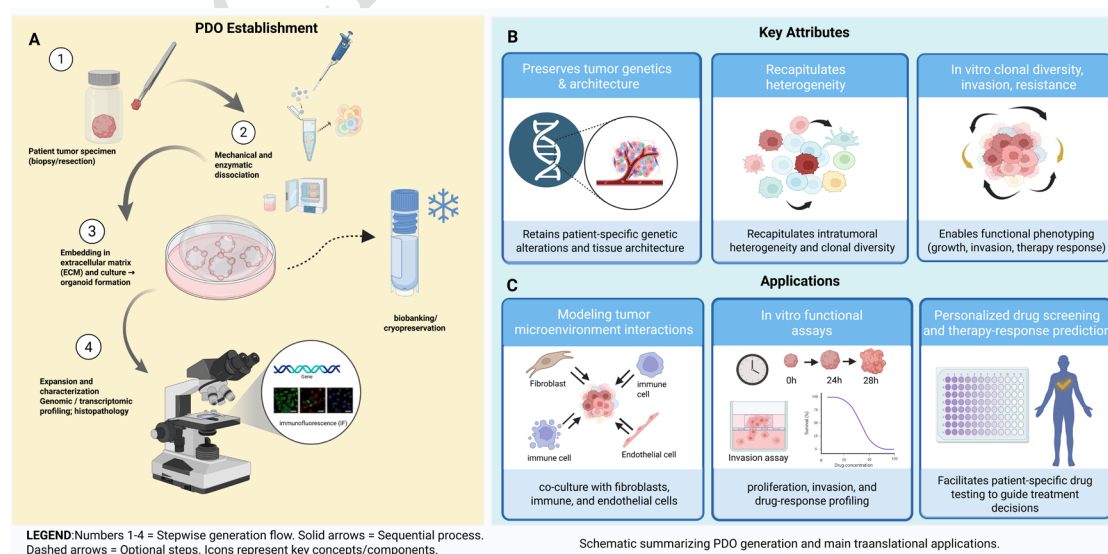


Figure 1. Generation workflow, key characteristics and main applications of PDOs. **A. Basic**

workflow for PDOs construction: (1) Acquisition of patient-derived tumor tissues (via biopsy or surgical resection); (2) Embedding of cell masses into ECM for 3D culture to promote organoid formation; (3) Diagnosis and characterization of the established organoids, including genomic/transcriptomic profiling and histopathological examination; (4) Morphological observation via imaging and electron microscopy, with concurrent cryopreservation and downstream analysis performed as required. **B. Core biological characteristics of PDOs:** Preservation of the genetic features and tissue architecture of the patient's primary tumor; faithful recapitulation of tumor heterogeneity; support for in vitro investigations into clonal diversity, invasion, and drug resistance of tumors. **C. Primary applications of PDOs:** Simulation of multicellular interactions within the tumor microenvironment (TME); performance of in vitro functional assays for tumor cell proliferation, invasion, and drug response; use in personalized drug screening and therapeutic response prediction, which serves as a robust platform for precision oncology.

Despite these advances, organoid construction still faces challenges in standardization, structural reproducibility, and microenvironment recreation. The self-assembly of conventional organoids is partly stochastic, which can lead to limited structural control and batch-to-batch variability. The absence of functional vascular networks limits organoid size because oxygen and nutrients can only diffuse over short distances. It also prevents accurate modeling of vascular drug delivery. Moreover, current organoid systems often do not fully capture the spatial heterogeneity of the in vivo microenvironment, including physical cues such as matrix stiffness gradients and oriented cell alignment^{9,10}. Bioprinting is a 3D biofabrication strategy that helps overcome these limitations by precisely depositing cells, biomaterials, and bioactive factors in predefined spatial patterns to generate organized 3D constructs¹¹. This technology further supports multi-cell co-culture and the functionalization of biomimetic ECM, substantially improving tumor model functionality^{12,13}. Recent advances in volumetric bioprinting and FRESH 2.0 suspension printing have enabled the rapid fabrication of centimeter-scale living constructs, with some approaches producing vascularized tissue models within minutes. The convergence of bioprinting and organoid technologies thus provides a powerful platform for constructing physiologically relevant TME models with controlled spatial heterogeneity and dynamic cellular interactions^{14,15}.

Building on previous reviews of 3D bioprinted tumor models, tumor organoids, and tumor-on-a-chip systems, we provide a systematic review of bioprinting for tumor organoid construction and evaluate its applications in tumor biology and precision medicine. Rather than cataloging all possible combinations of bioprinting technologies and organoid models, this review conceptualizes 3D bioprinted tumor organoids as programmable TME reconstruction platforms, with a central thesis that the major value of bioprinting lies in programmable

reconstruction of the tumor microenvironment (TME). Compared with conventional self-organized organoids, bioprinted tumor organoids allow key TME variables to be predefined, tuned, and interrogated for their biological and clinical consequences. This programmability is achieved through predefined spatial architectures, tunable mechanical and biochemical cues, and perfusable vascular networks. Accordingly, we evaluate this field from four interconnected perspectives: engineering controllability, biological fidelity, predictive value, and translational readiness. Specifically, we first evaluate how mainstream and emerging bioprinting approaches differ in their capacity to program spatial resolution, cell viability, construct scale, and vascular architecture. We then propose a hierarchical bioink design framework as the material basis of programmability, spanning ECM mimicry, functional responsiveness, and vascular integration. Next, we compare the evidence for drug-sensitivity prediction across different technological platforms, with particular attention to whether programmable TME reconstruction improves predictive value beyond conventional PDO systems. Finally, we discuss key challenges, including batch-to-batch consistency, limited clinical validation, and the need to determine whether programmable features confer measurable advantages over conventional organoids. We also outline future directions, such as Good Manufacturing Practice (GMP)-compliant automation, artificial intelligence (AI)-assisted quality control, and spatial multi-omics integration. Together, these analyses provide a conceptual and technical foundation for the further development of bioprinted tumor organoid platforms.

2. Bioprinting Technology Platform for Tumor Organoids

2.1 Principles and Classification of Bioprinting Technology

3D bioprinting is the core technology for the precise construction of tumor organoids. Based on their working principles, bioprinting techniques can be broadly categorized into extrusion-based, inkjet-based, and photopolymerization-based printing, alongside other emerging methods. Each approach involves trade-offs among resolution, cell viability, material compatibility, and fabrication scale. Therefore, the printing strategy should be selected according to the intended application. In this review, bioprinting technologies are not compared merely as fabrication tools, but are evaluated according to their capacity to program multicellular organization, matrix architecture, biochemical patterning, and vascular-like structures (**Figure 2**).

Bioprinting Mechanisms

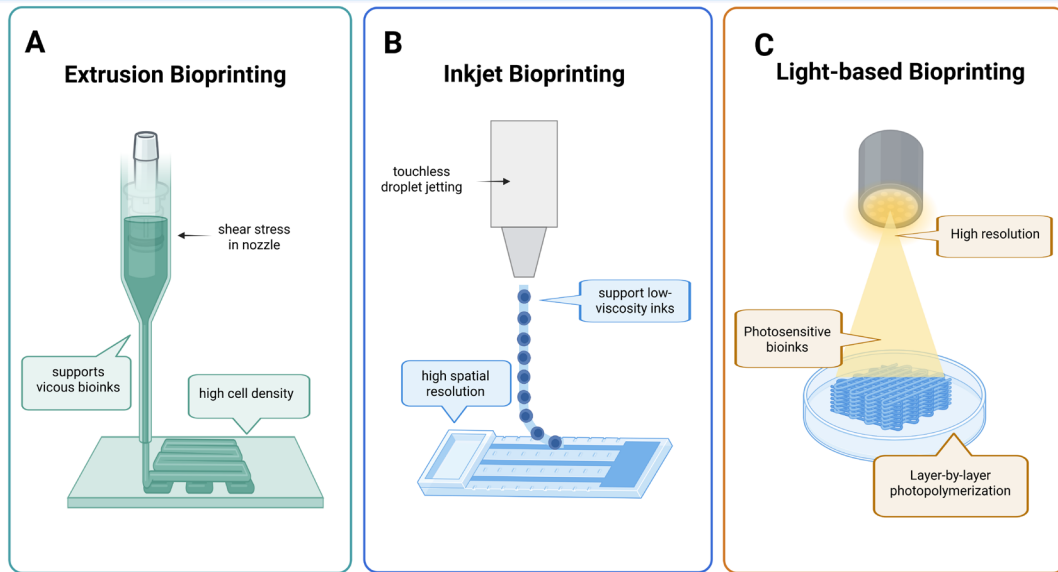


Figure 2. Schematic diagram of three mainstream bioprinting technologies. This figure illustrates the working principles and representative performance differences of extrusion-based bioprinting, inkjet-based bioprinting, and photopolymerization-based bioprinting. Extrusion-based bioprinting is compatible with high-viscosity bioinks, yet may induce high shear stress during the printing process; inkjet-based bioprinting utilizes low-viscosity bioinks to achieve high spatial resolution, but may cause thermal or shear-induced cell damage; photopolymerization-based bioprinting delivers superior high spatial resolution, yet it is prone to interlayer stress accumulation and structural deformation.

Extrusion-based bioprinting is the most prevalent bioprinting technique. It continuously extrudes bioink or cell suspensions through a nozzle via mechanical force, depositing material layer-by-layer to construct 3D structures. This method accommodates high-viscosity bioinks, making it suitable for fabricating large-volume, architecturally complex tissues with high cell density. It also supports the co-printing of multiple cell types and materials¹⁶. However, shear stress during extrusion can reduce cell viability and impair cellular function. Therefore, printing parameters, including extrusion pressure, nozzle diameter, and printing speed, as well as bioink formulations, should be optimized¹⁷.

Inkjet bioprinting is based on a non-contact droplet ejection principle, in which thermal, piezoelectric, or bubble-driven mechanisms are used to deposit bioink droplets onto a substrate or scaffold. This technique is mainly suitable for low-viscosity bioinks and is commonly used to print cell suspensions, growth factors, and other bioactive agents. It provides high spatial resolution and positioning accuracy, allowing precise cell placement with relatively low mechanical stress-induced damage¹⁸. Thermal inkjet approaches have been used to co-deposit

endothelial cells and biomaterials, supporting the formation of microvascular-like structures. This suggests that inkjet bioprinting can balance spatial positioning with the preservation of cellular function¹⁹. However, inkjet bioprinting requires strict control of bioink viscosity and droplet stability, including resistance to drying during jetting and storage. These requirements limit the range of printable materials²⁰.

Photopolymerization-based bioprinting uses light of specific wavelengths to rapidly crosslink and polymerize photosensitive bioinks into defined structures. Key sub-technologies include stereolithography, digital light processing, and computed axial lithography. In this process, a bioink formulated with photoinitiators and photosensitive biomaterials is exposed to a computer-controlled light pattern. Photoinitiators absorb light energy and generate reactive species that initiate biomaterial crosslinking. This process forms stable 3D constructs through either layer-by-layer fabrication or volumetric curing. Its main advantages include high spatial resolution for reproducing tissue microarchitecture and shortened fabrication time through area-based exposure or volumetric curing. These processes can be performed under mild, cell-compatible conditions, helping to preserve cell viability. This technology also offers strong material adaptability and structural controllability. However, it demands bioinks with appropriate viscosity and high optical transparency, as high-viscosity or opaque inks exhibit poor printability. Furthermore, photopolymerization printing involves a trade-off between resolution and build volume: current resolution is insufficient for nanoscale precision, while large-scale constructs are prone to interlayer stress accumulation, structural deformation, and cell death²¹⁻²⁴.

Beyond the mainstream technologies discussed above, a series of emerging methods are pushing the physical boundaries of conventional bioprinting, opening new frontiers in the field. These approaches further extend the programmability of tumor organoid construction by improving shape fidelity, spatial control, cell preservation, and construct scale. Suspension bioprinting prevents the gravitational collapse of soft bioinks, a major limitation of conventional extrusion bioprinting, by depositing the bioink within a yield-stress support bath. As exemplified by the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) technique, this approach uses a thermoreversible support bath to provide immediate mechanical support. This allows soft hydrogels that cannot maintain their shape in air to be printed into complex 3D structures with high fidelity. Its significance lies in shifting the paradigm of resolution enhancement from simply reducing nozzle size to the synergistic design of material, environment, and curing²⁵. FRESH 2.0 improves support-bath design and rheological tunability, enabling the embedded printing of soft ECM-based bioinks, such as unmodified collagen. This approach supports the fabrication of complex and perfusable tissue-like constructs with improved resolution and shape fidelity²⁶. In situ bioprinting aims to deposit bioinks directly

into tissue defects or target sites *in vivo*, offering a potential strategy for on-site tissue repair and regeneration. By integrating patient-specific medical imaging data with robot-assisted printing systems, this strategy may support more precise and personalized intraoperative fabrication. However, these systems remain largely under development²⁷. Imaging-guided deep-tissue *in vivo* sound printing further extends the concept of *in situ* bioprinting. It enables noninvasive and spatially controlled formation of functional biomaterials within deep tissues under real-time ultrasound guidance, suggesting potential applications in localized drug delivery and tissue repair²⁸. Acoustic bioprinting uses focused sound waves to position cells and biomaterials without physical contact. This non-contact strategy can support high cell viability and spatial resolution²⁹. By reducing shear stress, this method may help preserve sensitive cells and support the construction of multicellular structures. Combined numerical and experimental studies have shown precise patterning at both millimeter and single-cell scales, while maintaining cell morphology, proliferation, and differentiation capacity³⁰. Furthermore, owing to its nozzle-free and contactless design, acoustic bioprinting enables the spatial arrangement of multiple cell types and supports cell–cell interactions, thereby helping to reproduce key features of TME organization³¹. Laser-assisted bioprinting uses laser pulses to generate transient bubbles that propel bioink droplets, allowing high-resolution cellular patterning with relatively high cell viability³². Its nozzle-free design avoids nozzle clogging and reduces mechanical stress, making it suitable for shear-sensitive cell types. Integration with microfluidic platforms can further improve multi-material and multi-cell-type printing capabilities³³. For instance, embedded bioprinting addresses the gravitational instability of soft bioinks by depositing them within a supportive matrix, enabling the fabrication of complex 3D structures with improved shape fidelity³⁴. Collectively, these technologies expand the bioprinting toolkit and support the development of more diverse and functional tumor organoid models.

Volumetric bioprinting (VBP) has emerged as an important advance in light-based biofabrication. Unlike conventional layer-by-layer photopolymerization, VBP enables layer-free 3D curing within seconds to minutes by projecting dynamic light patterns from multiple angles into a rotating bioink reservoir. This volumetric curing strategy reduces interlayer defects and stair-step effects, thereby improving fabrication speed while supporting cell-laden constructs with high cell density. Bernal et al. used VBP to fabricate centimeter-scale, metabolically active liver-like constructs using liver organoids as building blocks within seconds^{35,36}. Although VBP remains at an early stage in tumor modeling, its rapid and layer-free fabrication capacity may support the construction of larger and more patient-specific tumor models.

In summary, the emergence of these technologies has greatly expanded the repertoire of bioprinting methods, but has also introduced complexity in the selection process. From an engineering trade-off perspective, the main advantage of extrusion-based bioprinting lies in its ability to construct large-volume, multicellular models. However, shear stress-induced cell damage and relatively low resolution, typically on the order of hundreds of micrometers, remain major limitations. Optimization should focus on bioink rheology rather than simply reducing nozzle size. Inkjet bioprinting is suitable for high-precision patterning of cells, growth factors, and other bioactive agents. Photopolymerization-based bioprinting, particularly volumetric bioprinting, offers advantages in resolution and fabrication speed. However, the potential cytotoxicity of photoinitiators and the difficulty of multi-material printing limit its broader application. Suspension bioprinting, represented by FRESH 2.0, enables high-fidelity fabrication through a support bath. However, its relatively complex workflow makes it more suitable for shape-accurate replication of complex structures than for high-throughput screening. Acoustic bioprinting enables gentle single-cell-level positioning, but its relatively low throughput may restrict its use to specialized applications, such as rare cell pairing. Therefore, technology selection should be guided by the biological question being addressed and the programmable TME features required by the model. Different strategies prioritize different capabilities, such as multicellular construction, high-resolution vascular-like patterning, or complex soft-matrix fabrication. To provide a structured overview of this selection process, major bioprinting strategies are summarized according to their programmable capabilities, applicable TME features, representative applications, and practical limitations (**Table 1**). Future progress will likely depend on the integration of multiple printing strategies with intelligent process optimization and real-time monitoring. Such advances may further promote the development and clinical translation of bioprinted tumor organoid platforms.

Table 1: Technology–application decision matrix for programmable TME reconstruction in bioprinted tumor organoids

Bioprinting strategy	Main programmable capability	Suitable TME features to model	Best-matched applications in tumor organoids	Key limitations / decision caveats	Reference
Extrusion-based bioprinting	Continuous deposition of high-viscosity, cell-laden bioinks; supports large-volume and multi-material construction.	Multicellular organization; stromal compartments; tumor-ECM architecture; high-cell-density constructs.	Large-volume tumor models; drug-resistance models; multicellular TME reconstruction; patient-derived constructs requiring high cell density.	Shear stress may reduce cell viability and impair function; resolution is relatively limited and depends on pressure, nozzle size, speed, and bioink rheology.	[16,17]
Inkjet-based bioprinting	Non-contact droplet deposition of low-viscosity bioinks, cells, and bioactive factors.	Localized cell placement; growth-factor patterning; endothelial-cell deposition; early microvascular-like organization.	High-precision cell/factor positioning; small-volume screening platforms; patterning of vascular or biochemical cues.	Requires strict control of bioink viscosity and droplet stability; printable material range is limited; thermal or shear-related cell damage may occur.	[18-20]
Photopolymerization-based bioprinting	Light-controlled crosslinking of photosensitive bioinks with high spatial resolution and rapid fabrication.	Tissue microarchitecture; stiffness patterning; thin-walled structures; vascular-like channels; spatially defined ECM regions.	High-resolution vascularized constructs; microstructure-sensitive TME models; tumor models requiring fast and controllable curing.	Requires optically transparent and appropriately viscous bioinks; photoinitiator-related cytotoxicity and structural deformation may occur; multi-material printing remains challenging.	[21-24]
Suspension / embedded bioprinting	Deposition of soft bioinks within a yield-stress support bath to maintain shape fidelity.	Soft ECM-like matrices; complex 3D topology; patient-specific structures; perfusable tissue-like constructs.	Tumor organoids requiring soft hydrogels, collagen/ECM-like matrices, complex architectures, or shape-accurate reconstruction.	Workflow is relatively complex; support-bath removal and process standardization may limit high-throughput use.	[25,26,34]
Acoustic bioprinting	Nozzle-free, contactless positioning of cells or biomaterials using focused sound waves.	Gentle cell positioning; rare-cell pairing; multicellular interfaces; tumor-stroma or tumor-immune spatial interactions.	Patient-derived organoid assembly; fragile-cell patterning; precise construction of multicellular TME organization.	Throughput is relatively low; specialized equipment may restrict broad high-throughput application.	[29-31]
Laser-assisted bioprinting	Laser-induced droplet transfer for high-resolution cellular patterning without nozzle clogging.	High-resolution cellular positioning; shear-sensitive cell patterning; multi-cell-type arrangement.	Models requiring precise deposition of sensitive cells or high-resolution cell patterns.	Equipment and workflow complexity may limit routine use; integration with other platforms may be needed for complex multi-material models.	[32,33]
Volumetric bioprinting	Layer-free 3D curing within seconds to minutes using dynamic light projection.	Large-scale cell-laden constructs; organoid-laden hydrogels; reduced interlayer defects; rapid fabrication of patient-specific models.	Rapid fabrication of larger organoid-based constructs and high-cell-density tissue-like models.	Tumor-modeling applications remain at an early stage; optical and material constraints still limit broad use.	[35,36]

Abbreviations: TME, tumor microenvironment; ECM, extracellular matrix. Supporting references correspond to the manuscript reference list. The application-oriented decision logic is synthesized from the reported capabilities and limitations of each printing strategy.

2.2 Core Process Parameters for Bioprinting

The process parameters of 3D bioprinting should be considered as a coupled system involving materials, equipment, printing settings, and culture conditions, rather than as isolated variables. In the context of programmable TME reconstruction, these parameters determine whether predefined spatial patterns, mechanical cues, biochemical signals, and vascular-like architectures can be faithfully translated into living constructs. The main optimization objectives include geometric precision and resolution, cell viability and functional maintenance, and structural stability during long-term culture. These objectives are interdependent and should be balanced during process optimization³⁷.

Accuracy and resolution are often conflated but refer to distinct concepts. Accuracy describes how closely the printed construct matches the digital design, as reflected by dimensional and positional errors. In contrast, resolution denotes the smallest reproducible feature size, such as line width or layer thickness. Because soft materials may flow, fuse, collapse, or shrink after deposition, resolution cannot be adequately described by printer specifications alone. Therefore, increasing attention has been given to geometric fidelity, which describes the extent to which the intended design is reproduced in the final construct. Standardized metrics are needed to improve comparability across studies³⁸. In extrusion-based bioprinting, resolution is not determined by nozzle diameter alone. It also depends on yield stress, shear-thinning behavior, and rapid post-deposition recovery or crosslinking. In photopolymerization-based systems, resolution is mainly governed by optical and reaction-related factors. These include exposure dose, light absorption and scattering, radical diffusion, and reaction kinetics, which together determine the sharpness of the cured boundary. Biocompatible light-absorbing strategies, such as the use of food-grade dyes to suppress overcuring, can improve the fabrication of complex microvascular networks and thin-walled structures. These strategies may enhance geometric fidelity while imposing limited additional stress on cells³⁹.

Strategies for maintaining cell viability should extend beyond immediate post-printing survival to include proliferation, differentiation capacity, and stable gene expression and functional phenotypes. A major challenge in bioprinting is that living materials are highly sensitive to process-induced perturbations. Therefore, process optimization must balance geometric accuracy with biological performance³⁷. In extrusion-based bioprinting, a major risk arises from shear stress within the nozzle and near the nozzle exit⁴⁰. In photopolymerization-based bioprinting, improving initiation efficiency and enabling crosslinking at milder wavelengths and lower initiator concentrations are important strategies for reducing photochemical stress⁴¹. Water-soluble photoinitiators, such as lithium phenyl-2,4,6-

trimethylbenzoylphosphinate, show rapid polymerization kinetics, favorable cytocompatibility, and compatibility with visible-light photocuring. These properties support process designs with lower initiator doses, shorter exposure times, and better preservation of bioactivity⁴².

Long-term culture requires printed constructs to maintain morphology and mechanical integrity while undergoing controlled changes over time. This depends on the coordination of material degradation, ECM deposition, and tissue remodeling. Crosslinkable gelatin-based systems, such as gelatin methacryloyl (GelMA), provide cell-adhesive cues and support cell migration. They can offer initial structural support while allowing cell-mediated tissue remodeling over time⁴³. Sacrificial writing into functional tissue (SWIFT) creates perfusable channels by writing sacrificial templates within high-cell-density living matrices. This technique provides a scalable strategy to promote the maturation and functionalization of dense tissue constructs⁴⁴.

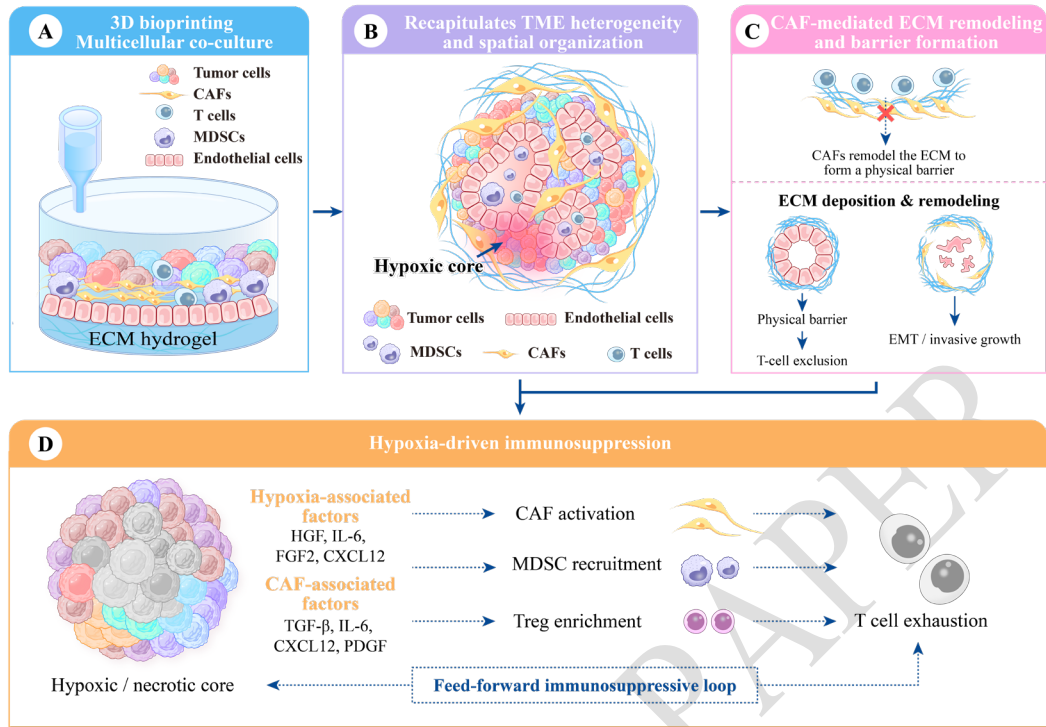
A central trade-off in current process optimization is that geometric accuracy, cell viability, and the fidelity of programmed TME features are difficult to optimize simultaneously. Extrusion-based methods often trade resolution for the ability to pattern multiple cell types. Photopolymerization-based methods provide high-resolution patterning but are more limited in multi-material printing. Suspension printing improves the shaping of soft materials but usually has lower throughput. Therefore, technology selection and process optimization should be guided by the key programmable features required for the target tumor microenvironment, such as spatial organization, mechanical gradients, biochemical signals, or vascular networks. Rather than seeking a single universal solution, researchers should match the printing strategy to the biological question and model requirements.

3. Bioprinting and Construction Workflows for Reconstructing the Tumor Microenvironment

3.1 Overview of TME

The TME is characterized by a highly heterogeneous, multicellular composition. Through mechanical, biochemical, and metabolic signaling, it regulates tumor proliferation, invasion, metastasis, and therapeutic response. Therefore, reconstructing key TME components in vitro is important for improving the physiological relevance and translational potential of tumor models^{14,45-47}. For bioprinted tumor organoids, these TME features should be viewed not only as biological components to be included, but also as programmable variables that can be spatially organized, mechanically tuned, and functionally interrogated.

Cellular diversity is a defining hallmark of the TME. Beyond cancer cells, the TME includes cancer-associated fibroblasts (CAFs), immune cells, and vascular endothelial cells. These cell types interact through soluble factor signaling, ECM-mediated mechanical regulation, and direct cell–cell contact. These interactions shape tumor biology and influence tumor initiation and progression^{48,49} (**Figure 3**). 3D organoid models can partially recapitulate the spatial organization and functional interactions of this cellular community through multicellular co-culture. For example, CAF-derived ECM components, including collagen IV, provide structural support for gland-like tumor organization. CAF-mediated matrix remodeling and paracrine signaling may also promote epithelial–mesenchymal transition (EMT)-like changes and invasive phenotypes in subsets of cancer cells⁵⁰. 3D organoid systems have also improved the modeling of the tumor immune microenvironment by incorporating or retaining immunosuppressive populations, such as myeloid-derived suppressor cells (MDSCs). This enables the study of T-cell suppression and immunotherapeutic responses⁵¹. In immune checkpoint inhibitor therapy, PD-L1 expression and the spatial organization of immune cell infiltration are important factors associated with treatment response. Accordingly, immune-competent 3D organoid models provide a useful platform for investigating tumor–immune spatial heterogeneity and evaluating immunotherapeutic responses⁵². Moreover, interactions between tumor cells and stromal cells, as illustrated by CAF transcriptional reprogramming in BRCA-mutated tumors, highlight how tumor genetic background can influence TME heterogeneity and therapeutic response⁵³. By controlling the ratios and spatial arrangement of distinct cell types, 3D bioprinting can help tumor organoids reproduce physical and biochemical interactions within the TME. This capability supports mechanistic studies of tumor initiation and progression and may facilitate the development of personalized therapeutic strategies.



3D bioprinted tumor organoids recapitulate TME heterogeneity, spatial organization, and multicellular crosstalk for mechanistic studies and therapy evaluation.

Figure 3. Multicellular organization and immunosuppressive crosstalk of the TME in 3D bioprinted tumor organoid models. (A) 3D bioprinting enables the construction of multicellular tumor organoids by spatially organizing tumor cells, cancer-associated fibroblasts (CAFs), T cells, myeloid-derived suppressor cells (MDSCs), and vascular endothelial cells within an ECM hydrogel matrix. (B) The resulting organoid model partially recapitulates key features of TME heterogeneity and spatial organization, including heterogeneous tumor cell clusters, stromal components, endothelial cells, immune cells, and the formation of hypoxic core regions. (C) CAF-mediated extracellular matrix remodeling contributes to ECM deposition and barrier formation, which can support gland-like tumor organization while restricting immune cell infiltration and promoting invasive tumor phenotypes. (D) Hypoxia-associated and CAF-associated factors collectively promote an immunosuppressive microenvironment by modulating stromal cells, suppressive immune populations, and T-cell dysfunction. These interactions may establish a feed-forward immunosuppressive loop that influences tumor progression and therapeutic response. Together, this schematic illustrates how 3D bioprinted multicellular organoid models can be used to reconstruct spatially organized tumor–stroma–immune interactions and to investigate mechanisms of TME-driven tumor progression and therapy resistance.

The metabolic and physical characteristics of the TME also influence tumor biology. Stromal cells, including fibroblasts and vascular endothelial cells, contribute to tumor

progression through ECM remodeling and angiogenesis⁵⁴⁻⁵⁷. In parallel, metabolic stressors such as hypoxia, acidic pH, and nutrient deprivation induce adaptive metabolic changes in tumor cells that support proliferation and metastasis^{58,59}. For example, under hypoxic conditions, tumor cells activate the hypoxia-inducible factor (HIF) pathway, which promotes glycolysis and lipid metabolism to support growth and stress adaptation⁶⁰. Endoplasmic reticulum stress, induced by metabolic perturbations and oncogenic signaling, can act as an integrative mechanism linking multiple TME-derived signals. It supports tumor cell adaptation and metastasis and can also reshape the immune landscape, thereby contributing to malignant progression⁶¹.

The TME influences drug responsiveness primarily through three mechanisms: immune modulation, restricted drug delivery, and metabolic reprogramming. First, the abundance and functional status of tumor-infiltrating lymphocytes, the expression of immune checkpoint molecules, and the accumulation of immunosuppressive cells are closely associated with the efficacy of immune checkpoint inhibitors^{62,63}. Second, stromal cell activation and ECM remodeling can impair drug penetration and intratumoral distribution, thereby contributing to resistance to chemotherapy and targeted therapy^{64,65}. Third, metabolic byproducts such as lactate can suppress immune cell function and reduce therapeutic activity, thereby promoting immune evasion and treatment resistance^{66,67}. Aberrant activation of specific signaling pathways, such as Notch3 upregulation in tumor cells, may further promote immunosuppressive cell infiltration and reduce therapeutic efficacy⁶⁸. Building on these mechanistic insights, new strategies are being developed to target or remodel the TME. Lifestyle interventions, including exercise, may enhance therapeutic responses by modulating inflammation and vascular function within the TME⁶⁹. Meanwhile, nanomedicine-based approaches and bioprinting technologies provide complementary tools for modulating or reconstructing the TME, with potential applications in drug screening and therapeutic optimization⁷⁰.

In summary, the compositional heterogeneity and functional complexity of the TME remain central challenges in tumor research and therapy. Traditional models often fail to fully capture these multidimensional interactions, limiting mechanistic studies and drug-response prediction. Therefore, developing in vitro models that better recapitulate the cellular diversity, spatial architecture, and dynamic functions of the TME is essential. In this context, the value of bioprinting lies not simply in incorporating more TME components, but in converting cellular composition, ECM organization, biochemical gradients, and vascular-like structures into controllable design parameters. Bioprinting enables the three-dimensional organization of multiple cell types and biomaterials, offering a promising strategy for tumor microenvironment reconstruction.

3.2 Bioink Systems: From Structural Support to Functional Biomimetics

Bioinks are essential for 3D bioprinting because their composition and physicochemical properties determine not only the fidelity, stability, and biological function of printed constructs, but also the extent to which TME features can be programmed. For tumor model fabrication, an ideal bioink should combine printability with the ability to preserve tumor cell phenotypes. Commonly used bioinks are predominantly based on natural and synthetic hydrogels. For example, collagen and hyaluronic acid can mimic key features of the tumor ECM, and process optimization can improve their printability and cytocompatibility. Alginate, gelatin methacryloyl (GelMA), and gelatin provide tunable mechanical properties and flexible crosslinking strategies, making them useful for supporting the structural requirements of 3D tumor organoid construction (Table 2).

3.2.1 Physical, Chemical, and Biological Properties

The performance of a bioink depends on multiple characteristics, including physicochemical, biological, mechanical, and printability-related properties. Among these, biocompatibility and printability are key attributes for practical application. Biocompatibility requires the bioink to be non-cytotoxic and to support cell survival, proliferation, and functional activity. Printability, in turn, encompasses rheological behavior, structural stability, and achievable resolution.

Rheological properties play a central role in bioprinting quality. They determine how bioinks deform and flow under applied forces, which affects filament continuity, structural stability, print resolution, and cell viability. Optimizing bioink rheology can reduce shear stress during extrusion, thereby limiting mechanical damage and improving post-printing cell viability and functional maintenance^{38,71}.

For instance, GL gel stability is mainly derived from electrostatic interactions between lactic acid and glycosaminoglycan nanoparticles, allowing self-assembly without support baths or chemical crosslinking. Its viscoelastic properties support shape retention and reduce diffusion or deformation of the deposited layer⁷². This shape fidelity shortens the initial transition zone in the deposition area and increases printing speed. However, it may also increase the risk of lateral buckling of printed filaments on the substrate⁷³. In alginate/CMC/TO-NFC composite hydrogels, increasing the TO-NFC concentration increases ink viscosity and yield stress, reduces filament spreading, and improves shape fidelity. Representative formulations have achieved cell survival rates above 86%, suggesting that rheological optimization can be achieved without compromising biocompatibility⁷⁴. The solid-like

behavior of the ink, such as storage modulus G' , and its yield stress are also important for maintaining printed morphology. For example, pre-crosslinked inks containing 15 mM calcium exhibit solid-like properties and suitable yield stress, facilitating the fabrication of dimensionally accurate and morphologically stable scaffolds⁷⁵. Bioink rheology affects not only flowability and shape fidelity during printing but also the behavior and viability of encapsulated cells by modulating the shear-stress environment. Therefore, optimizing key rheological parameters, such as shear viscosity, storage modulus, and yield stress, is important for balancing print fidelity and biocompatibility⁷¹. pH also regulates crosslinking kinetics and ink rheology. Adjusting pH can tune crosslinking rates and thereby improve printability and cell survival⁷⁶.

Different bioink components can influence rheological behavior. Polymer matrices, nanoparticles, crosslinkers, and extracellular vesicles regulate bioink viscoelasticity and structural stability. For example, incorporating methacrylated hyaluronic acid (MeHA) into gelatin combines the rapid photopolymerization capacity of MeHA with the cell-adhesive properties of gelatin. This combination can improve rheological behavior and printing accuracy while supporting cell proliferation and differentiation⁷⁷. The addition of polyhydroxybutyrate-polyhydroxyvalerate particles increases the storage modulus and yield stress of the ink, thereby enhancing the mechanical strength and morphological stability of printed scaffolds⁷⁵. Inorganic fillers, such as nanoclay, can improve rheological and mechanical properties and support cell proliferation, adhesion, and differentiation⁷⁸. Metal ions, including Al^{3+} and Fe^{3+} , form dynamic coordination bonds with functionalized polyethylene glycol (PEG) derivatives, allowing modulation of ink relaxation time and viscosity. These changes can affect print fidelity and cellular compatibility⁷⁹. Rheological modifiers such as Carbopol can impart shear-thinning behavior to polymers with poor extrudability, thereby expanding the range of printable materials⁸⁰. Finally, extracellular vesicles can be incorporated into bioinks to maintain suitable rheological properties while enabling the sustained release of bioactive molecules⁸¹.

Cell-supporting capability is a decisive factor governing the viability and functional maintenance of printed structures. Beyond adequate printability, the ink must provide a cell-friendly microenvironment conducive to survival, proliferation, and phenotypic retention. Cell viability serves as the most fundamental indicator of biocompatibility. Bioinks composed of GelMA and nano-cellulose, for instance, sustain over 90% viability in HepG2 liver cancer cells while preserving active metabolic function⁸². Likewise, incorporating graphene oxide into alginate/gelatin composite inks⁸³ or employing fish-derived gelatin electrospun fiber⁸⁴ markedly improves cell affinity, effectively supporting the adhesion, proliferation, and survival of adipose-derived stem cells.

The chemical crosslinking strategy directly impacts cell viability. For example,

horseradish peroxidase-mediated enzymatic crosslinking enables rapid gelation under mild conditions and maintains cell viability above 90%⁸⁵. Regulating gel stability via PEG crosslinking (PEGX) enhances both the survival of extruded cells and the mechanical integrity of the construct⁸⁶. Similarly, photoinitiator-based visible-light crosslinking strategies can achieve mechanical stability while maintaining cell viability⁸⁷. Gelatin-based inks can support osteoblast differentiation and functional gene expression by adjusting concentration, crosslinking density, or the alginate-to-gelatin ratio^{88,89}. Alginate-based inks modified with bioactive peptides, such as RGD, can improve cell adhesion, spreading, and proliferation, thereby helping to maintain cellular phenotypes⁹⁰.

Cell density is equally critical. High-cell-density inks promote intercellular communication and tissue-specific differentiation but pose greater printing challenges. To address this challenge, researchers have combined high-cell-density bioinks with biodegradable microgels or self-healing hydrogel scaffolds, enabling the fabrication of complex tissue structures while maintaining cellular function⁹¹.

Recent studies have explored multicomponent composite bioinks and dynamic crosslinking strategies to regulate cell organization and functional differentiation through mechanical and microenvironmental cues. Low-viscosity aqueous two-phase systems, for instance, enable high-speed printing while ensuring high cell viability and robust phenotypic maintenance⁹². Microfluidics-assisted multi-ink mixing offers additional avenues for orchestrating complex cellular behaviors⁹³. Furthermore, incorporating nanoparticles into polymer-based bioinks, together with dynamic covalent crosslinking, can improve the fabrication fidelity and mechanical stability of complex structures. This strategy also supports the functional maturation of chondrocytes⁹⁴.

3.2.2 Design of Tumor-Specific Inks

The evolution of bioinks is shifting from passive scaffolds toward programmable active materials⁹⁵. Unlike conventional tissue engineering bioinks that mainly provide structural support and promote differentiation, bioinks for TME modeling should allow dynamic interactions with cells. The continuous crosstalk between tumor cells and the ECM, including reciprocal remodeling, contributes to malignant behaviors such as invasion, metastasis, drug resistance, and immune evasion. Therefore, tumor-specific bioink design can be viewed as a stepwise strategy involving ECM biomimicry, dynamic responsiveness, and vascular integration. First, ECM stiffness, degradability, and cell-adhesive cues regulate cell fate and organoid morphogenesis, making ECM-mimetic bioinks a foundational component for reconstructing tumor-like microenvironments^{96,97}. In tumor models, combining patient-derived

cells with 3D bioprinting and tissue-specific microenvironments can help preserve tumor phenotypes and improve the prediction of therapeutic responses⁹⁷. Second, TME-associated signals, including acidic pH, aberrant protease activity, and altered redox states, have been used as responsive triggers in materials engineering, providing a rationale for functionalized bioink design^{96,98}. Third, functional vascular networks improve the exchange of oxygen, nutrients, and metabolites, which is important for developing larger and more physiologically relevant tissue models³⁹. In tumor modeling, perfusable 3D-bioprinted glioblastoma models have shown structural features and drug-response profiles that better resemble the *in vivo* TME⁹⁹. Overall, integrating ECM biomimicry, dynamic responsiveness, and vascularization into a single model remains technically challenging, and successful examples remain limited.

3.2.2.1 ECM-Mimetic Bioinks: Recapitulating Stiffness and Composition

The ECM consists of structural and functional macromolecules, including collagen, elastin, glycosaminoglycans, and proteoglycans. It provides physical support for tissue architecture and regulates tumor cell proliferation, migration, and invasion¹⁰⁰. During tumorigenesis, ECM composition and mechanical properties are frequently remodeled, including excessive collagen deposition and fiber realignment, which contribute to tissue stiffening and altered cellular behavior. In solid tumors such as hepatocellular carcinoma and colorectal cancer, the ECM protein profile differs from that of surrounding normal tissue, and matrix stiffness and collagen organization are associated with malignant progression^{89,101}. Therefore, bioinks that recapitulate ECM remodeling and spatial heterogeneity are important for constructing tumor organoids with improved physiological relevance¹⁰².

Decellularized ECM (dECM) derived from tumor tissue provides a direct strategy for recapitulating native ECM features. Tumor-derived dECM hydrogels retain tissue-specific biochemical and structural cues, thereby improving model fidelity and the physiological relevance of drug screening^{103,104}. The use of dECM materials in 3D bioprinting offers advantages because hydrogel-based systems are biocompatible, tunable, and suitable for modeling tumor ECM features. By modulating bioink composition and crosslinking density, local matrix stiffness and biochemical cues can be spatially controlled within printed constructs. This enables partial reconstruction of TME heterogeneity *in vitro*^{105,106}.

Precise control over matrix stiffness is pivotal for achieving ECM biomimicry. GelMA-based bioinks, for example, allow stiffness tuning by adjusting polymer network density, which can influence tumor cell invasion and gene expression profiles¹⁰⁷. Matrix stiffness can also be adjusted by modulating photocrosslinking density, polymer concentration, and crosslinking duration, covering stiffness ranges relevant to both normal tissues and solid tumors¹⁰⁸.

Incorporating stiffness gradients into a single construct can mimic mechanical changes at the tumor invasion front and provide a controllable model for studying stiffness-mediated epithelial–mesenchymal transition¹⁰⁹. At the molecular level, ECM stiffness regulates tumor behavior through the integrin–FAK–YAP/TAZ mechanosignaling axis^{110,111}. A stiff matrix increases cytoskeletal tension, promotes YAP/TAZ nuclear translocation, and activates pro-proliferative and anti-apoptotic gene programs. In contrast, a compliant matrix reduces YAP/TAZ nuclear retention¹¹⁰. Hydrogel-based platforms allow spatial control of local stiffness, enabling in vitro validation of this mechanobiological coupling and evaluation of mechano-targeted therapeutics^{108,109,111}. At the therapeutic level, the integrin inhibitor ATN-161 and the YAP inhibitor verteporfin have been shown to attenuate stiff-matrix-induced stemness-associated phenotypes¹¹⁰.

Controlling collagen fiber alignment provides another important strategy for ECM biomimicry. During tumor progression, collagen fibers not only increase in density but also undergo orientation remodeling. In particular, tumor-associated collagen signature-3 alignment, which is oriented perpendicular to the tumor boundary, is closely associated with enhanced invasiveness and poor prognosis and provides contact-guided routes for tumor cell migration¹¹². Magnetic field-assisted bioprinting can align collagen fibers within bioinks, thereby mimicking this structural feature in vitro and providing a tool to investigate fiber orientation-mediated tumor invasion¹¹³.

3.2.2.2 Functionalized Bioinks: Dynamic Signaling and Cellular Regulation

ECM-mimetic bioinks provide a basis for structural reconstruction of the TME. However, the TME is a dynamic system, and structural biomimicry alone cannot fully capture processes such as invasion and metastasis. The bidirectional interplay between tumor cells and the stroma is often mediated by physicochemical cues in the TME. Among these cues, acidic pH and protease activity are commonly used stimuli for designing responsive bioinks.

The core principle of pH-sensitive bioinks is to use the mildly acidic tumor pericellular environment (pH 6.5–6.9) as a trigger for programmed changes in the bioink network, including swelling, softening, accelerated degradation, ligand exposure, or drug release. This design converts acidity into cell-perceptible mechanical and biochemical cues, thereby reshaping the local matrix environment and modulating tumor-related signaling pathways. In this way, pH-sensitive bioinks provide a material platform for recapitulating acid-driven tumor invasion in vitro¹¹⁴.

A more advanced strategy introduces a secondary crosslinking network, such as a

photocrosslinked network, into a dynamic amide- or imide-based network. This design helps decouple the functional requirements of the printing and culture windows. The printing window requires sufficient flowability and extrudability for fine-structure fabrication, whereas the culture window requires long-term structural stability together with localized dynamics that allow cell-mediated matrix remodeling. By temporally regulating mechanical properties before and after printing through controlled network kinetics, this approach has been applied to dynamic bioink design for 3D breast cancer invasion models. These studies highlight the importance of dynamic crosslinking in supporting invasive protrusion formation and tumor cell migration into the surrounding matrix¹¹⁵.

The value of pH-responsive bioinks extends beyond matrix degradation. By responding to the mildly acidic tumor microenvironment, these materials can alter network mechanics, release bioactive factors, and modulate pore structure, thereby enabling controlled functional activation¹¹⁶. In tumor models, pH responsiveness is important not only because it can trigger structural breakdown but also because it induces functional changes in the local matrix. Acid-induced matrix remodeling may accelerate local stress relaxation and create a mechanical environment that supports invasive path formation, even without changes in bulk stiffness. This process may involve increased cytoskeletal tension and TRPV4 activity, or leader-cell-mediated hyaluronidase secretion that generates microchannels for follower cells^{117,118}. Bioactive factor release can also be achieved through acid-sensitive masking–unmasking strategies. Cleavage of acid-labile bonds or conformational changes can reduce steric hindrance and charge shielding, allowing adhesion ligands, chemokines, or protease substrates to be exposed at the tumor margin and enhance migration-related signaling³⁶. In addition, acid-induced microstructural rearrangement can increase network pore size and permeability, thereby affecting oxygen and drug diffusion. This provides a useful framework for studying how pH- or hypoxia-responsive nanocarrier dimensions regulate cargo release and transport within acidic tumor niches¹¹⁹.

Enzyme-sensitive bioinks are closely linked to the biological mechanisms of tumor invasion. Tumor and stromal cells secrete various proteases, among which matrix metalloproteinases (MMPs) play important roles in ECM remodeling, matrix-bound factor release, cell migration, and angiogenesis. Because MMP activity is a key feature of the TME, incorporating protease-cleavable motifs into bioink design can help model active tumor cell invasion into the surrounding matrix^{120,121}. A classic strategy is to incorporate both integrin-binding motifs and MMP-cleavable sequences into an otherwise inert polymer scaffold, typically polyethylene glycol. This design enables cells to locally degrade the matrix and migrate through the scaffold in response to MMP secretion. Seminal studies have shown that adhesion signals alone are insufficient to drive invasive behavior; protease-cleavable sites are

also required for effective invasion and matrix remodeling. By coupling material degradation to cell-secreted enzymes, this strategy provides a foundational framework for constructing tumor invasion models¹²².

In bioprinting, this strategy allows the spatial programming of matrix degradability. A degradation-resistant network can be printed in the tumor core region, whereas a network with lower crosslinking density or greater enzymatic susceptibility can be printed at the simulated invasion front. This design helps model the spatial features of the tumor invasion zone. For cancer research, matrix materials should not only mimic the structure of the TME but also show reproducibility and compatibility with high-throughput screening platforms. 3D bioprinting platforms based on tunable synthetic hydrogels, such as PEG-4MAL, illustrate this capability. By regulating matrix stiffness, degradability, and bioactive ligand density, these platforms can generate well-defined and customizable tumor-mimetic microenvironments. These platforms support real-time imaging of cell invasion and drug-response evaluation in multiwell plate formats. They also help address key limitations of natural matrices such as Matrigel, including batch-to-batch variability and limited control over matrix properties¹²³.

3.2.2.3 Vascularization Construction Bioink

Although ECM-mimetic and functionally responsive bioinks have improved the physiological relevance of tumor models, solid tumor growth, progression, and therapeutic response remain highly dependent on interactions with the vasculature. Tumor angiogenesis supports oxygen and nutrient supply, facilitates distant metastasis, and regulates immune cell infiltration within the TME¹²⁴. Traditional two-dimensional culture systems, and even simple 3D models, cannot fully recapitulate the complex intratumoral vascular network and its dynamic interactions with tumor cells^{125,126}. Consequently, constructing functional vascular networks at the organoid scale remains a key barrier to advancing tumor models from tissue-like constructs toward organ-level models.

Sacrificial bioprinting is an established strategy for constructing perfusable vascular networks. Early studies showed that vascular structures could be engineered through the spatial organization of cells and hydrogel-based materials. For example, Leong et al. assembled cell-hydrogel filaments to generate ordered, endothelialized vascular structures that established functional connections with the host circulation after implantation. These findings support the feasibility of engineering perfusable vascular networks for vascularized tissue models¹²⁷. Building on this foundation, Skylar-Scott et al. developed SWIFT technology to extend vascularized construct fabrication toward tissue-scale models. By embedding sacrificially printed perfusion channels within a high-cell-density organoid matrix, SWIFT enabled the

formation of perfusable networks in constructs with near-physiological cell densities. It also demonstrated the capacity to support perfusion and functional maturation in cardiac organoid models, providing an important strategy for organoid vascularization⁴⁴.

Translating these strategies to cancer research requires vascular structures that are not only geometrically defined but also functionally responsive to pathological stimuli. By combining nano-engineered bioinks with multicellular printing, multilayer vascular structures containing endothelial and smooth muscle cells can be constructed. These engineered vessels can support long-term perfusion and reproduce inflammation-induced pathological responses, such as thrombosis. Such platforms may help investigate metastatic events at the organoid scale, including tumor cell breaching of the vascular barrier and thrombosis-associated inflammation¹²⁸.

Tumor-specific vascularization strategies should integrate stromal components to improve physiological relevance. For example, a vascularized lung cancer organoid model was constructed using lung-derived decellularized ECM bioink, lung fibroblasts, and a perfusable vascular network. This model captured dynamic interactions between tumor cells and vascular endothelial cells and provided insights into how a fibrotic microenvironment contributes to resistance to targeted therapies¹⁰³.

Vascularized tumor organoids improve the physiological relevance of in vitro models by introducing a perfusable, hemodynamic microenvironment. This feature expands their utility in drug development and mechanistic studies. Perfusable vascular networks allow in vitro simulation of drug transport from the bloodstream into the tumor stroma, supporting quantitative assessment of drug delivery efficiency and barrier penetration¹⁰⁵. These models also enable real-time observation and analysis of tumor–endothelial adhesion, transendothelial migration, and vascular-mediated immune cell infiltration during metastasis (**Figure 4**).

Vascularized Tumor Organoids: From Engineering to Functional Modeling

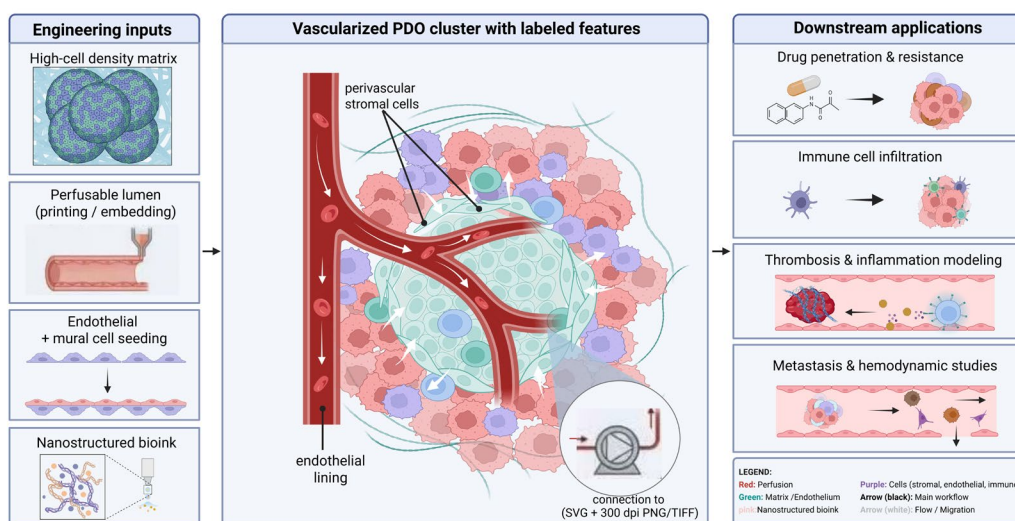


Figure 4. Schematic overview of the integrated workflow from engineering construction to functional applications of vascularized tumor organoids. The left panel illustrates the core engineering inputs for constructing vascularized organoids, including high-cell-density matrices, printing or embedding of perfusable channels, co-seeding of endothelial cells and pericytes, as well as the application of nanostructured bioinks. The middle panel depicts PDOs with a vascular network, highlighting the endothelial lining, perivascular stromal cells and tumor cell clusters. The right panel presents the typical applications of vascularized organoids, including drug penetration and drug resistance research, immune cell infiltration, thrombosis and inflammation modeling, as well as metastasis and hemodynamic analysis. Color legends: red represents perfusion; green represents matrix/endothelium; purple represents cells (stromal, endothelial, immune cells); white arrows indicate the workflow direction; black arrows indicate blood flow/migration direction. This figure summarizes the engineering strategies of vascularized organoids and their advantages and application potential in TME research.

The TME is characterized by multiple concurrent stimuli and dynamic interactions among cellular, stromal, vascular, and immune components. Accordingly, tumor-specific bioinks are evolving toward the integration of ECM biomimicry, functional responsiveness, and vascularization. ECM-mimetic bioinks reproduce key features of native tumor matrices, including matrix composition, ligand density, and mechanical properties, thereby providing tumor cells with a physiologically relevant matrix context. Functionalized bioinks respond to tumor-associated metabolic and invasive cues through pH-, enzyme-, or redox-sensitive mechanisms. Vascularized bioink systems incorporate endothelialized and perfusable networks to support mass exchange, drug transport, and immune cell infiltration^{46,48,129}. These bioink systems collectively support the controlled reconstruction of mechanical, metabolic, and

immunosuppressive features within the TME. ECM stiffness regulates tumor proliferation, migration, and drug resistance through pathways such as integrin–FAK–YAP/TAZ signaling. Bioprinting platforms with programmable local stiffness gradients allow investigation of this mechanobiological coupling^{46,130}. Hypoxia stabilizes HIF signaling and promotes metabolic reprogramming, efflux pump activity, and therapeutic tolerance. In parallel, acidic microenvironments can activate survival pathways, including NF- κ B signaling. Spatially resolved gradient platforms therefore provide useful systems for studying drug resistance driven by metabolic heterogeneity¹³¹. Vascularized models allow tumor cells, stromal cells, and endothelial or immune populations to be spatially separated, enabling more direct assessment of their individual contributions to immunosuppression and drug response. However, key material parameters, including stiffness, degradation kinetics, ligand density, and growth factor release profiles, are often coupled and remain difficult to regulate independently. Multi-nozzle, coaxial, and gradient printing technologies enable the spatial assembly of ECM-mimetic layers, responsive layers, and vascular channels, thereby supporting the reconstruction of TME spatial heterogeneity^{46,129}. Future progress will therefore depend not only on the development of new materials but also on establishing quantitative relationships among material parameters, microenvironmental signals, and tumor functional phenotypes. Such efforts may shift bioink design from an empirical practice toward a more predictive and standardizable engineering framework.

Table 2: Bioink design matrix for programmable reconstruction of tumor microenvironmental variables

Bioink design strategy		Programmable variable	TME	Representative materials / implementations		Biological function modeled	Useful readouts / applications		Main limitation / caution	Refs.
Rheology and printability tuning	Flowability; shape fidelity; shear exposure	yield stress	GL gel; alginate/CMC/TO-NFC; pre-crosslinked Ca ²⁺ inks; pH-regulated crosslinking	Stable deposition with reduced printing stress	Filament continuity; stability; print resolution; viability	Higher viscosity may improve fidelity but increase shear stress or buckling risk	38, 71-76			
Cell-supportive bioinks	Cell adhesion; phenotype retention	survival; mechanical stability; high-cell-density integrity; organization	GelMA/nanocellulose; GO-alginate/gelatin; fish-derived gelatin fibers; RGD-modified alginate	Cytocompatible maintenance of cell function	Viability; proliferation; activity; adhesion/spreading	Bioactivity must be balanced with printability and stability	82-84, 90			
Crosslinking and high-cell-density support	Gel integrity; high-cell-density organization	mechanical stability; high-cell-density integrity; healing scaffolds	HRP-mediated enzymatic crosslinking; PEGX stabilization; visible-light curing; microgels; self-healing scaffolds	Cell survival and mechanical integrity	Viability; mechanical stability; tissue-like organization	Excessive crosslinking or cell density may impair diffusion or viability	85-91			
ECM-mimetic and bioinks	ECM composition; density; tissue-specific biochemical cues	ligand stiffness; stiffness gradients; mechanotransduction	Collagen; alginate/GelMA/gelatin; tumor-derived or tissue-specific dECM	Native-like matrix context and tumor phenotype preservation	Matrix fidelity; drug-screening relevance; tumor fidelity	dECM improves biomimicry but may introduce batch variability and limited controllability	96, 97, 100-106			
Matrix stiffness programming	Local stiffness; degradation; exposure; pore structure; release	stiffness gradients; mechanotransduction	GelMA photocrosslinking density; dynamic networks; acid-labile masking/unmasking	Stiffness-driven invasion, EMT-like behavior, and YAP/TAZ signaling	Localization; mechano-targeted response	Stiffness often couples with degradation, ligand density, and diffusion	107-111			
Collagen fiber alignment	Fiber orientation; guidance architecture	contact-bioinks	Magnetic field-assisted collagen alignment within bioinks	Oriented ECM routes for tumor migration and invasion	Fiber alignment; directional invasion; contact-guided migration	Alignment control should be linked to quantitative invasion readouts	112, 113			
pH-responsive and dynamic bioinks	Acidity-triggered changes; degradation; exposure; pore structure; release	mechanical stiffness gradients; mechanotransduction	pH-sensitive photocrosslinked dynamic networks; acid-labile masking/unmasking	Acidic TME, invasion-path formation, and matrix remodeling	Swelling/softening; migration; pore size; drug and factor release	pH response may affect multiple material parameters simultaneously	114-119			
Enzyme-sensitive degradable bioinks	MMP-sensitive degradability gradient; local matrix remodeling	degradation; exposure; pore structure; release	PEG-based scaffolds with integrin-binding and MMP-cleavable motifs; tunable PEG-4MAL platforms	Protease-dependent invasion and invasion-front remodeling	Real-time degradation; drug remodeling	Requires independent control of ligand density, stiffness, and degradation	120-123			
Vascularization and bioinks	Perfusible channels; endothelial barrier; mass transport; stromal-vascular interface	channels; mass transport; vascularized models	Sacrificial printing; SWIFT; lung dECM; vascularized models	Oxygen/nutrient exchange, drug transport, immune-cell infiltration, and metastasis-related events	Perfusion; barrier function; migration; tumor-endothelial adhesion	Functional vascularization remains technically demanding at the organoid scale	39, 44, 99, 103, 105, 124-129			
Multicomponent and bioinks	Material heterogeneity; multi-ink mixing; responsive layers; spatial assembly	Material heterogeneity; multi-ink mixing; responsive layers; spatial assembly	Aqueous two-phase systems; microfluidics-assisted multi-ink mixing; covalent networks; printing	Coordinated reconstruction of mechanical, metabolic, and immunosuppressive TME features	Cell organization; spatial heterogeneity; phenotype mapping	Quantitative links between material parameters and tumor phenotypes remain insufficient	46, 92-94, 129-131			

Abbreviations: TME, tumor microenvironment; ECM, extracellular matrix; dECM, decellularized extracellular matrix; GelMA, gelatin methacryloyl; CMC, carboxymethyl cellulose; TO-NFC, TEMPO-oxidized nanofibrillated cellulose; GO, graphene oxide; RGD, arginine-glycine-aspartic acid; HRP, horseradish peroxidase; PEGX, PEG crosslinking; MMP, matrix metalloproteinase; SWIFT, sacrificial writing into functional tissue. Reference numbers correspond to the manuscript bibliography.

3.3 The Complete Process of Tumor Organoid Generation

Tumor organoid construction is an end-to-end process that includes cell sourcing, bioink formulation, printing optimization, and post-printing maturation. In bioprinted tumor organoids, this workflow translates programmable design into a living model by defining cellular composition, matrix context, spatial organization, and maturation conditions. Careful control at each stage is critical for the physiological relevance and functional reliability of the final model.

3.3.1 Upstream Design: Cell Sources and Construction of Multicellular Systems

The creation of physiologically relevant tumor organoids begins with careful upstream design. Cell source selection and the establishment of multicellular systems are the primary determinants of the model's clinical relevance and complexity.

Cell sources fall broadly into two categories: patient-derived tumor tissue and established tumor cell lines. Patient-derived cells retain the genomic features and heterogeneity of the original tumor, making them suitable for personalized research and clinically relevant modeling. Established cell lines, in contrast, offer high reproducibility and ease of expansion, making them ideal for mechanistic studies and high-throughput screening. Regardless of the source, standardized culture and expansion protocols are essential to ensure cell viability, purity, and phenotypic stability.

Solid tumors are not simply clusters of malignant cells but complex ecosystems composed of multiple cell types. Consequently, single-cell monocultures cannot fully capture the complexity of the TME. To better model tumor heterogeneity and cell–cell interactions, current studies increasingly use multicellular co-culture systems incorporating tumor cells, CAFs, immune cells, and endothelial cells^{3,12}. Cancer types differ in cellular composition and microenvironmental features, which should inform the choice of bioprinting strategy. For example, pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense fibrotic stroma with increased matrix stiffness. Biomimetic PDAC models may therefore benefit from mechanically tunable bioinks and co-culture with CAFs. Embedded bioprinting can rapidly generate CAF-containing PDAC models with enhanced chemoresistance, providing a useful platform for drug screening^{132,133}. In glioblastoma multiforme (GBM), the TME includes astrocytes, microglia, and other glial populations, and ECM stiffness influences tumor behavior. Accordingly, GBM models may benefit from hydrogel bioinks that match the mechanical properties of brain tissue and from co-culture with relevant glial cells to better model the GBM microenvironment¹³⁴⁻¹³⁷. For liver cancer, tumor–microvascular interactions are closely associated with disease progression and drug response. This highlights the need for 3D models

that incorporate endothelial components to better represent stage-specific features of the TME^{138,139}.

3.3.2 Midstream Control: Ink-Cell Compatibility and Printing Process Optimization

Once upstream design is finalized, the midstream phase focuses on integrating cells with bioinks and fabricating them into 3D structures through bioprinting. This stage includes bioink selection and optimization, adjustment of printing parameters, and control of the printing environment. Together, these factors influence the initial quality of printed constructs and cell viability.

The selection and formulation of bioinks are critical pre-printing steps. An ideal bioink for tumor organoids should balance printability and biocompatibility. It should exhibit suitable rheological properties to support smooth extrusion and structural fidelity while providing a microenvironment that supports cell survival, proliferation, and functional activity. Common matrix materials include GelMA, hyaluronic acid (HA), and collagen, which are typically crosslinked through physical or chemical methods to provide mechanical strength and structural stability. GelMA-based bioinks, for example, allow matrix stiffness to be tuned by modulating polymer network density, thereby influencing tumor cell invasion behavior¹⁰⁷.

Advances in materials engineering continue to push the boundaries of bioink performance. For example, incorporating a small amount of proanthocyanidin as a crosslinking agent can enhance the crosslinking density and mechanical properties of collagen-based photopolymerizable bioinks. This formulation enables micron-scale patterning on digital light processing platforms, supports cell loading and uniform distribution, and shows favorable biocompatibility¹⁴⁰. Once a material system is selected, bioink rheology should be tuned to match nozzle requirements, thereby supporting process reliability and structural fidelity^{141,142}.

Printing equipment and environmental preparation are equally critical. The printer should be calibrated to ensure accuracy and batch-to-batch reproducibility. The printing environment should also be controlled for temperature, humidity, and sterility to reduce cellular stress and contamination risk. Furthermore, printing parameters, including printing speed, nozzle pressure, and layer thickness, should be adjusted according to the modeling requirements of different tissue types^{6,143}. Overall, pre-printing preparation aims to optimize the compatibility among cells, bioinks, and printing equipment, thereby supporting the construction of physiologically relevant tumor organoid models.

Control of key printing parameters is critical for maintaining the morphological integrity

of printed constructs and the biological function of encapsulated cells. Printing speed and nozzle pressure affect both resolution and cell viability. Therefore, these parameters should be calibrated according to the rheological properties of the bioink to preserve cell viability during printing. Layer thickness and interlayer adhesion are also important. Appropriate layer thickness helps ensure continuous fabrication and uniform cell distribution, while reducing the risk of structural collapse or delamination. Interlayer adhesion should be monitored and optimized to ensure adequate bonding between successive layers, thereby supporting structural integrity, intercellular communication, and subsequent tissue integration⁵⁶. Temperature control is also important, particularly when temperature-sensitive bioinks are used, because it helps maintain bioink performance and cell viability¹⁴³.

3.3.3 Downstream Processing: Crosslinking and Curing, Dynamic Cultivation, and Functional Characterization

Completion of the printed construct represents only the initial step in tumor organoid fabrication. Subsequent downstream processing and maturation culture are required for organoids to develop stable biological functions.

The crosslinking method and timing are important determinants of the mechanical properties of printed constructs. Physical crosslinking, such as temperature-responsive gelation, and chemical crosslinking, such as photoinitiated crosslinking, should occur sufficiently rapidly during or immediately after printing to maintain structural stability and morphological fidelity. For GelMA-based bioinks, photocrosslinking density influences matrix stiffness, which in turn affects the invasive phenotype and gene expression programs of tumor cells¹⁰⁷. Matrix porosity and microstructure should also be regulated through crosslinking parameters and printing conditions. These architectural features influence cell–cell interactions and the transport of oxygen, nutrients, and metabolic waste, thereby affecting tissue maturation and cellular function¹⁴⁴.

Dynamic culture techniques are important for promoting organoid maturation. After printing, organoids require further culture in systems that support cell proliferation, differentiation, and self-organization, thereby promoting the development of physiologically relevant TME models^{6,8}. At this stage, perfusion bioreactors can mimic aspects of the *in vivo* hemodynamic environment, improve mass transport, and introduce fluid shear stress. These conditions can enhance organoid maturation and physiological relevance.

Systematic functional characterization is an essential step in validating tumor organoid models. Beyond morphological assessment, it should include evaluation of cell proliferation

and viability, invasion and migration, tumor-associated gene and protein expression, and drug sensitivity. These characterization data serve as quality-control benchmarks for assessing whether the model preserves key histological, molecular, and functional features of the source tumor. They also provide a foundation for mechanistic studies, drug screening, and precision oncology applications^{145,146}.

In summary, tumor organoid construction can be viewed as an end-to-end engineering process involving upstream cell design, midstream printing control, and downstream maturation culture. Coordinated optimization of cellular composition, bioink properties, printing parameters, and culture conditions is essential for generating physiologically relevant tumor organoids. These models provide useful platforms for mechanistic studies and drug screening. Representative studies further show how engineered tumor organoid platforms connect model construction with functional validation, including spatial organization, fluorescence or histological assessment, and drug-response analysis (**Figure 5**).

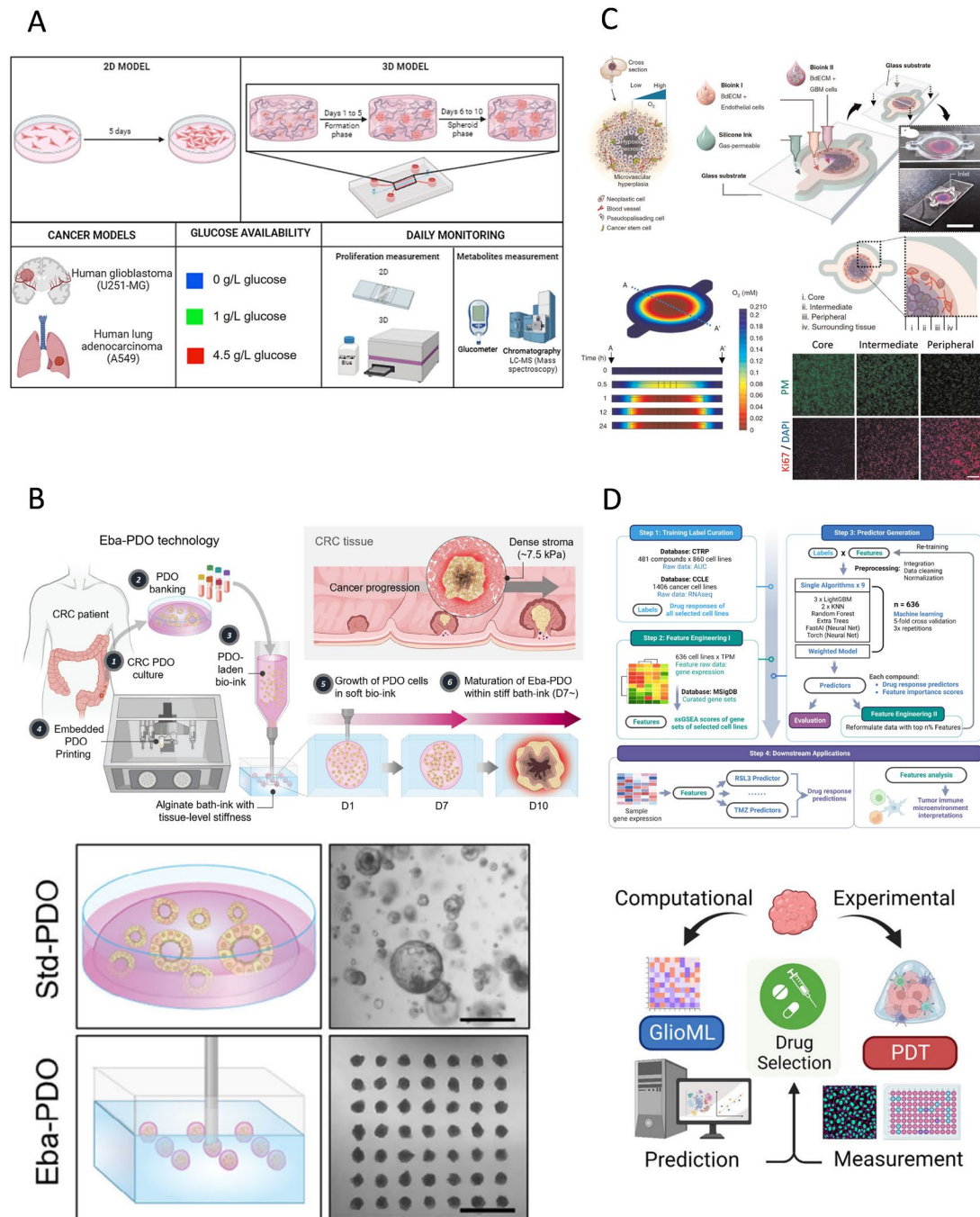


Figure 5. 3D bioprinted tumor model system: from necessity validation to clinical personalized prediction. (A) Microfluidics-based 2D/3D tumor model comparison validates that 3D models better recapitulate tumor metabolism and microenvironment (adapted and modified from Ref. ¹, Copyright 2025, licensed under CC BY 4.0); (B) Embedded bioprinting enables high-throughput patient-derived organoid array for standardized drug screening

(adapted and modified from Ref. ⁸, Copyright 2025, licensed under CC BY 4.0);(C) Conceptual framework of 3D bioprinting for tumor microenvironment (TME) reconstitution (adapted and modified from Ref. ⁴⁶, Copyright 2020, licensed under CC BY 4.0; adapted and modified from Ref. ⁹⁷, Copyright 2019, licensed under CC BY 4.0);(D) Artificial intelligence integrated with 3D models predicts patient-specific drug response for precision therapy (adapted and modified from Ref. ¹³⁴, Copyright 2024, licensed under CC BY 4.0).

4. Translational Value and Applications of Tumor Organoid Models

The translational value of tumor organoid models is a central consideration in their development. Building on the technical frameworks and microenvironment reconstruction strategies discussed in the previous sections, this section focuses on the major applications of bioprinted tumor organoid models. Rather than emphasizing structural biomimicry alone, we examine whether programmable TME reconstruction can generate clinically meaningful readouts, including patient-specific drug sensitivity, TME-mediated resistance, immune-cell infiltration, and drug delivery barriers. This section outlines the application of these models in three major domains: personalized medicine, drug discovery, and immunotherapy evaluation. It also discusses their potential transition from laboratory tools to platforms that support clinical decision-making.

4.1 Preclinical Translation and Personalized Medicine

Tumor organoid models are increasingly being translated from laboratory tools into platforms that support clinical decision-making, particularly in personalized medicine. The following sections discuss their applications across three major domains: patient-specific drug sensitivity screening, drug discovery, and immunotherapy evaluation.

4.1.1 Personalized Medicine: PDOs for Drug Sensitivity Screening

With the advancement of precision medicine, PDOs have emerged as valuable platforms for personalized therapy because they retain key genetic, phenotypic, and functional characteristics of the primary tumor^{147,148}. Through 3D culture, PDOs can partially recapitulate the spatial architecture and tumor cell–cell interactions of parental tumors. They also largely preserve genetic diversity and intratumoral heterogeneity, making them physiologically relevant platforms for personalized therapeutic testing^{8,126}.

Bioprinting enhances PDO models at multiple levels. First, it improves physiological relevance. For example, a colorectal cancer PDO array constructed by embedded bioprinting (Eba-PDOs) reproduced key TME features, including matrix stiffness and hypoxia. This model

also captured patient-specific differences in tumor marker expression, such as CEACAM5, which was associated with therapeutic responses to 5-fluorouracil⁸. Bladder cancer PDOs similarly capture tumor heterogeneity and drug sensitivity. When integrated with microfluidics and 3D bioprinting, these models may improve the accuracy and efficiency of drug screening, thereby supporting more precise personalized treatment¹⁴⁹. Bioprinted renal cell carcinoma organoids can preserve intratumoral heterogeneity, providing a useful platform for preclinical drug testing¹⁵. Second, bioprinting can improve construction throughput and model uniformity. HA–collagen composite hydrogels combined with immersion bioprinting enable high-throughput PDO fabrication and reduce surface-tension-driven two-dimensional spreading in conventional multiwell plates. This approach improves the consistency of organoid volume and morphology, thereby supporting the stability and reliability of screening data¹⁴³. Extrusion-based high-throughput bioprinting has been used to rapidly generate patient-derived renal cell carcinoma organoids with uniform size. These organoids retain key pathological and molecular features of the parental tumors while preserving interpatient and interorganoid heterogeneity. This approach facilitates automated batch construction, quality control, and preclinical patient-specific drug screening¹⁵. Third, bioprinting facilitates seamless integration with advanced analytical technologies. A pipeline combining high-speed live-cell interferometry with machine learning algorithms enables rapid screening of personalized treatment regimens¹⁴. Acoustic bioprinting enables controlled spatial arrangement of patient-derived tumor and normal organoids, supporting the construction of 3D tissue architectures that recapitulate tumor invasion behavior. These models may further assist treatment-response prediction and surgical decision-making²⁹.

Growing clinical evidence supports the use of PDOs for drug-sensitivity prediction. By retaining key genetic and phenotypic features of primary tumors, PDOs can model patient-specific drug sensitivity and resistance profiles in vitro and support the development of personalized treatment regimens¹⁵⁰. For instance, colorectal cancer Eba-PDOs captured variations in CEACAM5 expression that predicted differential patient responses to 5-fluorouracil⁸. High-grade serous ovarian cancer PDOs have been used to longitudinally model patient-specific drug responses, including resistance to carboplatin and PARP inhibitors, supporting their use in precision drug screening¹⁵¹. In colorectal cancer, an organoid score derived from drug sensitivity profiling correlated significantly with patient treatment response and progression-free survival, supporting its utility as a personalized decision-making tool¹⁵². In PDAC, the HOPE pilot feasibility trial provided prospective proof-of-principle evidence that PDO drug-sensitivity profiling was associated with clinical response. A normalized dose–response area under the curve (AUC) cutoff of approximately 0.56 helped distinguish disease control from progressive disease. These findings support further investigation of organoid

pharmacotyping as a tool for guiding treatment selection¹⁵³. In lung cancer, integrating PDOs with artificial intelligence and multi-omics technologies may help model individual tumor heterogeneity and microenvironmental features, thereby improving the prediction of responses to targeted therapies and immunotherapy¹⁵⁴. Breast and bladder cancer PDOs have also shown utility in drug screening and efficacy prediction, with progress in modeling the tumor immune microenvironment and drug-resistance mechanisms^{149,155}.

Despite these advances, the clinical translation of bioprinted PDOs remains limited by important evidence gaps. Conventional PDO studies have shown clinically relevant drug-response prediction. For example, gastric cancer PDOs reproduced patient responses to chemotherapy in 91.7% of clinically evaluable cases, suggesting that non-printed PDOs can serve as functional platforms for precision medicine¹⁵⁶. Meanwhile, recent bioprinted PDO models have shown promising capabilities, including the incorporation of perfusable tumor vasculature, tissue-specific dECM bioinks, stromal components, and rapid ex vivo drug testing within approximately two weeks^{157,158}. However, these studies mainly establish feasibility and clinical associations rather than demonstrating superior predictive performance over conventional PDO models. Direct head-to-head comparisons between conventional PDOs and programmable bioprinted PDOs remain lacking. Such comparisons should use identical patient samples, matched drug panels, and matched clinical endpoints. Therefore, the assumption that 3D printing improves drug-sensitivity prediction should be treated as a testable hypothesis rather than an established fact.

More fundamentally, it remains unclear whether features uniquely enabled by 3D printing, such as defined matrix architecture, spatial cell patterning, or integrated vascular networks, directly contribute to improved predictive performance. Current studies show that printed organoids can support drug testing and may recapitulate patient-specific tumor–matrix or tumor–vascular interactions^{157,158}. However, few studies have determined whether this increased engineering complexity provides a measurable predictive advantage over conventional PDO culture¹⁵⁶⁻¹⁵⁸. Future studies should therefore adopt prospective, multicenter, head-to-head designs in which conventional and programmable bioprinted PDOs are generated from the same biopsy specimens. Such studies should quantify not only drug-response concordance with patient outcomes but also turnaround time, batch-to-batch variability, key model-quality attributes, and process parameters. Establishing this mapping among programmability, model quality, and clinical predictive accuracy will be essential for moving bioprinted PDOs from promising decision-support tools toward empirically validated precision-oncology platforms.

4.1.2 Drug Discovery: High-Throughput Screening and Efficacy Evaluation

With the development of tumor organoid technology, integrating bioprinting with high-throughput screening has become an important approach for improving the precision and clinical relevance of cancer drug development. Compared with traditional two-dimensional cell culture, 3D tumor organoid models better preserve tissue architecture, genetic heterogeneity, and key features of the tumor microenvironment, thereby improving the predictive value of drug screening and supporting new drug development^{14,159}.

High-throughput drug screening platforms are essential for large-scale drug evaluation. Standardized printing processes using hydrogel bioinks and immersion bioprinting have enabled the batch production of uniform PDOs¹⁴³. Integrating high-speed live-cell interferometry with machine learning algorithms enables label-free, dynamic, and quantitative monitoring of thousands of individual organoids. This approach can distinguish drug-sensitive from drug-resistant tumor cells across different drug candidates¹⁴. In parallel, integrating microfluidic chips with bioprinting can support dynamic organoid culture and drug-exposure simulation, thereby advancing high-throughput, automated, and clinically relevant drug screening¹⁶⁰.

Evaluating drug efficacy, resistance, and preclinical predictability is central to drug development. Compared with traditional two-dimensional cultures, organoid models can better capture tumor heterogeneity and key mechanisms underlying drug response, thereby supporting personalized therapeutic decision-making^{6,161}. Bioprinted multicellular co-culture models may further improve TME reconstruction and support more reliable drug-response prediction. For instance, 3D-bioprinted colorectal cancer multicellular models incorporating tumor cells, tumor-associated macrophages, and endothelial cells show increased tumor-associated gene expression and chemoresistance compared with single-cell models. These models provide a more physiologically relevant system for drug-efficacy evaluation¹⁶². In preclinical screening, drug-sensitivity profiles obtained from PDOs have been shown to correlate with patient clinical responses¹⁶³. Moreover, 3D bioprinting allows the use of different bioinks, such as GelMA- and HA-based hydrogels, to tailor the mechanical and biochemical microenvironment of organoids. This may improve their physiological relevance and predictive value for drug response^{143,164}.

Drug toxicity assessment is a critical safeguard in drug development. Bioprinted human liver models can exhibit mature hepatic functions and sensitivity to drug-induced liver injury, supporting early hepatotoxicity screening and safety evaluation of new drug candidates¹⁶⁵. Furthermore, bioprinting enables co-culture of tumor organoids with immune cells, providing a platform for modeling the tumor immune microenvironment and evaluating the efficacy and potential toxicity of immunotherapeutic agents¹⁶⁶.

In summary, 3D bioprinting enables controlled spatial organization of cells and matrix components in patient-derived organoid models, supporting the development of high-throughput and more standardized drug-screening platforms. This framework can improve the physiological relevance of drug testing, facilitate drug discovery, and support the development of precision medicine.

4.1.3 Analysis of Mechanisms Underlying Immunotherapy Response and Resistance

A key challenge in incorporating immune cells into tumor organoid models is not simply achieving co-culture, but recapitulating the mechanistic steps of immune infiltration and suppression within a spatially defined microenvironment. From an engineering perspective, a relevant strategy is to couple printed tumor–stroma modules with perfusable vascular networks, enabling controlled immune cell perfusion and infiltration. For example, 3D-bioprinted vascularized tumor models have been shown to support CAR-T cell recruitment to perfused endothelium, subsequent infiltration into tumor tissue, and tumor volume reduction *in vitro*¹²⁹. Similarly, reproducible 3D-printed neuroblastoma models allow visualization and quantification of CAR-T cell infiltration into tumors, activation, and cytotoxicity, which are key readouts for functional immunotherapy evaluation beyond traditional 2D culture systems¹⁶⁷. Together, these studies suggest that engineered tumor models with defined perfusable barriers and stromal context can model key steps of T-cell migration from the vasculature into tumor tissue. They also provide controlled platforms for investigating immune suppression mechanisms and therapeutic responses.

Platforms for evaluating CAR-T efficacy have advanced considerably. The breast cancer-on-chip model developed by Maulana et al. integrates an endothelial barrier with a tumor compartment. This design enables CAR-T cells to migrate across the barrier and infiltrate the tumor under perfusion conditions, while also allowing continuous monitoring of cytokine release. By incorporating patient-derived breast cancer organoids, this platform supports the assessment of patient-specific CAR-T efficacy and safety margins¹⁶⁸. It also allows analysis of the relationship between T-cell infiltration efficiency and cytotoxic function, providing an *in vitro* tool for optimizing CAR-T cell engineering.

Investigating immune suppression requires models with greater architectural and cellular complexity. By introducing M2-like macrophages into a tumor-on-chip model, Bains et al. showed that these cells induced neighboring endothelial cells to upregulate PD-L1 and downregulate ICAM-1, thereby restricting CAR-T cell infiltration and effector function. Anti-PD-L1 treatment partially reversed this effect, suggesting that such platforms provide useful models for dissecting the molecular mechanisms of restricted CAR-T infiltration and screening

combination strategies to overcome immune suppression¹⁶⁹.

Modeling responses to immune checkpoint inhibitor (ICI) therapy increasingly aims to recapitulate clinically relevant response mechanisms. The lung cancer organoid–PBMC gel–liquid interface co-culture model developed by Li et al. reproduces ICI-induced T-cell recruitment and tumor regression in vitro, supporting clinical immunotherapy outcome prediction and biomarker identification. This organoid–systemic immune coupling design provides a useful framework for incorporating quantifiable immune-cycle readouts, including recruitment, activation, killing, and re-recruitment, into print-on-chip platforms¹⁷⁰. In tumor-on-a-chip systems designed to approximate organ-level architecture, Veith, Nurmik, and colleagues established a lung cancer model using patient-derived autologous cells to study tumor–immune interactions under a more physiologically relevant context. They evaluated anti-PD-1 responses and showed that the addition of FAP⁺ CAFs attenuated anti-PD-1 efficacy. This model therefore provides an experimentally tractable in vitro system for studying stroma- and fibroblast-mediated tolerance to immune checkpoint blockade and for testing therapeutic interventions¹⁷¹.

Thus, the value of incorporating immune components into tumor organoid models extends beyond co-culture alone. Engineering approaches can integrate perfusable vascular barriers, spatially organized stromal modules, and controlled immune cell perfusion. These systems support mechanistic studies of immune cell transit, infiltration, and cytotoxic activity within a structured microenvironment. For example, multilayered 3D stromal barrier models containing cancer cells, endothelium, and CAF-rich regions permit quantitative analysis of T-cell infiltration and antitumor cytotoxicity while recapitulating features of immune exclusion¹⁷². Similarly, high-throughput 3D tumor vasculature models demonstrate that natural killer cell extravasation and tumor-cell killing can be monitored in vitro within perfusable vascular networks¹⁷³. Furthermore, microfluidic 3D tumor models with endothelial barriers allow quantification of activated T-cell migration toward tumor tissue, highlighting the role of vasculature in immune trafficking¹⁷⁴. Although these platforms model key steps in tumor–immune interactions, systematic quantitative studies are still needed to determine which programmable vascular features, such as endothelial barrier properties, immune checkpoint ligand density, or ECM composition, most strongly influence effective immune infiltration. By enabling dynamic tracking of immune cell activation, migration, and exhaustion, these engineered models can support mechanistic studies of immunotherapy response and resistance in immuno-oncology research.

In summary, this review provides a systematic overview of the technical implementation and translational applications of bioprinted tumor organoids in three major areas: personalized

drug screening, high-throughput drug discovery, and immunotherapy evaluation. Representative studies further demonstrate that these platforms can generate functional readouts, including high-throughput drug-response profiling, immune-cell cytotoxicity, vascular immune trafficking, and spatial biomarker validation (**Figure 6**). Despite progress in these areas, model standardization, the predictive accuracy of high-throughput assays, and the matching of autologous immune components remain common challenges that limit clinical translation.

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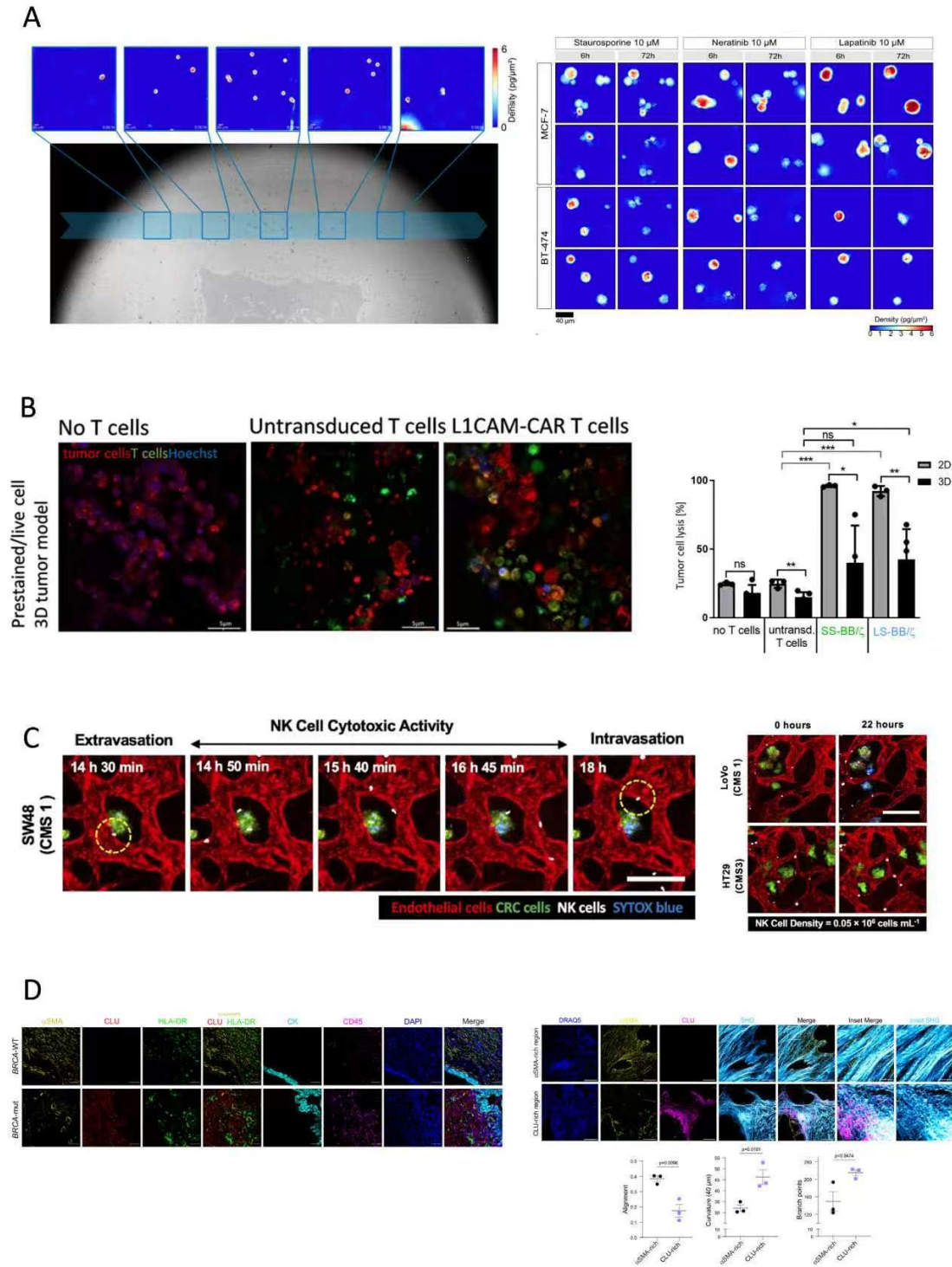


Figure 6. Advanced 3D tumor models for preclinical cancer research.1(a) High-throughput drug screening platform using bioprinted tumor organoid arrays. High-speed live-cell interferometry (HSLCI) enables label-free, real-time monitoring of drug responses in thousands of organoids, distinguishing sensitive and resistant phenotypes at single-organoid resolution (adapted and modified from Ref. ¹⁴, Copyright 2023, licensed under CC BY 4.0).(b) 3D

bioprinted tumor model for CAR-T therapy preclinical evaluation. Immunofluorescence imaging visualizes CAR-T cell infiltration into 3D tumor constructs, while quantitative cytotoxicity assays validate tumor-specific killing under physiological 3D conditions (adapted and modified from Ref. ¹⁶⁸, Copyright 2021, licensed under CC BY 4.0).(c) Vascularized tumor-on-a-chip model for dynamic NK cell immunotherapy monitoring. Time-lapse imaging tracks the full process of NK cell extravasation from vessels, tumor-directed migration, and subsequent tumor cell killing, recapitulating vascular-immune-tumor crosstalk (adapted and modified from Ref. ¹⁷⁴, Copyright 2021, licensed under CC BY 4.0).(d) Multicellular tumor microenvironment (TME) model of BRCA-mutant pancreatic cancer for radiotherapy resistance studies. Multiplex immunofluorescence shows spatial distribution of tumor cells and distinct cancer-associated fibroblast (CAF) subtypes; second harmonic generation (SHG) imaging reveals CAF-mediated collagen matrix remodeling linked to treatment resistance (adapted and modified from Ref. ⁵³, Copyright 2022, licensed under CC BY 4.0).

4.2 Model Standardization and Domain-Specific Challenges

Despite its potential for tumor organoid construction, 3D bioprinting still faces technical and translational barriers that limit clinical application. A central challenge is whether programmable TME features can be fabricated, measured, and reproduced across batches, laboratories, and clinical settings. Key challenges include reproducible fabrication, standardized process control, quality assurance, ethical governance, and regulatory alignment. Addressing these issues will be essential for advancing bioprinted tumor organoids from preclinical models toward clinically actionable platforms¹².

4.2.1 Technical Challenges: From Printable to Reproducible

Printing accuracy and repeatability are pervasive challenges across all 3D printing modalities¹⁷⁵. For bioprinted tumor organoids, the challenge extends beyond structural fidelity to the controlled construction of a biologically functional cellular microenvironment. The deposition of cells, matrix materials, and bioinks should be carefully coordinated. Even small deviations in printability, shape fidelity, or post-printing stabilization can affect organoid behavior and drug response. These variations arise not only from hardware limitations but also from the coupling among bioink rheology, crosslinking behavior, printing parameters, and cell viability during printing³⁸.

Defining a robust process window is essential for improving reproducibility. During extrusion, small fluctuations in pressure, temperature, or dwell time can lead to variations in line width, interlayer fusion, and porosity. These variations may arise from thixotropic recovery,

pre-crosslinking reactions, and cell-density-dependent changes in viscosity. These structural variations can alter oxygen and drug diffusion profiles as well as cell–matrix mechanical feedback, thereby affecting the biological phenotype of the organoid¹⁷⁶. A quantitative process map linking bioink composition, rheology, and printing outcomes can be established by combining design of experiments with response surface methodology or machine learning. For instance, one study used a design of experiments framework to systematically screen alginate–gelatin–nanoclay formulations and identified a robust printing window with structural deviation below 5% and extrusion pressure below 30 kPa¹⁷⁶.

Process observability and closed-loop control represent essential pathways toward reproducible bioprinting. First, load or pressure sensors integrated at the extrusion end can monitor extrusion dynamics online. These sensors convert nominal flow settings into process-relevant readouts, such as extrusion pressure and model-inferred shear rate, viscosity, or wall shear stress. These signals can help detect potential anomalies, including nozzle clogging, phase separation, and localized pre-gelation. Second, in situ imaging combined with computer vision allows real-time quantification of line width, positional error, and layer-to-layer stacking fidelity. These measurements can guide automated adjustment of printing parameters, including speed, pressure, and nozzle–substrate gap height^{177,178}. For instance, a low-cost load cell has been integrated into a piston-driven extrusion system to measure pressure in situ. Based on capillary rheometry principles, this system can estimate shear rate and viscosity, thereby linking bioink rheology to the mechanical forces experienced during printing and helping define a more robust operating window¹⁷⁹. Similarly, a modular in situ monitoring platform combining vision transformer-based segmentation with artificial intelligence has been proposed for defect detection and parameter optimization. This approach illustrates how data-driven monitoring may improve bioprinting reproducibility and support automated process control¹⁸⁰.

The main bottleneck in large-scale manufacturing has shifted from organoid formation itself to the ability to design, verify, and reproduce batch-to-batch consistency. Quality objectives should therefore extend beyond morphological similarity to include structural, mechanical, and biological consistency. This requires a closed-loop mapping between Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs). CQAs should include organoid size distribution, necrotic core thresholds, matrix stiffness and stress relaxation, drug permeability coefficients, and biomarker stability. CPPs should extend beyond traditional parameters, such as flow rate, printing speed, and nozzle geometry, to include sensitive variables such as extrusion pressure, shear history, interlayer dwell time, temperature, crosslinking exposure, cell density, and aggregation state. Building a CQA–CPP knowledge base for complex systems, such as patient-derived organoids and high-density cell bioinks, can help clarify why identical equipment settings may produce different outcomes across batches

of cells or materials. It can also shift quality control from offline end-point inspection toward online release criteria, thereby supporting the transition of bioprinting toward GMP-compliant manufacturing¹⁷⁶.

A major challenge in this field is not whether increasingly complex structures can be printed, but whether organoids fabricated across different batches, laboratories, and cell sources exhibit comparable biological behavior. Without a standardized framework linking CQAs to acceptable CPP ranges, bioprinted tumor organoids may remain artisanal constructs rather than reliable tools for clinical decision-making¹⁸¹. However, specifying parameter windows alone is insufficient. A deeper challenge is to understand how small process fluctuations, such as temperature drift, extrusion-pressure variation, or changes in bioink residence time, lead to macroscopic structural differences and functional shifts. These effects are mediated by coupled processes such as thixotropic recovery, crosslinking kinetics, viscoelastic relaxation, and cell sedimentation³⁸. Addressing this issue requires a shift from empirical parameter screening toward mechanistic error-propagation modeling. Such modeling can clarify how process variability affects construct quality and organoid phenotype, thereby providing a basis for reproducible bioprinted organoid manufacturing^{38,181}.

4.2.2 Balancing Patient Rights and Technological Innovation

As emerging experimental platforms¹⁶⁰, bioprinted tumor organoids combine 3D bioprinting with microfluidic organ-on-a-chip systems and CRISPR-based gene editing to better model the structural and functional complexity of tumors. This approach helps address some limitations of conventional models and supports applications in drug development, personalized therapy, and immunotherapy^{44,160,182,183}. However, the clinical translation of these models raises several ethical challenges.

Foremost among the ethical concerns are the provenance of patient-derived cells and the adequacy of informed consent. Because tumor organoids are constructed from patient tissues, their collection, storage, and use must comply with rigorous ethical review and data protection standards to protect patient privacy and prevent misuse¹⁸⁴. The patient-specific nature of bioprinted products further blurs the boundary between research and clinical treatment, complicating clinical trial design and ethical review¹⁸⁵. Before such technologies are introduced into clinical practice, their safety, efficacy, and the robustness of patient consent must be critically evaluated to mitigate the risk of therapeutic misconception¹⁸⁶. Equitable access is another societal concern, as the high cost and technical complexity of bioprinted organoids may exacerbate existing disparities in healthcare resource distribution¹⁸⁷. Therefore, ethical considerations in clinical applications should address not only patient safety and rights but also

the broader social impact and equity of technology adoption. A multidisciplinary ethical oversight framework is therefore needed to guide responsible clinical translation.

Given the ethical and regulatory requirements for bioprinting, researchers and clinicians should receive ongoing ethics training to ensure responsible application of this technology while balancing patient welfare with scientific progress^{186,188}.

4.2.3 Regulatory Framework Out of Step with Technological Advances

The rapid development of bioprinting technology presents new challenges for existing regulatory frameworks. Because bioprinting integrates cell biology, materials science, and engineering, it does not fit neatly within conventional regulatory categories for medical devices or pharmaceuticals. Regulatory frameworks for tissue-engineered medical products and 3D-bioprinted products are still evolving and must address raw materials, cell sources, manufacturing processes, and final-product safety assessments¹⁸⁹.

Regulatory lag remains a major barrier to clinical translation. Some countries and regions have begun to develop bioprinting-specific regulatory frameworks. For example, Australia revised relevant regulations in 2021 to better accommodate the unique characteristics of bioprinting technologies. However, unclear product classification and insufficient evidence standards remain unresolved, limiting the capacity of current regulatory systems to keep pace with technological innovation¹⁸⁵. Surveys indicate that the lack of clear regulatory guidance and ethical standards remains a major barrier to widespread adoption, highlighting the need for professional training and regulatory refinement¹⁸⁶. Regulatory lag may hinder the clinical translation and commercialization of bioprinting technologies, thereby limiting patient access to innovative treatments.

Quality control and safety assessment are central to regulatory concerns. Because bioprinted products typically involve living cells and biomaterials, their manufacturing processes must comply with GMP, and systematic evaluation of biocompatibility, toxicity, and clinical efficacy is required^{190,191}. Regulations also require ethical review and informed consent for tissue-engineered products in clinical trials to protect patients' rights.

In summary, the development and refinement of regulatory frameworks are important for standardizing the research, development, and clinical application of bioprinting technologies. Future regulatory efforts should balance technological innovation with safety oversight. Multistakeholder collaboration will also be needed to establish flexible and scientifically sound frameworks that support the standardized development and clinical translation of advanced technologies such as bioprinted tumor organoids^{189,192}.

5. Multimodal Fusion and Interdisciplinary Innovation

5.1 Scenarios for Multimodal Fusion Technology

The convergence of bioprinting with microfluidics, advanced imaging, and programmable perturbation technologies is moving tumor organoid models beyond static structural reconstruction toward dynamic biomimicry and functional analysis. This shift expands organoid engineering along three programmable axes: from static spatial patterning to temporally responsive constructs, from single-tissue models to organ-on-a-chip systems that capture perfusion and inter-organ interactions, and from passive scaffolds to actively tunable microenvironments. Such multimodal integration improves the precision and controllability of *in vitro* TME reconstruction. It can generate platforms with improved physiological relevance and translational potential for mechanistic studies, drug screening, and personalized therapy^{141,193}.

The integration of bioprinting with microfluidics has generated synergistic opportunities for constructing tumor organoid models with dynamic microenvironments. Bioprinting enables controlled spatial positioning of cells and ECM components, making it well suited for modeling tumor cell heterogeneity and TME interactions¹⁴¹. However, bioprinting alone cannot fully capture the dynamic physical and chemical gradients present *in vivo*. Microfluidic technology addresses this limitation by using microscale fluid dynamics to regulate the transport of cytokines, drugs, and nutrients. It can also help model dynamic changes in perfusion and metabolism. Integrating these two technologies into a unified tumor-on-a-chip platform enables dynamic regulation and real-time monitoring of the TME. It may also improve the throughput and precision of drug screening and tumor immune microenvironment research^{141,149} (**Figure 7**). In bladder cancer research, for example, PDOs constructed using combined bioprinting and microfluidic platforms have modeled intratumoral heterogeneity and tumor-immune cell interactions, providing useful tools for drug-sensitivity assessment and personalized therapy¹⁴⁹. Similarly, bioprinted liver tumor spheroids cultured under dynamic microfluidic conditions show improved nutrient exchange and drug responsiveness, thereby enhancing model physiological relevance and drug-response assessment¹⁶⁰. Collectively, this integrated strategy supports the programmable reconstruction of TME dynamics across temporal and spatial scales, providing an *in vitro* platform for linking perfusion, transport, and cellular interactions to tumor behavior and therapeutic response¹⁹⁴.

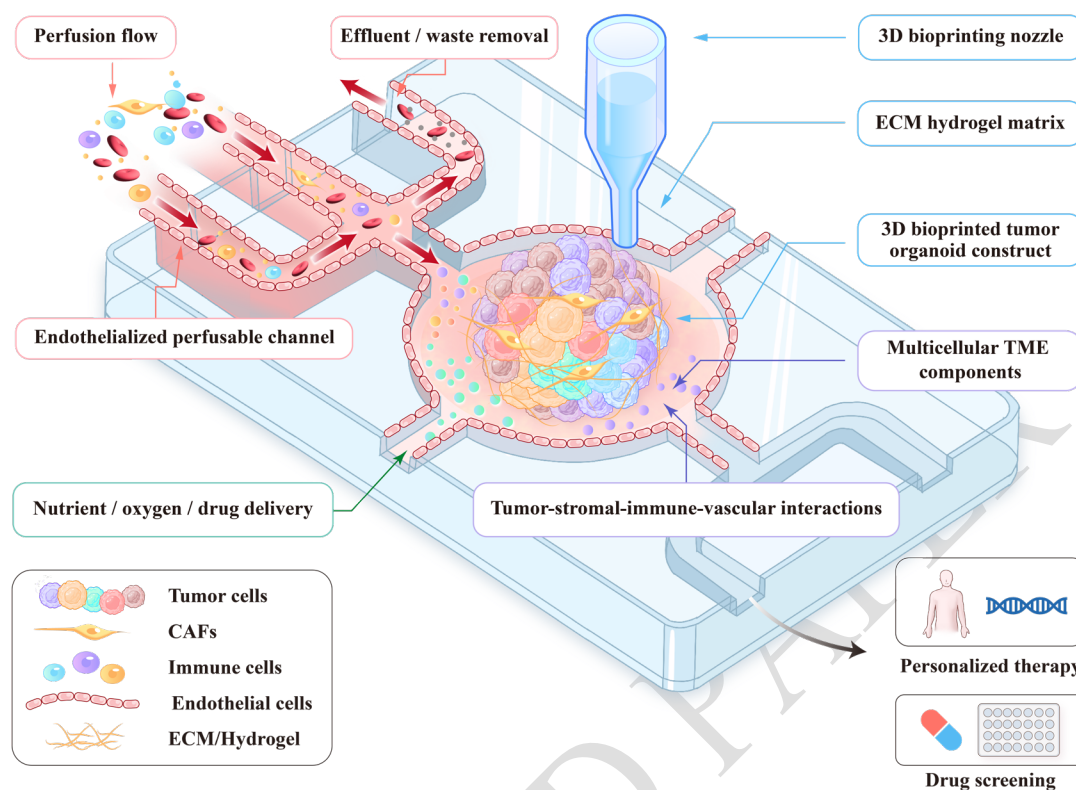


Figure 7. Integrated tumor-on-a-chip platform combining 3D bioprinting and microfluidics for dynamic TME reconstruction. This schematic illustrates a microfluidic tumor-on-a-chip system integrated with 3D bioprinting. A bioprinting nozzle deposits cell-laden ECM hydrogel to construct a multicellular tumor organoid containing tumor cells, CAFs, immune cells, endothelial cells, and ECM/hydrogel components. The surrounding endothelialized perfusable channels provide controlled perfusion flow, nutrient/oxygen/drug delivery, and effluent/waste removal, thereby recapitulating dynamic transport processes and microenvironmental gradients. Within the central culture chamber, the 3D bioprinted tumor organoid enables spatial organization of multicellular TME components and modeling of tumor–stromal–immune–vascular interactions. This integrated platform supports programmable reconstruction of tumor heterogeneity and TME dynamics, providing a physiologically relevant *in vitro* model for drug screening, tumor immune microenvironment research, and personalized therapy.

The introduction of gene editing technologies, particularly the CRISPR/Cas9 system, has enabled precise genomic manipulation within tumor organoid models (**Figure 8**). CRISPR/Cas9 can be delivered as ribonucleoprotein complexes to induce site-specific cleavage of target DNA, allowing targeted knockout, knock-in, or base editing. Edited cells can then be expanded in 3D dynamic tumor cultures and organoid platforms for mechanistic studies, including apoptosis, proliferation regulation, and genetic screening. These platforms can also support personalized drug testing and the assessment of culture-associated genetic drift during

long-term expansion. This approach can help clarify mechanisms of tumor initiation, progression, and drug-resistance evolution, thereby supporting personalized medicine^{6,195}. In glioma models, gene knockout strategies that mimic the loss of key regulatory genes allow mechanistic investigation of their roles in TME interactions and therapeutic response^{194,196}. Beyond loss-of-function studies, gene editing can also be used to modulate immune regulatory genes such as Eomes, enabling controlled analysis of immune evasion in vitro. This approach helps connect molecular regulation with responses to immune checkpoint inhibitors, such as anti-PD-1 therapy, and may guide immunotherapy optimization^{197,198}. CRISPR technology can help address genetic instability in organoid culture by enabling the targeted validation or correction of specific genetic alterations, thereby improving model stability and reproducibility⁶. One example is a high-throughput liver toxicity screening platform that integrates CRISPR/Cas9 with pluripotent stem cell-derived human liver organoids. This platform supports the prediction of drug-induced liver injury and genetic susceptibility across diverse genetic backgrounds, thereby improving the clinical relevance of the model¹⁹⁹. In summary, CRISPR gene editing has expanded the use of organoid models in mechanism studies, target discovery, and personalized therapy.

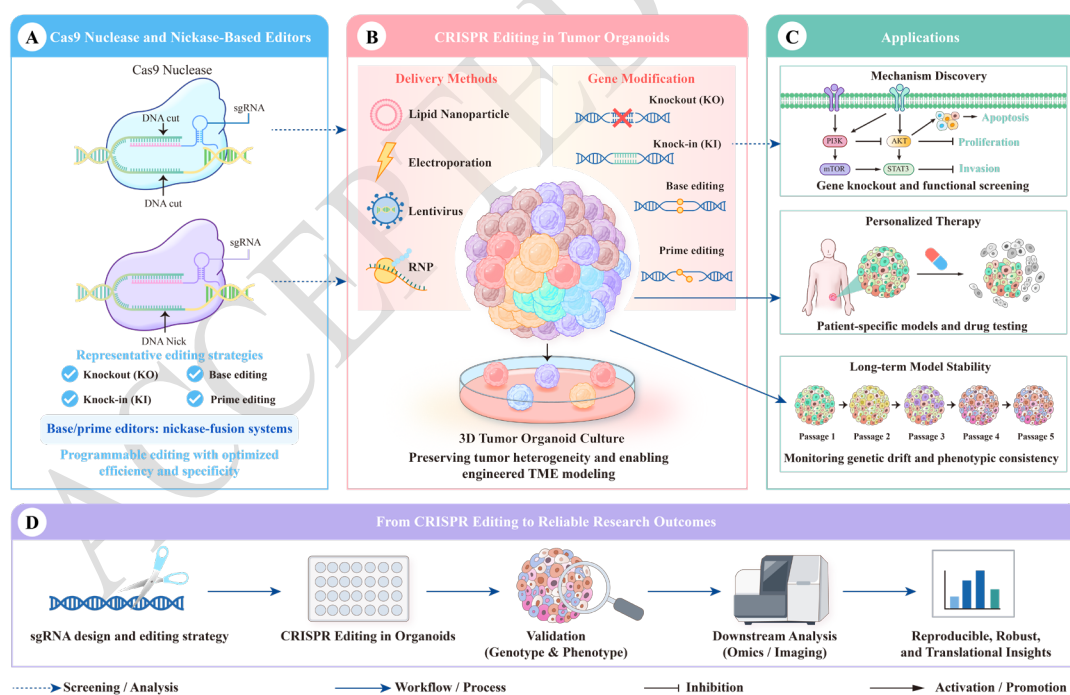


Figure 8. CRISPR-based genome editing in tumor organoids: editing strategies, delivery methods, applications, and validation workflow. CRISPR technologies enable programmable genomic manipulation in tumor organoid models. Cas9 nuclease induces site-specific double-strand DNA breaks for conventional knockout or knock-in editing, whereas nickase-based systems provide the basis for base editing and prime editing with improved precision. CRISPR

components can be introduced into tumor organoids using lipid nanoparticles, electroporation, lentiviral vectors, or ribonucleoprotein delivery. These approaches support gene knockout, knock-in, base editing, and prime editing while maintaining tumor heterogeneity in 3D organoid culture. Edited tumor organoids can be used for mechanism discovery, functional genetic screening, personalized drug testing, and evaluation of long-term model stability. The lower workflow highlights key steps from sgRNA design and editing strategy selection to organoid editing, genotype and phenotype validation, downstream omics or imaging analysis, and generation of reproducible and translationally relevant research outcomes.

Advances in imaging technology now support multiple stages of the bioprinted tumor organoid workflow, from construct design and process monitoring to functional validation. Medical imaging modalities, such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound, can non-invasively capture patient-specific 3D tumor architecture, including tumor morphology and spatial heterogeneity. Integrating these imaging data with bioprinting supports more faithful reconstruction of the TME and may improve predictions of drug penetration and treatment response²⁰⁰⁻²⁰². For instance, imaging-guided approaches can convert anatomical structures captured by CT or MRI into computer-aided design models. When combined with FRESH printing, this strategy enables the fabrication of patient-specific scaffolds that recapitulate tumor topology and reduce morphological distortion associated with conventional methods. These scaffolds support the generation of organoids that better reflect the boundaries and spatial architecture of the patient's tumor²⁰³. During subsequent culture, non-destructive imaging techniques, including optical coherence tomography and fluorescence microscopy, can be integrated with deep learning-based segmentation algorithms. This enables continuous 3D monitoring of bioprinted organoid clusters and quantification of key process parameters, such as volume growth, cavity formation, and fusion^{204,205}. These non-invasive modalities shift organoid research from static endpoint assessment toward dynamic process analysis, improving the visualization and quantitative evaluation of model development.

Multimodal 3D volumetric imaging and fluorescent labeling further expand organoid characterization beyond structural assessment by enabling functional and spatial analysis. For example, the OrBITS image analysis platform combines bright-field imaging with fluorescent labeling to monitor organoid development and drug-induced cell death in real time²⁰⁶. Meanwhile, a 3D imaging workflow combining viral transduction-based fluorescent labeling with fluorescence micro-optical sectioning tomography enables high-resolution mapping of cellular composition and functional distribution within intact brain organoids²⁰⁷. The integration of imaging with bioprinting nonetheless faces persistent challenges, including the trade-off between spatial resolution and penetration depth, material-induced optical interference, multimodal data registration, and phototoxicity. As high-resolution imaging, AI-

driven image analysis, and multimodal data fusion continue to advance, image-guided bioprinted organoid platforms may support the development of an image–model–response closed-loop framework. This framework could enable data-driven drug selection and mechanistic validation.

The integration of bioprinting with AI and machine learning is advancing tumor organoid engineering from experience-driven fabrication toward data-driven intelligent manufacturing and predictive analysis. For printing-parameter optimization, machine learning can build surrogate models from limited experimental data to map relationships among process parameters, material rheology, and printing quality. When coupled with Bayesian optimization algorithms, this approach can accelerate the identification of optimized process windows, thereby improving efficiency and reproducibility²⁰⁸. For process quality control, in situ imaging combined with deep learning enables automated segmentation of printed structures and real-time defect detection. This supports cross-batch stability control¹⁸⁰. AI can further quantify cell dose and spatial distribution. For example, optical measurements of droplet velocity can be used to train models that predict the number of cells in individual droplets, thereby providing a quantitative basis for linking cell dose and spatial distribution to phenotype and drug sensitivity²⁰⁹. For drug-response prediction, the integration of bioprinting and AI can be organized into a two-stage strategy. First, bioprinting standardizes organoid preparation, thereby minimizing experimental noise. Second, multimodal AI integrates dynamic morphological changes, molecular profiles, and drug response data to construct predictive models with genuine extrapolative capacity. To address the limited availability of organoid drug-sensitivity data, approaches such as PharmaFormer use Transformer-based transfer learning, with pre-training on large-scale cell-line datasets followed by fine-tuning with organoid-specific data. This strategy improves the prediction of drug responses in organoid-based screening²¹⁰. Prospective evidence has also begun to substantiate the predictive utility of organoids in real clinical cohorts and their translational value in biomarker discovery^{211,212}. When further integrated with standardized 3D printing platforms and AI-driven rapid readout technologies, this paradigm is poised to shorten the timeline from tissue acquisition to treatment recommendation, thereby advancing clinical applicability.

5.2 Emerging Technologies and Future Directions

5.2.1 Dynamic Remodeling of Time-Responsive Structures

4D bioprinting can be defined as programmable spatiotemporal biofabrication. After 3D deposition, the printed construct undergoes time-dependent changes in morphology, mechanical properties, or the presentation of bioactive signals in response to external or

endogenous cues. These cues may include hydration or exposure to physiological fluids, temperature, light, magnetic fields, ionic or biochemical microenvironments, enzymatic remodeling, and cell-generated traction forces. By embedding transformation pathways into the initial design, 4D bioprinting treats the printed construct as an intermediate state rather than a final structure. The construct then evolves according to predefined triggering conditions, deformation kinetics, reversibility, and spatial propagation rules²¹³⁻²¹⁶. This dynamic behavior can better mimic tissue remodeling processes during development, regeneration, and disease progression^{214,216}.

Two representative strategies have emerged for stimulus-responsive morphological remodeling. The first relies on exogenous stimuli. For example, magnetoresponsive nanoparticles incorporated into cell-laden hydrogels or bioinks can induce magnetic-field-driven actuation, thermal responses, and mechanical regulation of cell behavior, enabling remote and time-controlled 4D deformation²¹⁵. The second strategy harnesses endogenous cell-generated forces. Printable scaffolds or granular hydrogel systems can be designed to allow stress relaxation, softening, degradation, or matrix remodeling during culture. These material changes can translate collective cell contractility into predictable bending, curling, folding, or other programmed shape transformations^{214,216}. Historically, Kirillova and Ionov's group developed an early 4D biofabrication strategy using shape-morphing hydrogels to generate hollow, self-folding, cell-laden tubular structures. This work provided a basis for later studies on self-organizing geometries driven by material transformation and cell-matrix interactions²¹³.

Recent studies have further developed the concept of deformation-driven maturation. Pramanick et al. established an embedded 4D bioprinting platform using collagen-hyaluronic acid bioinks printed within yield-stress granular support hydrogels. In this system, cell-driven shape morphing could be programmed by print geometry, cell phenotype, bioink composition, and bath viscoelasticity. Finite element modeling was used to predict deformation patterns, and programmed morphing enhanced structural and functional maturation in induced pluripotent stem cell-derived cardiac tissues²¹⁴. Extending this paradigm, a recent study demonstrated designable deformation kinetics through material engineering strategies such as thickness gradients, advancing 4D deformation from phenomenological demonstration toward parametric engineering design²¹⁷.

Despite its promise for constructing dynamic tissue models, 4D bioprinting still faces key challenges in disease modeling and organoid applications. Although current studies have shown that printed constructs can undergo programmed deformation, the field must move beyond phenomenological shape change toward frameworks that explain, reproduce, and quantitatively link deformation dynamics to biological function. These frameworks should incorporate

multidimensional dynamic readouts, including time-resolved geometry, mechanical properties, mass-transfer conditions, and cell–matrix interactions throughout the deformation process^{214,216}. More importantly, these biological readouts should be systematically linked to physical design parameters, such as stimulus dose and threshold, deformation kinetics, reversibility, material viscoelasticity, and spatial propagation rules. This linkage could establish a transferable modeling framework for 4D biofabrication²¹⁴. Programmable softening, stiffening, stress relaxation, and shape transformation can then be used to simulate matrix-stiffness evolution and deformation-induced strain-field reorganization. In this way, environmentally responsive remodeling can be translated into testable biological hypotheses for development, regeneration, and disease modeling^{214,216}.

5.2.2 Organoids and Integrated Platforms Combining Microfluidics and Sensing/Detection

Tumor-on-a-chip and organoid-on-a-chip technologies can shift organoids from static droplet cultures toward microphysiological systems with controlled perfusion, defined gradients, and longitudinal monitoring. Microfluidic systems can impose shear stress, interstitial flow, and microscale gradients of nutrients, drugs, oxygen, and metabolites. Vascular or endothelial barrier modules further support physiologically relevant modeling of drug administration, distribution, permeation, and action. These platforms may generate drug-response profiles that better reflect *in vivo* pharmacokinetic/pharmacodynamic conditions while improving the standardization of culture geometry, perfusion strategies, chamber volume, and material interfaces²¹⁸⁻²²⁰. Recent translational platforms have moved beyond simply placing organoids in flow channels. Instead, they increasingly integrate TME architecture design with readout modalities, including automated imaging, algorithm-assisted analysis, molecular profiling, and integrated sensors, to generate more reproducible and mechanistically informative drug-screening workflows^{218,220-222}.

At the architectural level, stable organoid positioning and high-throughput analysis remain key challenges. Microfluidic platforms with microwell arrays, traps, capture structures, or modular chambers enable individual organoid positioning, automated imaging, and multi-condition comparisons^{165,218,221}. In colorectal cancer, Lin et al. developed a high-density microwell array for single-cell-derived tumor organoids (STOs), positioning thousands of STOs at predefined locations within a single focal plane for automated high-content analysis. By integrating phenotypic drug screening, RNA sequencing of resistant subpopulations, computational drug prediction, and on-chip validation, this platform created a closed-loop workflow linking heterogeneity-resolved screening with molecular interpretation and therapeutic revalidation¹⁶⁵. Similarly, Grouped-seq integrates imaging-based phenotyping with

genome-wide transcriptomic readouts on a microwell chip. This approach reduces sample and reagent consumption while enabling mechanistic interpretation of drug responses in patient-derived tumor organoids²²¹.

At the detection level, multimodal sensing is shifting tumor-on-chip systems from endpoint assays toward longitudinal and minimally perturbative analytical platforms. Integrated physical and barrier readouts, such as trans-epithelial/endothelial electrical resistance (TEER) and impedance, can monitor epithelial or endothelial barrier integrity and drug-induced barrier disruption. Chemical sensors for oxygen and pH can report hypoxia and metabolic remodeling. Metabolic or functional readouts, such as ATP/ADP ratios, provide dynamic information on cellular stress and viability^{222,223}. Izadifar et al. developed a sensor-integrated Organ Chip incorporating TEER, oxygen, pH, and metabolic activity measurements, which enabled continuous and non-invasive monitoring during prolonged culture²²². Extending such multimodal sensing to tumor organoid-on-a-chip systems could provide dynamic process indicators of drug penetration, tolerance development, and microenvironmental remodeling, thereby addressing some limitations of endpoint assays such as live/dead staining^{222,223}.

At the process level, an important trend is to integrate molecular diagnostics, functional drug-sensitivity testing, and automated execution within a single platform, especially when clinical turnaround times and sample availability are limited. Zhang et al. developed a dual-functional microfluidic chip that combined rapid EGFR mutation detection using DNA nanorulers with drug testing in lung cancer organoids. DNA nanosensors also enabled in situ ATP monitoring during organoid growth and drug response, supporting further automation of the system²²³. These advances suggest that chip-based organoid platforms can couple molecular stratification with functional response testing, thereby supporting more clinically actionable precision-oncology workflows^{220,223}.

At the application level, immunotherapy evaluation has promoted the integration of chip-based platforms with live imaging and quantitative image analysis. Veith, Nurmik, and colleagues established patient-derived lung tumor-on-chip models containing autologous primary cells. Using live-cell imaging and advanced image analysis, they quantified anti-PD-1-induced, T-cell-mediated tumor killing. Notably, the addition of FAP⁺CAFs impaired the anti-PD-1 response, suggesting that tumor-on-chip systems can model stroma-dependent immunotherapy resistance¹⁷⁰. In a complementary study, Bains et al. developed a vascularized tumor-on-chip model incorporating an endothelial-lined perfusable channel, tumor cells, M2-like macrophages, and CAR-T cells. This model showed that M2-like macrophages induced PD-L1 expression and suppressed ICAM-1 expression in adjacent endothelial cells, thereby limiting CAR-T extravasation and effector function. Anti-PD-L1 treatment partially reversed

this inhibitory cascade¹⁶⁹. Together, these studies show that chip-based tumor models can integrate vascular barriers, immune suppression, cell therapy, and quantitative imaging. They provide a practical framework for incorporating cytokine, metabolic, and barrier sensing into unified platforms for mechanistic analysis^{169,170,222}.

5.2.3 Development of a New Bioink System

Bioink performance strongly influences the geometric fidelity, biological activity, and functional feasibility of bioprinted constructs. Conventional hydrogel bioinks, including alginate-, GelMA-, and silk fibroin-based systems, have improved biocompatibility and printability. However, they often cannot simultaneously meet the competing requirements of high-fidelity fabrication, suitable mechanical properties, printing stability, and cell-supportive microenvironments. Therefore, the development of next-generation bioinks that integrate mechanical robustness, bioactivity, printability, and clinical applicability is important for moving bioprinting from laboratory prototyping toward translational applications^{224,225}.

In terms of material systems, research is increasingly focused on multicomponent composite bioinks and functionalization strategies. Natural proteins and nanocomponents have been incorporated to improve mechanical, rheological, and bioactive properties. Fibroin, for example, has been used as a bioink component in cartilage tissue engineering because it offers shear-thinning behavior, cytocompatibility, biodegradability, printability, and structural support^{226,227}. Nanofibers, such as lysozyme nanofibers (LNFs) and cellulose oxalate nanofibers, can improve shear-thinning behavior, printing accuracy, mechanical strength, and structural stability of hydrogels while maintaining cell viability^{228,229}. Protein-based additives, including egg white, have also been used to functionalize alginate-methylcellulose bioinks, supporting cell adhesion, proliferation, and osteogenic differentiation²³⁰. Collectively, multicomponent composites offer a viable pathway for integrating printability, mechanical support, and biological guidance within a single ink platform.

Performance enhancement strategies mainly involve rheological control, dynamic crosslinking, and additive incorporation, and can be organized into three principal approaches. The first is rheological optimization for print fidelity. Organic-inorganic hybrid hydrogels combining thermogel-forming copolymers, alginates, and inorganic clays substantially improve thermogelation and shear-thinning behavior, enhancing both shape retention and layer-by-layer stability²³¹. The second approach employs composite microspheres to tailor mechanical and biological performance. Gelatin-hydroxyapatite microspheres, for example, modulate ink mechanics and cell migration to promote osteogenic differentiation²³². Similarly, mesenchymal stem cell-loaded hydrogel microspheres can be assembled into organoids through

digital light processing and stepwise induction. These organoids model endochondral ossification in vitro and promote the repair of large bone defects after implantation²³³. The third strategy harnesses dynamic crosslinking systems, such as boronate-crosslinked hyaluronic acid-based hydrogels, to regulate chemical modification and mechanical properties in a spatiotemporally controlled manner, thereby expanding the design space for 4D bioprinting²³⁴. Beyond ink formulation, auxiliary materials, including support baths and sacrificial inks, help address challenges such as shape collapse and insufficient resolution. These materials help preserve the structural fidelity of printed fibers and enable the fabrication of complex, biologically relevant architectures^{235,236}.

For cellular biocompatibility, the incorporation of platelet concentrates into alginate-gelatin bioinks enables sustained release of growth factors, preserving cell viability and promoting tissue regeneration^{237,238}. Computational methods, including machine learning and finite element modeling, can further optimize ink formulations and printing parameters, thereby improving printing accuracy and functional performance while reducing experimental burden^{239,240}. In summary, the development of high-performance bioinks requires coordinated optimization of rheological properties, mechanical strength, biological activity, and process compatibility. This provides a material and manufacturing foundation for bioprinting applications in tumor organoids and broader tissue engineering.

5.2.4 Interdisciplinary Collaborative Innovation

Interdisciplinary technological convergence is advancing tumor organoid models from static biomimetic constructs toward dynamic, programmable microphysiological systems. By integrating microfluidic platforms with phenotypic imaging, transcriptomic profiling, and functional perturbation, researchers can investigate tumor heterogeneity and microenvironmental interactions under controlled and reproducible conditions. For example, microwell chip-based organoid platforms can couple high-throughput drug-response phenotyping with genome-wide transcriptomic readouts, allowing drug-response mechanisms to be inferred rather than merely observed²²¹. Similarly, single-cell transcriptomic mapping combined with tumoroid co-culture can functionally reconstruct and validate computationally predicted tumor-TME interactions in vitro. This moves organoid research from phenomenological description toward mechanistic attribution²⁴¹.

The introduction of engineered bacteria as living modulators of the microenvironment is emerging as a strategy that may advance organoid culture from static nutrient supplementation toward more programmable niche regulation. The value of incorporating bacteria extends beyond modeling infection or symbiosis. Microbiome-compatible organoid systems provide an

experimental basis for studying localized host–microbe interfaces. In addition, synthetic biology enables bacteria to be engineered as programmable living sensors and actuators^{242,243}. For example, hypoxia-tolerant apical-out intestinal organoids provide direct access to the epithelial surface and support co-culture with anaerobic bacterial strains. This platform enables investigation of bacterial colonization and epithelial niche responses under physiologically relevant oxygen conditions²⁴². In engineered systems, bacteria can be programmed to sense disease-associated molecular cues. For example, engineered *Acinetobacter baylyi* has been shown to detect tumor-derived DNA from colorectal cancer cells, organoids, and tumors through a modular genetic biosensing strategy²⁴³. More broadly, synthetic gene circuits enable the modular assembly of sensing, logic, and output functions, allowing effector release to be coupled to defined environmental or temporal cues²⁴³. Such designs may help address some limitations of exogenously supplied soluble factors, including rapid diffusion within 3D matrices, limited spatial selectivity, and difficulty in maintaining long-term gradients. In TME research, engineered attenuated *Salmonella* strains have been developed as tumor-localizing delivery vehicles for immunomodulatory payloads. A recent study engineered attenuated *Salmonella* Typhimurium to release ClyA and FlaB, thereby inducing immunogenic cancer cell death, DAMPs and tumor antigen release, macrophage remodeling, and tumor-specific T-cell responses in mouse tumor models. Although this strategy has not yet been validated in tumor organoid or bioprinted organoid platforms, it may provide transferable design modules and a functional evaluation framework for future integration into controllable 3D tumor models²⁴⁴.

Achieving stable and reproducible bacterial effects in organoids—particularly in bioprinted constructs—requires a shift from simple co-culture toward controlled loading, exposure, and clearance. The central challenge is not merely introducing bacteria into the system, but engineering three key interfaces. The first is loading and spatial addressing: precisely confining bacteria to cavity surfaces, defined hydrogel layers, or microfluidic branch channels. The second is exposure and dose control: the contact area, residence time, and bacterial density at the interface with epithelial or tumor cells must be quantitatively regulated. The third interface is biosafety and clearance: bacterial survival and activity should be controllable through suicide switches, inducible autolysis systems, or exogenous triggering mechanisms that permit on-demand elimination. At the methodological level, organoid–microorganism co-culture has become increasingly standardized. Common introduction strategies include intracavitary microinjection, extracavitary exposure, reverse-polarity culture, and open epithelial monolayers. Quality control checkpoints are typically centered on barrier-function integrity, microbial-load stability, and host-cell stress status^{244,245}. Remotely triggered genetic circuits further extend gene-expression control from chemically induced regulation to physically triggered regulation. For example, ultrasound-triggered expression can provide

improved spatiotemporal selectivity and reversibility within 3D constructs, thereby reducing the risk of system destabilization caused by prolonged transgene expression²⁴⁶. At the evaluation level, high-throughput bacteria–3D tumor tissue co-culture platforms enable parallel comparison of different bacterial strains, effector payloads, and genetic circuits within 3D microtissues. By quantifying bacterial dynamics, therapeutic efficacy, and safety profiles, these platforms provide a scalable framework for evaluating engineered bacteria in organoid-based drug screening and mechanistic studies²⁴⁵.

Building on controlled loading and dynamic regulation, single-cell and spatial multi-omics technologies can convert the predefined architectures of bioprinted models into interpretable maps of cellular heterogeneity. These approaches allow spatiotemporal relationships between microenvironmental variables and cellular states to be analyzed at single-cell resolution. The core strength of bioprinting lies in its ability to predefine spatial structures, including cell arrangement, matrix composition, mechanical gradients, and perfusion pathways. However, post-printing phenotypic evolution is often assessed only at endpoint. Integrating spatial transcriptomics with single-cell omics can link cell states, spatial locations, and microenvironmental variables within printed constructs, thereby converting structural control into mechanistically interpretable biological insight. To address challenges such as difficult organoid sectioning and limited cell numbers, layered organoid spatial transcriptomics processes samples into analyzable near-monolayer architectures. This approach reduces spatial information loss, improves procedural consistency, and provides a practical histological interface for aligning printing coordinates with spatial omics coordinates²⁴⁷. Concurrently, scalable high-resolution 3D spatial transcriptomics workflows have enabled transcriptomic profiling across 3D volumes, with subcellular resolution and 3D reconstruction. These approaches allow direct testing of a key hypothesis: whether predefined gradients of oxygen, nutrients, drugs, and stiffness can drive the intended cell states and spatial partitioning within printed models²⁴⁸.

The application of spatial multi-omics tools provides critical support for mechanistic studies centered on reactive oxygen species (ROS) and metabolic heterogeneity. Both ROS levels and metabolic states show temporal variability and spatial heterogeneity. Within a single tissue construct, hypoxic core regions, proliferative peripheral zones, and stroma-rich compartments may display distinct redox states and metabolic pathway preferences. To overcome the limitations of static molecular endpoint assays in capturing dynamic biological processes, a two-step strategy can be adopted: nondestructive functional imaging followed by spatial molecular attribution. First, label-free live imaging provides reproducible functional readouts during early drug exposure. Second, spatial omics analysis at selected time points enables molecular attribution and spatial localization of the mechanisms underlying drug

response. Label-free microscopy, which leverages endogenous fluorescence and multi-harmonic signals, can help predict DNA damage responses in 3D non-small-cell lung cancer organoids using indicators such as the optical redox ratio. These findings suggest that metabolic and redox states may serve as early readouts of tumor heterogeneity, subpopulation divergence, and emerging drug resistance²⁴⁹⁻²⁵¹. At the spatial metabolic level, desorption electrospray ionization mass spectrometry imaging combined with co-section multiplex immunofluorescence resolves the spatial distribution of metabolites—including lipids—while preserving tissue architecture and immune cell context. This approach links metabolic signatures with cellular phenotypes and invasive behavior, providing spatial evidence for investigating metabolic crosstalk among tumor cells, immune cells, and stromal populations²⁵². Incorporating this strategy into a bioprinting workflow can establish an iterative research loop. First, models with predefined gradients or microstructures are fabricated. Live redox or metabolic functional imaging is then used to track the emergence of heterogeneity. Spatial transcriptomic or metabolomic analysis can be performed at selected time points to localize the underlying molecular changes. Finally, these findings can be traced back to material and structural parameters for causal validation, for example, by altering perfusion conditions, matrix stiffness, or engineered bacterial output to test whether heterogeneity reorganizes in the predicted direction. This iterative cycle helps move bioprinted organoid research from descriptive correlation toward causal testing.

In summary, the integration of synthetic biology, microfluidic chips, spatial multi-omics, and functional imaging is transforming tumor organoid models from static structural mimics into dynamic, programmable, and analyzable in vitro microphysiological systems. Engineered bacteria provide living regulatory tools for spatiotemporal intervention, whereas microfluidic and 3D-printing platforms establish quantifiable interfaces for drug loading, exposure, and clearance. Spatial omics further aligns structural coordinates with cellular state maps, enabling validation of the relationships between microenvironmental variables and phenotypic heterogeneity. Together with functional imaging-based molecular attribution, these approaches help connect dynamic phenotypes with underlying mechanisms and support reversible validation. This interdisciplinary framework is expected to improve the mechanistic analysis of tumor heterogeneity, metabolic reprogramming, and immune evasion, while providing a technological basis for next-generation personalized drug screening and therapeutic optimization.

6. Conclusions and Outlook

In vitro tumor modeling is undergoing a paradigm shift from traditional two-dimensional, static, and spatially unstructured culture systems toward more dynamic and spatially organized

models. 3D bioprinted tumor organoids provide a promising platform for engineering cellular arrangement, extracellular matrix microenvironments, and multicellular interactions, thereby advancing tumor models from structural mimicry toward functional biomimicry¹¹⁻¹⁵. Rather than viewing these models as merely another category of 3D tumor models, this review frames bioprinted tumor organoids as programmable TME reconstruction platforms. The major value of this technology lies in its ability to engineer predefined cellular spatial organization, tunable mechanical and biochemical cues, and perfusable vascular networks that can be designed, measured, and functionally validated. These features help address key limitations of conventional self-assembled organoid models and support studies of tumorigenesis, drug-resistance evolution, and precision oncology applications.

Despite these advances, the clinical translation of bioprinted tumor organoids remains constrained by several persistent bottlenecks. Technically, the central challenge has shifted from demonstrating printability to achieving reproducible fabrication. The nonlinear coupling among material rheology, crosslinking kinetics, and cellular mechanics can generate difficult-to-control batch-to-batch variability. Without standardized CQA frameworks and corresponding CPP ranges, these models may remain artisanal constructs rather than reliable clinical decision-support tools^{17,21-24}. Biologically, current models still have limited ability to recapitulate the coordinated interplay of mechanical, chemical, and spatiotemporal signals within a single construct. Moreover, whether programmable features, such as stiffness gradients and defined vascular architectures, causally improve drug-response prediction remains to be rigorously validated through head-to-head comparisons with conventional organoids. Concurrently, ethical concerns related to patient tissue use and genetic manipulation, together with persistent regulatory lag in frameworks tailored to living cell-based products, present additional obstacles to clinical translation and adoption.

Looking ahead, three interconnected research directions may shape the next phase of development. The first is the establishment of a standardized and clinically translatable technology framework. This will require GMP-compliant automated bioprinting platforms integrated with quantitative CQA–CPP mapping databases. In parallel, well-designed prospective, multicenter clinical trials are needed to validate the clinical utility of bioprinted organoid-guided drug screening, using endpoints such as progression-free survival or overall survival. These efforts may support its future development as a companion diagnostic tool. The second direction is to develop physiologically relevant model systems with improved predictive fidelity. Future efforts should prioritize immunocompatible and vascularized tumor models incorporating autologous immune cells and functional endothelial barriers. In parallel, spatial multi-omics technologies should be deeply integrated to align printing coordinates with molecular state maps, enabling causal validation of microenvironmental regulation rather than

correlative observation alone. The third direction is the advancement of intelligent process control and predictive analytics. This will require integrating advanced printing modalities, including suspension and coaxial printing, with real-time in situ monitoring. AI-driven algorithms can also be incorporated for process optimization and drug-response prediction. Together, these approaches may reduce technical variability and shorten the timeline from patient biopsy to actionable therapeutic recommendations.

Ultimately, the value of bioprinted tumor organoid technology lies not in engineering complexity per se, but in whether such complexity can be made reproducible, verifiable, and clinically translatable while helping dissect the spatiotemporal complexity of the TME. With continued advances in materials science, engineering technologies, synthetic biology, and spatial multi-omics, bioprinted tumor organoids may become important platforms for next-generation precision oncology. Their continued development is expected to improve mechanistic cancer research, therapeutic evaluation, and personalized clinical decision-making, ultimately contributing to better patient-centered cancer care.

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Conflict of interest

The authors declare no competing interests.

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