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Advances in three-dimensional bioprinted tumor organoids: From model construction to clinical translation

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Abstract

Traditional cancer research models face inherent limitations, as two-dimensional cell cultures fail to capture the complexity of tumor biology, while animal models are confounded by species-specific discrepancies. The integration of tumor organoids with three-dimensional (3D) bioprinting has recently emerged as a transformative strategy. This approach combines the histological and genetic fidelity of organoids with the spatial precision and structural controllability of 3D bioprinting, thereby enabling the fabrication of biomimetic tumor models. Such models more faithfully recapitulate critical features of the tumor microenvironment (TME), addressing major gaps in conventional experimental systems. This review systematically examines the principles, recent advances, and translational applications of 3D bioprinting-enabled tumor organoids, including the biological basis of organoids, key bioprinting strategies, and technical considerations. Major applications include constructing heterogeneous TMEs with immune interactions, engineering vascularized tumor structures, enabling high-throughput drug screening, validating bioprinted organoids using clinical samples, and advancing clinical translation, regulatory frameworks, and Good Manufacturing Practice-compliant manufacturing of tumor organoids. Despite substantial progress, several challenges remain, including limited printing resolution, bioink instability, difficulties in sustaining long-term cultures,

and gaps in standardization. Nevertheless, integration with emerging technologies, such as microfluidics, artificial intelligence, big data analytics, and standardized biomanufacturing platforms, is anticipated to bridge the gap between basic tumor research and clinical translation. Ultimately, these synergistic advances may accelerate the development of personalized cancer therapies and improve patient outcomes.

Keywords: Clinical translation; Drug screening; Three-dimensional bioprinting; Tumor microenvironment; Tumor organoids

1. Introduction

Cancer is among the leading causes of morbidity and mortality worldwide. Its complex pathogenesis and pronounced interpatient and intratumoral heterogeneity have long posed major challenges in both clinical treatment and basic research.[1] To address the challenges of cancer treatment, establishing research models that can accurately simulate the biological characteristics of human tumors has become crucial. An ideal tumor model must meet three core requirements: structural mimicry, functional relevance, and clinical translatability. However, traditional research models have insurmountable limitations.[2]

Although two-dimensional (2D) cell culture systems enable high-throughput experimentation, they lack three-dimensional (3D) structure and interactions with the extracellular matrix (ECM).[3] This limitation makes it impossible to reproduce the polar organization, nutrient gradient distribution, and heterogeneous phenotypes of tumor cells.[4,5] Animal models can partially simulate the *in vivo* microenvironment; however, due to species differences, significant deviations exist in metabolic pathways and immune responses. For instance, the rate of drug metabolism in rodents often differs significantly from that in humans, and the activation patterns of immune cells, such as T lymphocytes, show notable species-specific variations. This discrepancy contributes to the extremely high failure rate of candidate drugs that are effective in animal models during clinical trials.[6,7] These dilemmas highlight the urgency of developing new tumor models, and the cross-integration of organoid technology with 3D bioprinting is providing a revolutionary solution to this problem.[6,8]

Organoid technology enables the formation of self-organizing, miniaturized organ structures through *in vitro* 3D culture of stem cells or primary tumor cells, which closely resemble the source tumors in terms of gene expression profiles, histopathological features, and drug response patterns.[5,6,9–11] The foundational development of organoid technology can be traced back to the establishment of intestinal organoids by Sato *et al.*[12] in 2009, which demonstrated that single $Lgr5^+$ stem cells could self-organize into crypt–villus structures *in vitro* without a mesenchymal niche—a breakthrough that laid the groundwork for subsequent tumor organoid models. Subsequently, tumor organoid technology rapidly expanded to multiple cancer types, such as breast cancer[13] and colorectal cancer,[14] providing a "patient avatar" model for personalized medical research. However, because organoids form spontaneously, it is difficult to precisely control the spatial distribution of cells and microenvironmental components, especially when simulating complex biological processes such as tumor–stroma interactions and vascular infiltration.[6,10,15]

The emergence of 3D bioprinting technology has addressed these limitations. Through computer-aided design and the precise deposition of biological materials, this technology enables the spatial positioning and assembly of cells and matrices, allowing the construction of complex tissue models with biomimetic structures.[6] When combined with tumor organoids, 3D bioprinting not only preserves tumor-derived characteristics of organoids but also endows models with a precise structural hierarchy and functional compartmentalization through controlled printing, forming a "bioengineered tumor microenvironment (TME)".[9] This innovative approach can simulate dynamic processes such as tumor invasion and metastasis and facilitate the construction of multicellular interaction networks involving blood vessels, immune cells, and fibroblasts. Consequently, it provides an unprecedented research platform for investigating TME regulatory mechanisms and developing novel targeted therapies.[4,15]

This review systematically examines the integrated application of tumor organoids and 3D bioprinting technology, comprehensively analyzing technical advances and existing challenges, from model construction principles to clinical translation potential, with the aim of

providing theoretical insights and practical guidance to advance precision oncology research.

2. Overview of tumor organoids

2.1. Definition and biological characteristics of tumor organoids

Tumor organoids are miniature tumor structures formed through the self-renewal and differentiation of stem cells/progenitor cells isolated from tumor tissues or cancer cell lines in a 3D culture system that simulates the *in vivo* microenvironment.[6,15,16] Their core characteristics include: histological fidelity (retention of the glandular structure and cellular heterogeneity of the original tumor), genetic stability (maintenance of gene mutation profiles during long-term culture), and functional relevance (exhibition of tumor biological behaviors such as proliferation, invasion, and drug response).[15,17]

Tumor organoids can be divided into three categories based on their origin: patient-derived organoids (PDOs),[17] cell line-derived organoids,[18–20] and genetically engineered organoids[21] (Figure 1). Among these, PDOs are directly derived from clinical samples and most accurately reflect individual tumor characteristics, making them the gold standard model for personalized medical research.[22]

2.2. Comparison of tumor organoids with conventional tumor models

2D cell culture systems form monolayers of cells through adherent growth. Although they enable large-scale expansion, they often disrupt the native spatial organization of cell–cell junctions and reduce interaction with the ECM, resulting in significant differences in gene expression profiles compared with *in vivo* tumors.[15,16,23]

Patient-derived xenograft (PDX) models, a branch of animal models, transplant patient tumor tissues into immunodeficient mice, and can better preserve aspects of the TME.[24] However, these models present several limitations. For example, their construction success rate is relatively low, and human stromal cells are gradually replaced by murine cells during serial passaging.[19,25] This replacement distorts the original TME, leading to inaccurate experimental outcomes, especially in studies focusing on stromal–tumor cell interactions. As a result, the application of PDX models in personalized precision medicine is severely restricted, limiting their ability to effectively guide clinical treatment decisions.

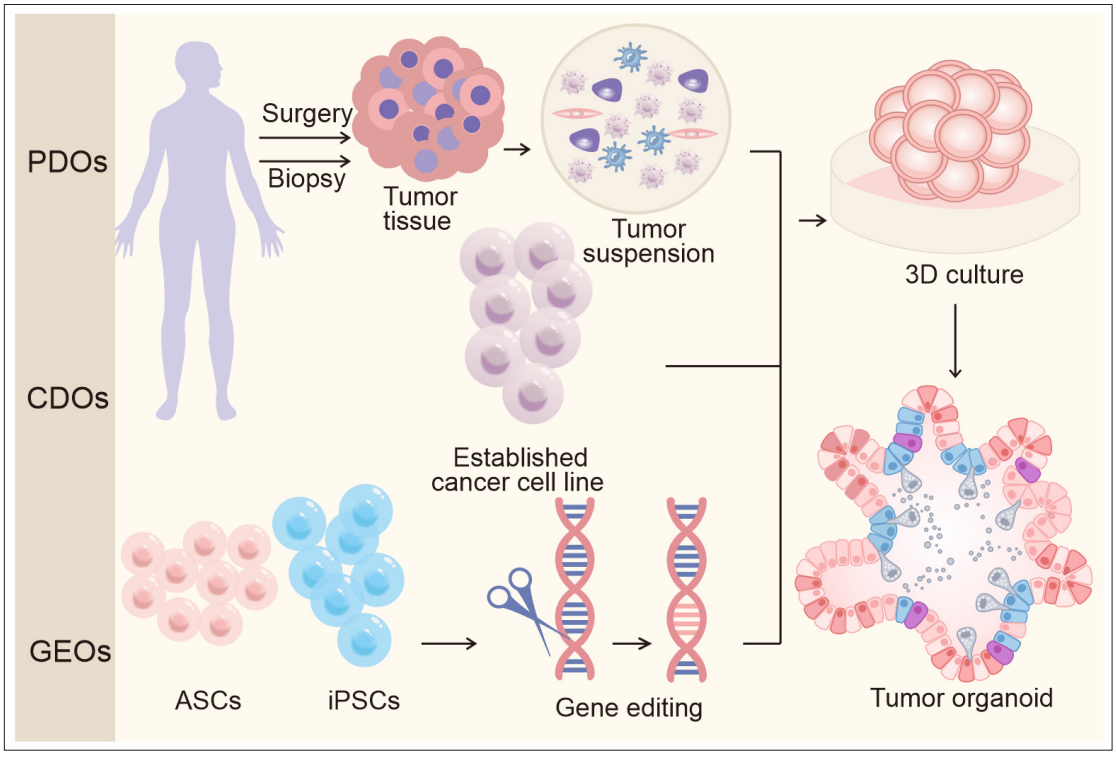

Figure 1. Generation of tumor organoids. Tumor organoids are categorized into PDOs, CDOs, and GEOs. All types are cultured in 3D systems to form tumor organoids, with PDOs being the most effective at capturing individual tumor characteristics. Created using CorelDRAW 2021 (Corel Corporation, Ottawa, Canada). Abbreviations: ASCs, adult stem cells; iPSCs, induced pluripotent stem cells; PDOs, patient-derived organoids; CDOs, cell line-derived organoids; GEOs, genetically engineered organoids.

Another branch of animal models is the cell line-derived xenograft (CDX) model, which involves inoculating *in vitro*-passaged tumor cell lines into immunodeficient mice to induce tumor formation.[26] Compared with PDX models, CDX models exhibit a higher construction success rate and shorter experimental cycles. They are widely used for preliminary *in vivo* drug efficacy evaluation because standardized cell lines yield relatively consistent experimental results.[27] However, prolonged *in vitro* passaging leads to the loss of certain *in vivo* characteristics, and the resulting TME is relatively simplistic, lacking the diverse cellular interactions and stromal components present in patient tumors.[28]

Tumor organoids represent a balance between 2D cell cultures and animal models (Table 1). They simulate ECM interactions through matrices such as Matrigel, thereby maintaining cell polarity and signaling pathway activity.[19] Moreover, long-term stable passaging can be achieved by optimizing culture conditions, such as supplementing epidermal growth factor.[17,22] To consolidate these advantages within a unified theoretical framework, the 2014 perspective by Lancaster and Knoblich[29] provided a formal definition of organoids as self-organizing 3D structures that recapitulate organ-specific cell types and tissue-level functions, thereby establishing benchmark criteria against which tumor organoids are now evaluated.

While tumor organoids already provide an intermediate platform between 2D cultures and animal models, 3D bioprinted tumor organoids further distinguish themselves from other 3D systems, such as multicellular spheroids and organ-on-a-chip devices. Unlike spheroids, which self-assemble without spatial control, bioprinted organoids enable precise, layer-by-layer deposition of patient-derived cancer cells, stromal components, and ECM mimetics, thereby preserving intratumoral heterogeneity and spatial architecture.[30] A representative example of spheroids is neurospheres, a non-bioprinted 3D model used to support neural progenitor cell expansion and investigate early neural development; however, neurospheres exhibit inherent limitations, including batch-to-batch variability, limited clonality, and lack of complex tissue architecture.[31] Furthermore, compared with microfluidic tumor-on-a-chip models—which often require cleanroom fabrication and are limited to 6–10 parallel replicates per run—3D bioprinted tumor organoids can be produced at 96- to 384-well throughput in a standard biosafety cabinet and are already integrated with automated high-content imaging platforms.[32–34]

2.3. Application advantages and challenges of tumor organoids

In basic research, tumor organoids provide an ideal tool for analyzing mechanisms of carcinogenesis. Through clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9-mediated gene editing in PDOs, the functions of driver genes can be accurately verified.[16] Using organoid-on-a-chip technology, the invasion trajectories of tumor cells can be observed in real-time, revealing the spatiotemporal dynamics of the epithelial–mesenchymal transition (EMT) process.[17,35]

In clinical settings, PDOs have been applied to individualized drug sensitivity testing. For instance, they have been used to screen the efficacy of aurovertin B in patients with triple-negative breast cancer (TNBC).[19] They also play a role in elucidating drug resistance mechanisms, such as investigating how Hedgehog signaling mediates sorafenib resistance in hepatocellular carcinoma PDOs.[35] Additionally, PDOs contribute to new drug development,

Table 1. Comparison of tumor research models

Model type	Physiologic fidelity	Reproducibility	Cost	Application scenarios	Limitations	References
Two-dimensional cell lines	Low	High	Low	High-throughput drug screening	Lack of 3D structure; simple microenvironment; significant genetic drift	[15,16,23]
PDX models	High	Low	High	Individualized treatment	Long construction cycle (8–12 weeks); species differences; ethical restrictions	[19,25]
CDX models	Medium	High	Medium	Preliminary *in vivo* drug efficacy verification; potential drug screening	Lack of patient tumor stromal components/cell interactions; reduced sub-clonality; not suitable for immunotherapy	[26–28]
Organoids	Medium-high	Medium-high	Medium	Drug screening; mechanism research	Insufficient vascularization; lack of immune components; structural randomness	[17,19,22]

Abbreviations: CDX: cell line-derived xenograft; PDX: patient-derived xenograft.

exemplified by the verification of the efficacy of the bispecific antibody MCLA-158 (an anti-B7-H3 humanized monoclonal antibody) using colorectal cancer PDOs.[35]

To strengthen translational relevance, a milestone for clinical translation was achieved in 2015, when van de Wetering *et al.*[36] established a living biobank of 55 colorectal cancer PDOs and demonstrated >80% concordance between organoid drug responses and patient outcomes, thereby consolidating the "patient avatar" concept. Moreover, Vlachogiannis *et al.*[37] conducted a large-scale clinical trial involving patients with metastatic gastrointestinal cancers and reported that PDOs predicted patient responses to targeted therapies and chemotherapy with over 80% accuracy. Together, these studies provide robust evidence supporting the clinical utility of PDOs in guiding personalized treatment decisions.

In July 2022, the United States Food and Drug Administration (FDA) for the first time allowed organoid-on-a-chip data to support the initiation of a clinical trial for a new drug (Sutimlimab) without requiring traditional animal data, marking a regulatory milestone for organoid applications in drug development.[35,38]

More recently, a translational study further validated the clinical predictive power of PDOs. In a cohort of 128 patients with locally advanced rectal cancer, organoid responses to combined chemoradiotherapy achieved 93.75% accuracy and 96.3% sensitivity in predicting patient outcomes, with synergistic and antagonistic treatment effects faithfully recapitulated *in vitro*.[39]

Despite these advances, tumor organoids continue to face multiple interconnected challenges. Organoids derived from the same patient sample often exhibit structural heterogeneity, including variations in size and morphology, which undermine experimental reproducibility.[35] In addition, their microenvironment remains oversimplified, lacking key components like blood vessels, nerves, and immune cells; thereby limiting the simulation of dynamic tumor–host interactions.[17] Long-term culture stability is another concern; organoids from certain cancer types, such as pancreatic cancer, are prone to phenotypic drift during passaging, resulting in the loss of original tumoral characteristics.[35]

These overlapping limitations have collectively driven the need for innovation, particularly the integration of organoid technology with advanced engineering approaches capable of addressing inherent drawbacks.[18,40] Tumor organoids excel at recapitulating intrinsic tumor biology but lack structural uniformity and precise spatiotemporal control over microenvironmental components.[41] In contrast, 3D bioprinting offers unparalleled capabilities for engineering complex, spatially defined constructs with tailored microenvironments.[41] This complementary relationship creates a powerful synergy: 3D bioprinting can "upgrade" tumor organoids by reducing structural heterogeneity and microenvironmental oversimplification, while tumor organoids provide 3D bioprinting with biologically relevant building blocks to generate more clinically translatable models.[42] This synergy motivates the in-depth exploration of 3D bioprinting principles, technologies, and materials in the following section.

3. Core aspects of three-dimensional bioprinting: from technology classification to bioinks

3.1. Classification and working mechanisms of three-dimensional bioprinting technologies

3D bioprinting technology constructs 3D scaffolds through the layer-by-layer deposition of biological materials and living cells.[43] This process involves the precise positioning and assembly of materials and cells under computer control, enabling the fabrication of complex structures that mimic the architecture and function of native tissues. Based on their forming principles, mainstream 3D bioprinting technologies can be categorized into three primary types (Table 2), each with distinct mechanisms and applications in tumor model construction (Figure 2; Table 3).

Table 2. Comparison of three mainstream three-dimensional bioprinting technologies

Printing technology	Precision	Speed	Cell damage	Suitable materials	Typical application scenarios	References
Extrusion-based	Medium	Medium	Low	Alginate–gelatin; GelMA; collagen	Tumor–stroma co-culture models; breast tumor spheroid construction	[44,45]
Inkjet-based	High	High	Medium	PEG-4MAL; low-viscosity hydrogels	High-throughput drug screening arrays; real-time cell movement monitoring	[44]
Photocurable	High	High	High	GelMA; PEGDA; methacrylated hyaluronic acid	High-precision vascular networks; micro-patterned TME	[44,46]

Abbreviations: GelMA: gelatin methacryloyl; PEGDA: polyethylene glycol diacrylate; PEG-4MAL: 4-arm polyethylene glycol maleimide; TME: tumor microenvironment.

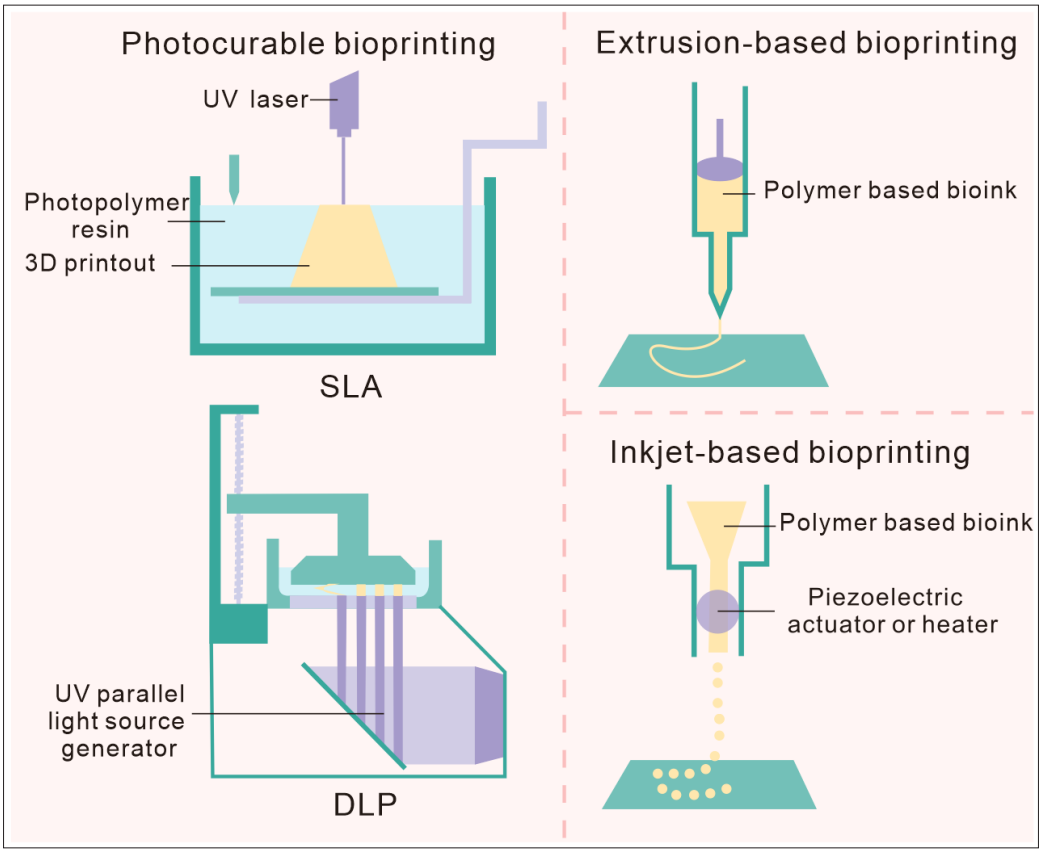

Figure 2. 3D bioprinting technologies applied in tumor model construction. Photocurable bioprinting (SLA and DLP) utilizes light to solidify bioinks, whereas extrusion-based bioprinting deposits bioinks through pressure, and inkjet-based bioprinting dispenses microdroplets of bioinks. Each approach exhibits unique mechanisms and applications for constructing tumor-relevant structures. Created using CorelDRAW 2021 (Corel Corporation, Ottawa, Canada). Abbreviations: UV: ultraviolet; 3D: three core three-dimensional; SLA: stereolithography; DLP: digital light processing.

Table 3. A comparison of three-dimensional bioprinting technologies in tumor organoid engineering

Technology	Typical spatial resolution	Cell survival after printing	Strengths for tumor modeling	Major limitations	References
Extrusion-based	100–300 μm	85–95%	Multi-cell/type & ECM deposition; scalable to several mm^3; compatible with high-viscosity composite hydrogels	Comparatively low resolution; risk of nozzle clogging; axial heterogeneity when printing branched vascular trees	[47–51]
Inkjet-based	50–100 μm	75–90%	High speed, low reagent volume; 96-well plate-compatible mini-tumor arrays for high-throughput screening; real-time live-cell imaging after dispensing	Limited to low-viscosity bioinks (<10 mPa·s); difficulty in maintaining vertical orientation; 3D architecture; possible heat damage to cells	[52–55]
Photocurable	10–50 μm	90–98%	Ultra-high resolution for micro-vessels & hypoxic niches; rapid layerless curing; compatible with stimuli-responsive or multi-material resins	Requires photo-initiator (cytotoxicity risk); shallow construct height (mm scale) without z-stage translation; post-print stiffness may hinder cell spreading	[56,57]

Abbreviations: 3D: three-dimensional; ECM: extracellular matrix.

Extrusion-based bioprinting operates by applying mechanical or pneumatic pressure to extrude bioink through a nozzle, which is then deposited onto a platform following a preprogrammed path to build structures layer-by-layer.[6] It employs sacrificial (temporary, dissolvable frameworks), support (temporary rigid supports for sag-prone parts), and embedded (printing in soft protective gels) strategies to facilitate the fabrication of large-scale constructs and complex organ models,[58] making it particularly suitable for constructing multicellular structures,[59] such as heterogeneous TMEs composed of tumor cells, stromal cells, and immune cells.[6,60] This technology is compatible with a wide range of hydrogels and has been used to generate tumor–stroma coculture models using cell-laden bioinks, such as collagen.[60] Quantitative benchmarks indicate that filament diameters of 100–300 μm can be routinely achieved while maintaining 85–95% cell viability when nozzle diameters of ≥200 μm and extrusion pressures of ≤200 kPa are used. Moreover, extrusion-based bioprinting tolerates high-viscosity composite hydrogels and can be scaled to construct several mm^3 in size, offering the highest throughput among current bioprinting modalities.[50,51,61] However, its comparatively low resolution and susceptibility to nozzle clogging may introduce axial heterogeneity, particularly when printing branched vascular structures.[62,63]

Inkjet-based bioprinting is inspired by conventional inkjet printing and generates microdroplets of bioink through thermal bubble or piezoelectric actuation.[64] These droplets are precisely deposited at designated locations.[6] This technology features non-contact, nozzle-less operation with minimal cellular damage.[58] Drop-on-demand systems routinely achieve lateral resolutions of 50–100 μm and maintain 75–90% cell viability, provided the bioink viscosity remains <10 mPa·s and cell density $<10^6$ cells/mL.[55,65] These characteristics allow for the rapid production (in minutes) of 96-well-plate–compatible mini-tumor arrays for high-throughput drug screening, with real-time live-cell imaging performed immediately after dispensing.[52,66] Nevertheless, requirements for low-viscosity bioinks, challenges in maintaining vertical 3D architecture, and potential heat-induced damage in thermal modes restrict its application to relatively thin tumor models.[53,54]

Photocurable bioprinting employs ultraviolet or visible light[67] to initiate photocrosslinking reactions in photosensitive bioinks, rapidly solidifying the material to form the desired structures. Representative technologies include stereolithography and digital light processing.[68] This technique enables computer-assisted patterning of 3D, cell-laden constructs using photoactivatable bioresins, offering significantly enhanced resolution compared with extrusion-based methods.[48,58] Layer-free projection methods achieve the highest spatial resolution among the three modalities (10–50 μm) while maintaining 90–98% cell viability when photoinitiator concentrations are kept <0.25% (w/v) and irradiance <20 mW/cm^2.[69,70] These capabilities support the fabrication of ultra-high-resolution micro-vessels and hypoxic niches that closely mimic tumor neovasculature, and the rapid curing speed is compatible with stimuli-responsive or multimaterial resins.[71–73] However, the mandatory use of photoinitiators poses potential cytotoxicity risks, and limited light-penetration restricts construct height to the millimeter scale unless z-stage translation is employed.[57,74]

3.2. Component selection, cutting-edge designs, and printing parameter optimization of bioinks

3.2.1. Core constituents of bioinks

Bioinks are the core materials in 3D bioprinting and play a pivotal role in determining printing success and the functionality of the resulting constructs.[75] It must simultaneously meet the dual requirements of printability and biocompatibility. The careful selection and design of bioinks are therefore essential for achieving optimal printing results and biological performance.

The cellular component of bioinks is tailored to the specific requirements of the model. For example, liver organoid bioinks are supplemented with hepatocytes, hepatic stellate cells, and Kupffer cells to restore liver physiology,[76] whereas tumor bioinks ideally contain patient-derived cancer cells to preserve intrinsic heterogeneity, as demonstrated in colorectal cancer organoid arrays.[77] Once the appropriate cell population is selected, single-cell viability must remain ≥80% throughout printing and subsequent culture, because this fraction determines the physiological relevance of the final construct.[47] At the pipeline level, Schütte *et al.*[78] increased the overall success rate of establishing patient-derived colorectal organoid lines to above 70% by adding amphotericin B and a Rho-associated coiled-coil containing kinase II inhibitor during the first week—an optimization now widely adopted by academic biobanks. Ultimately, the precise combination of cell types determines how accurately the model can replicate tumor biology and its microenvironment.

Scaffold materials form the structural framework of the bioink and can be divided into natural and synthetic materials. Natural materials, such as collagen, gelatin,[79], and sodium alginate, offer excellent biocompatibility, as they are derived from biological sources and possess chemical compositions similar to the natural ECM. For instance, gelatin methacryloyl (GelMA), a photo-crosslinkable derivative of gelatin, retains cell-adhesive RGD motifs and

enzymatic degradation sites, promoting cell adhesion and proliferation.[80] However, pure GelMA hydrogels exhibit low mechanical strength, with compressive strengths as low as 2.98 kPa at 5% concentration, limiting their use in load-bearing applications.[76,81] Synthetic materials provide superior mechanical stability and controllable degradation rates.[82] Polyethylene glycol (PEG), for example, is FDA-approved and degrades into non-toxic, water-soluble byproducts, making it highly biocompatible.[83] However, PEG lacks cell-adhesive motifs, which may reduce cell attachment and tissue integration.[84] To overcome these limitations, composite scaffolds have been developed. For example, gelatin–PEG blends combine the biocompatibility of gelatin with the mechanical strength of PEG, resulting in hydrogels with tunable mechanical properties and improved cell compatibility.[85] Specifically, GelMA–PEG composite hydrogels have been shown to balance stiffness and remodeling, supporting both mechanical integrity and ECM deposition.[86] Thus, composite scaffolds such as gelatin–PEG blends represent the mainstream choice in current bioink design, offering balanced performance in biocompatibility, mechanical strength, and degradation kinetics.[76,82] For instance, Yue *et al.*[87] demonstrated that incorporating 5% (w/v) PEG diacrylate into GelMA hydrogels increased the compressive modulus from 3 to 12 kPa while maintaining over 85% cell viability.

Functional factors and physical cues are integrated into bioinks to simulate the TME. For example, hydrogel stiffness can mimic the mechanical properties of colorectal cancer stroma and thereby regulate carcinoembryonic antigen-related cell adhesion molecule 5 expression.[77] Geometric constraints can also guide neural axon sprouting or liver organoid polarization.[88] Soluble factors, such as growth factors, can also be added to modulate cell behavior.

3.2.2. Advanced bioink designs

Beyond static composite hydrogels, the last 5 years have seen three converging directions that transform bioinks into "active" biomaterials: multi-material patterning, dynamic self-healing networks, and stimulus-responsive systems.

(i) Multi-material patterning enables voxel-specific mechanical or biochemical gradients that single polymer inks cannot achieve. For example, an alginate–gelatin–nano-hydroxyapatite ink was co-printed with an RGD-functionalized alginate to generate a 250 μm mineralized shell around a soft, cell-rich core, driving site-specific osteogenesis of patient-derived bone marrow stromal cells without the need for exogenous osteogenic media.[89] Similarly, coaxial deposition of a GelMA core containing colorectal cancer organoids and an alginate–fibrin shell loaded with cancer-associated fibroblasts yielded a 500 μm-thick pseudocapsule that upregulated α-smooth muscle actin and interleukin (IL) 6 within 72 h.[90]

(ii) Dynamic, self-healing hydrogels replace permanent covalent networks with reversible supramolecular interactions. Host–guest hydrogels based on cyclodextrin–adamantane-modified hyaluronic acid shear-thin during extrusion yet instantly self-heal after deposition, producing ≤50 μm filaments with >95% post-printing viability of human induced pluripotent stem cell-derived hepatocytes.[91]

(iii) Stimulus-responsive bioinks undergo preprogrammed shape or functional changes after printing. For instance, Kirillova *et al.*[92] demonstrated that methacrylated alginate and hyaluronic acid hydrogels self-fold into perfusable tubular structures upon green-light illumination, enabling the formation of vascular-like conduits with high spatial resolution and cell compatibility. Similarly, Cao *et al.*[93] developed bilayer hydrogels composed of polycaprolactone and sodium alginate that undergo temperature-triggered shape transformation, rolling into tubular constructs mimicking native blood vessels, with tunable diameter and wall thickness controlled by layer composition and external stimuli.

3.2.3. Critical printing parameters for optimizing bioink performance

Optimizing the physical printing window is equally critical to the rational design of bioinks, as suboptimal printing conditions can significantly compromise the performance of bioinks. Extrusion pressure, nozzle speed, temperature, and crosslinking kinetics all play a role in determining post-printing cell viability, structural fidelity, and long-term culture stability. Excessive shear stress (>25 kPa) or high nozzle translation speeds (>15 mm/s) can reduce patient-derived tumor-cell survival to <70% within 24 h, whereas 15–20 kPa and 5–10 mm/s preserve ≥85% viability without sacrificing resolution.[49] Maintaining the bioink at 4–10°C delays premature gelation, and brief ultraviolet exposure (5–10 mW/cm^2, <10 s) ensures uniform crosslinking with minimal photoinitiator toxicity.[68] Using layer thickness ≤100 μm helps mitigate the effects of limited light penetration (1.5–2.0 mm at 405 nm in GelMA), thereby reducing the risk of hypoxic cores during extended culture.[57] Thus, parameter optimization is as critical as bioink composition for generating clinically relevant tumor organoids.

4. Applications of three-dimensional bioprinting in tumor organoids

4.1. Three-dimensional bioprinting for the construction of heterogeneous tumor microenvironments with immune interactions

4.1.1. Precise construction of heterogeneous tumor microenvironments

The heterogeneity of the TME is a key factor leading to treatment resistance.[94] The TME has a complex composition, encompassing a variety of cell types and ECM components (Figure 3). 3D bioprinting technology enables the precise arrangement of various TME components through spatial positioning techniques, overcoming the structural randomness inherent in organoid formation. This precision is reflected not only in the spatial distribution of different cell types but also in the simulation of physicochemical microenvironments analogous to those *in vivo*, thereby providing a more authentic model for in-depth investigations into tumor occurrence and progression[95] (Table 4).

The heterogeneous TMEs precisely constructed using 3D bioprinting technology have been extensively applied in the research of various tumor types, including lung cancer, glioblastoma (GBM), liver cancer, colorectal cancer, gastric cancer, and pancreatic cancer.

In lung cancer model construction (Figure 4A), researchers have optimized GelMA scaffolds by incorporating hyaluronic acid methacrylate, a strategy that enables the model to house multiple cell types, including lung epithelial cells, vascular endothelial cells, and mesenchymal cells.[99] This 3D bioengineered model effectively simulates cell–matrix interactions and matches the microenvironmental characteristics of lung tissues by regulating the scaffold composition,[100,101] highlighting the advantages of 3D bioengineering in constructing physiologically relevant TMEs.

In GBM models (Figure 4B), 3D bioprinting has been employed to reconstruct complex TMEs containing glioma cells, macrophages, and endothelial cells, with the printing of co-culture models featuring spatial zoning.[102,103] GBM is characterized by high invasiveness and drug resistance,[104] which are closely associated with interactions between tumor cells and immune cells in the TME. Utilizing GelMA-based bioink, researchers developed a "tumor–macrophage" co-culture model in which macrophages are polarized into a pro-tumor phenotype under the induction of glioma cells, secreting cytokines such as transforming

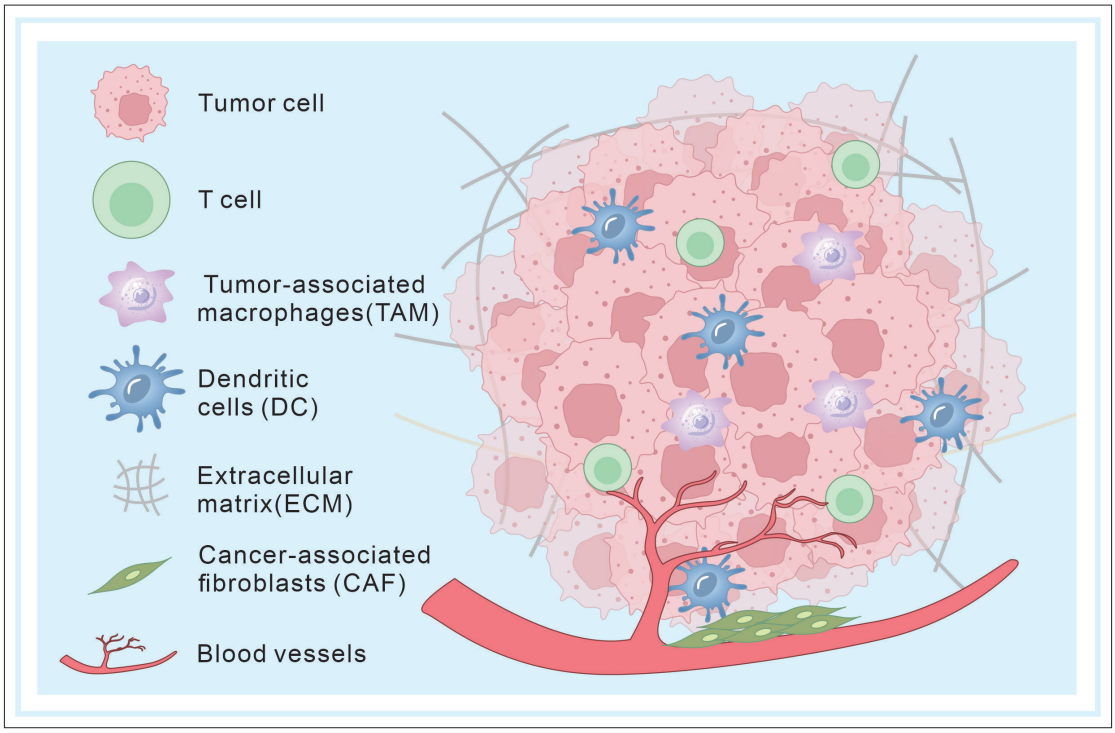

Figure 3. Components of the tumor microenvironment (TME), including tumor cells, immune cells (T cells, tumor-associated macrophages, dendritic cells), extracellular matrix, cancer-associated fibroblasts, and blood vessels, organized by 3D bioprinting to represent heterogeneous TMEs for cancer research. Created using CorelDRAW 2021 (Corel Corporation, Ottawa, Canada).

Table 4. Selected three-dimensional bioprinted tumor microenvironment models

Tumor type	Construction strategies	Key outcomes	References
Colorectal cancer	Build PDOs; recapitulate tumor structure and composition	Retain genetic heterogeneity; mimic TME; support tumor and drug study	[14,36,96–98]
Lung cancer	Optimize GelMA scaffolds with hyaluronic acid; house multiple cells	Simulate cell–matrix interactions; match lung microenvironment	[99–101]
GBM	Reconstruct TME with glioma, macrophage, and endothelial cells; print co-culture model	Simulate pro-tumor macrophages; reflect drug resistance with 25% higher survival than 2D culture	[102–105]
Liver cancer	Construct TME with hepatoma cells, hepatic stellate cells, and ECM; control stiffness to 4–10 kPa	Activate stellate cells for collagen secretion; enhance migration and invasion with 30% higher EMT	[106–109]
Gastric cancer	Build 3D model with GelMA via extrusion; design grid; optimize bioink mechanics	Mimic TME; provide biomimetic ECM for cell adhesion and proliferation	[110]
Pancreatic cancer	Control stroma proportion; simulate fibrotic TME	Represent proliferation and drug resistance; aid drug screening and mechanism study	[111,112]

Abbreviations: 3D: three-dimensional; ECM: extracellular matrix; EMT: epithelial–mesenchymal transition; GBM: glioblastoma; GelMA: gelatin methacryloyl; PDOs: patient-derived organoids; TME: tumor microenvironment.

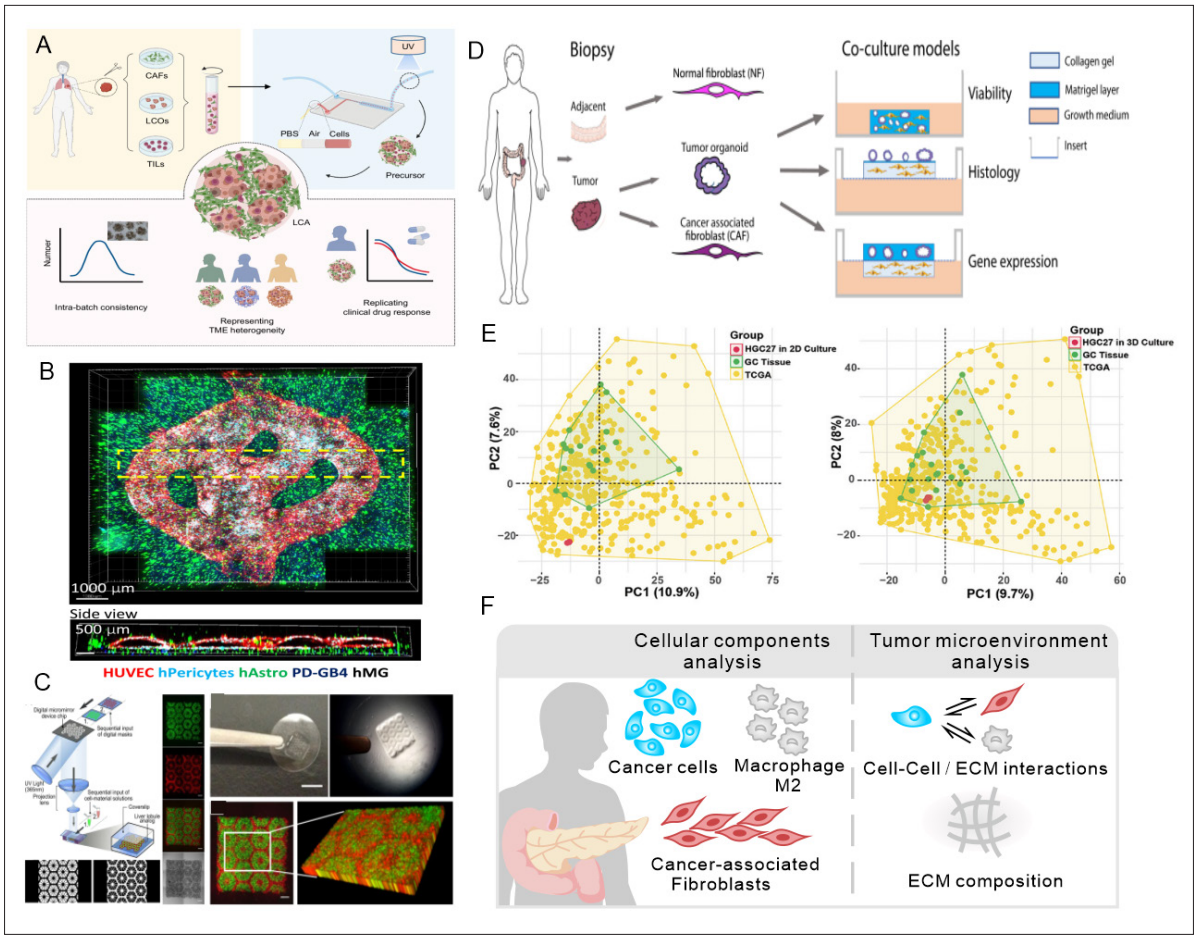

Figure 4. Applications of precisely engineered heterogeneous tumor microenvironment (TME) in three-dimensional bioprinted and organoid cancer models. Representative examples include (A) lung cancer (reprinted from Zhang *et al.*[113]); (B) glioblastoma (reprinted from Neufeld *et al.*[103]), scale bar: 500, 1000 μm; (C) liver cancer (reprinted from Datta *et al.*[114]); (D) colorectal cancer (reprinted from Atanasova *et al.*[115]); (E) gastric cancer (reprinted with permission from Ju *et al.*[110]; copyright © 2025, Wiley-VCH GmbH); and (F) pancreatic cancer (The image was created by the authors using CorelDRAW 2021 (Corel Corporation, Ottawa, Canada).

growth factor-β to promote glioma cell invasion.[105] When treated with temozolomide, the survival rate of glioma cells in this 3D bioprinted model was approximately 25% higher than that in 2D culture models, a result consistent with the *in vivo* drug resistance characteristics of GBM.[106,116]

In liver cancer models (Figure 4C), 3D bioprinting technology has been applied to construct a microenvironment containing hepatocellular carcinoma (HCC) cells, hepatic stellate cells, and liver decellularized ECM (dECM).[76] Liver cancer is often accompanied by cirrhosis, and the stiffness of the liver matrix is an important factor affecting tumor progression.[108] By adjusting the ratio of GelMA and dECM in the bioink, the stiffness of the model was controlled to 4–10 kPa, which is similar to the stiffness of cirrhotic liver tissues.[109] Experimental results showed that in this stiffness environment, hepatic stellate cells were activated to secrete a large amount of collagen. Meanwhile, the migration and invasion ability of HCC cells was significantly enhanced, with the expression of EMT-related markers increased by about 30% compared with the low-stiffness model.[106,107]

In colorectal cancer research (Figure 4D), the PDO construction strategy facilitates the precise establishment of heterogeneous TMEs by recapitulating the structural features and cellular composition of the original tumor tissue.[96] These organoids retain the genetic heterogeneity of the primary tumor, including somatic mutations, copy number variations, and gene expression profiles that are consistent with clinical colorectal cancer tissues.[14] For example, the "cystic versus solid" epithelial organization observed in primary colorectal tumors is generally preserved in these organoids.[36] Marker expression analysis reveals both inter-patient heterogeneity and heterogeneity among individual organoids within each culture, which accurately reflect the heterogeneous characteristics of the *in vivo* TME.[97] Such precise recapitulation enables organoids to better mimic the *in vivo* biological behavior of tumors, offering a reliable model for studying tumor progression and drug responses that traditional models, which often fail to preserve tissue-specific heterogeneity, cannot provide.[36,98]

In gastric cancer models (Figure 4E), researchers used GelMA hydrogel as bioink to construct a 3D bioprinted model via extrusion-based printing. This model accurately mimics the heterogeneous TME of gastric cancer. It is designed with a grid-like structure to simulate the spatial arrangement of tumor cells *in vivo*. The bioink formulation is optimized to match the mechanical properties of gastric cancer tissues, providing a biomimetic ECM microenvironment for cell adhesion and proliferation.[110]

In addition, in pancreatic cancer models (Figure 4F), 3D bioprinting precisely constructs a heterogeneous TME. Pancreatic cancer is known for rich stromal fibrosis, which is an important reason for treatment difficulty.[111] Researchers simulate its fibrotic microenvironment by controlling the proportion and distribution of stromal components via 3D bioprinting. These models represent cancer cell proliferation and drug resistance, aiding drug screening and mechanism research.[112]

In summary, this section provides foundational model support for the subsequent simulation of tumor–immune crosstalk (**Section 4.1.2**).

4.1.2. Spatial simulation of tumor–immune crosstalk

Beyond integrating immune cells into tumor-specific models, 3D bioprinting enables targeted replication of spatially dependent tumor–immune interactions—a critical feature lacking in 2D co-culture systems and traditional organoids.[117–119] By controlling the spatial arrangement of multiple cell types, bioprinted models more accurately reproduce the structure of the TME, allowing for the investigation of how spatial organization affects immune responses.

In breast cancer models, the integration of bioprinting with magnetic levitation technology enables the positioning of tumor cells, immune cells, and stromal cells in distinct scaffold regions, mimicking the layered structure of the breast tumor TME. This spatial arrangement recreates key TME crosstalk, such as macrophage secretion of IL-10 and the subsequent inhibition of $CD8^+$ T-cell activity, providing insights into how spatial separation of immune and tumor cells influences immune suppression.[120]

Composite bioinks are critical for maintaining the survival and functionality of both tumor and immune cells.[114] Unlike single-component bioinks, which may compromise either tumor organoid formation or immune cell viability, these blends ensure that immune cells retain their ability to migrate, activate, and interact with tumor cells, thereby replicating the functional dynamics of the *in vivo* TME.[121]

These integrated models overcome a core limitation of traditional organoids by recapitulating tumor–immune crosstalk events, such as immune checkpoint activation, macrophage-mediated immune suppression, and T-cell exhaustion.[98,122] Consequently, they serve as valuable tools for exploring immune escape mechanisms and screening immunotherapies. Ongoing optimization of bioink materials and printing precision is expected to further enhance their utility in guiding clinical precision treatments.[114]

4.2. Strategies for building vascularized tumor organoids

Tumor vascularization is crucial for nutrient supply and the development of distant metastasis. Traditional organoids often suffer from central necrosis due to the lack of a functional vascular network and cannot simulate hematogenous metastasis.[123–126] (Figure 5A). 3D bioprinting addresses this limitation through two strategies, each with distinct advantages that can be selected and optimized according to specific research needs.

Direct printing of vascular channels is one key approach. Photocurable technologies are used to fabricate vascular scaffolds with branched structures.[126] After seeding endothelial cells, a connected vascular network is formed. In GBM models, this vascularized structure recreates physiological interstitial fluid flow and provides

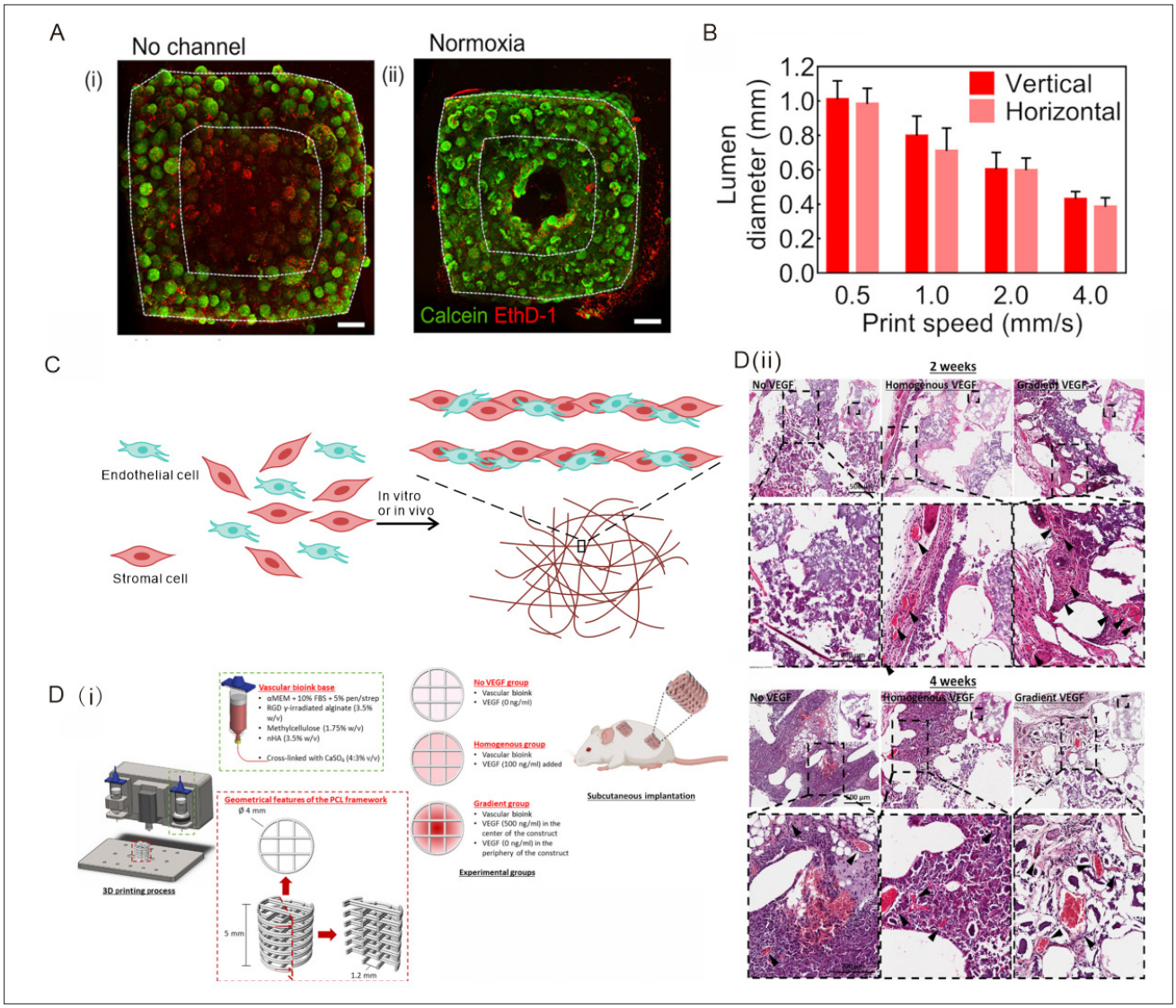

Figure 5. Strategies for engineering vascularized tumor organoids and their impact on angiogenesis and drug delivery. (A) (i) Vascularized perfusion abolishes central necrosis and sustains viability across 4 mm, whereas (ii) avascular organoids develop a necrotic core, demonstrating that functional vasculature is essential for nutrient delivery and metastasis modeling. Scale bar: 500 μm. (B) By adjusting the print speed, channel diameters can be precisely controlled between 400 and 1000 μm. Reprinted from Skylar-Scott *et al.*[126] (C) Endothelial cells form tubular structures in response to vascular endothelial growth factor (VEGF) gradients. Reprinted with permission from O'Connor *et al.*[132] Copyright © 2022, Springer Nature Limited. (D) Spatially controlled VEGF gradients within three-dimensional (3D)-printed constructs direct angiogenesis. (i) Schematic of 3D-printed scaffolds with varying VEGF gradients and (ii) Quantification of vessel density *in vivo* at 2- and 4-weeks post-implantation, demonstrating the influence of VEGF gradients on angiogenic guidance and density. Scale bar: 500 μm; magnification: 200 μm. Reprinted from Freeman *et al.*[127]

a microenvironment that supports tumor cell survival and phenotypic differentiation.[128] The diameter of the vascular scaffold can be precisely controlled to mimic *in vivo* tumor microvessels, which is essential for reconstructing the tumor vascular microenvironment (Figure 5B). Oxygen and nutrient transport efficiency can be further optimized by adjusting scaffold porosity.[129] In such vascularized models, tumor cells located in the perivascular regions exhibit higher viability and drug resistance compared to those in peripheral regions, reflecting the supportive role of vascular structures in tumor survival.[130]

To enhance endothelial cell adhesion and proliferation on scaffolds, researchers grafted GRGDSP peptides containing RGD sequences onto polycarbonate urethane (PCU) surfaces using photoactive 4-benzoylbenzoic acid under ultraviolet irradiation. Higher peptide concentrations significantly improved cell adhesion and spreading. For example, PCU200 (200 μg peptides/cm^2) exhibited increased cell adhesion and larger cell spreading areas. It also promoted proliferation and retained approximately 92% of cells under flow shear stress, whereas unmodified PCU retained only about 1% of cells. These results show its potential for use in small-diameter vascular grafts.[131]

Induction of angiogenesis represents the second major strategy. Sustained-release systems loaded with growth factors can be embedded within bioinks. Following bioprinting, endothelial cells migrate along stromal-derived vascular endothelial growth factor (VEGF) gradients and self-assemble into perfusable microvessels that anastomose with the surrounding environment[132] (Figure 5C). In lung cancer models, vascular networks constructed by this approach effectively support drug delivery, with distribution patterns highly consistent with those observed in clinical tumor tissue sections.[133] Additionally, the loading dose and release rate of growth factor sustained-release systems can be precisely regulated. Studies have shown that when the initial VEGF release rate is maintained within an optimal range, endothelial cell migration is significantly accelerated, enabling the formation of a preliminary vascular network within 7 days.[132]

Adjusting the composition and structure of bioinks enables control over VEGF gradient distribution, thereby guiding the directionality and density of angiogenesis[127,132] (Figure 5D). In lung cancer models, VEGF concentrations within the tumor area are approximately 2–3 times higher than in surrounding areas. This gradient promotes preferential endothelial cell migration toward the tumor, forming a tumor-centered vascular network.[134] Hemodynamic characteristics of the network, including blood flow velocity and flow rate, can be monitored using laser Doppler flowmetry, revealing similarities to vascular parameters observed in clinical lung cancer tissues.[132] In paclitaxel delivery experiments, these vascular networks effectively transport drugs into tumors, achieving drug concentrations approximately twice those of traditional models and a more uniform intratumoral distribution.[133]

Finally, vascularized organoids not only realistically simulate the delivery of chemotherapeutic drugs but also provide an ideal platform for evaluating anti-angiogenic therapies. Their drug response curves show high consistency with PDX models.[134,135] In evaluations of bevacizumab efficacy, tumor vascular density in vascularized organoid models decreases by ~40% following treatment, closely matching results observed in PDX models.[136] In contrast, traditional organoid models fail to accurately reflect anti-angiogenic effects due to the absence of vascular structures.[132]

4.3. High-throughput drug screening using three-dimensional bioprinted organoids

The integration of 3D bioprinting and microfluidic technologies enables the construction of miniaturized, perfusable platforms for drug screening.[137] These platforms recapitulate vascular network topology and hemodynamic cues, offering high throughput, high sensitivity, and low reagent consumption. Moreover, they enable the rapid screening of effective antitumor drugs to support clinical decision-making.[112,132,138]

Standardized tumor organoid arrays can be generated in 96-well plates using 3D bioprinting.[52] These arrays are subsequently integrated with microfluidic chips to achieve precise drug delivery under perfusable conditions, as well as real-time monitoring of vascular perfusion and organoid responses.[132,138] The bioprinted organoid arrays exhibit high consistency, characterized by uniform morphology and spatial distribution, which ensures the reliability of subsequent quantitative analysis.[138,139] Through microchannel design, the microfluidic chips enable precise control of drug concentration gradients and administration timing, with a minimum administration volume of 10 nL. When combined with perfusable, vascular-like networks that mimic *in vivo* drug transport, this approach substantially reduces drug consumption. Moreover, sensors and imaging modules integrated into the chips allow real-time monitoring of organoid viability, metabolic activity, and morphological changes, providing multi-parameter evaluation metrics for drug screening.[132]

In clinical studies of breast cancer[13] and colorectal cancer,[36] 3D bioprinting platforms constructed using GelMA/adipose dECM composite bioinks have been employed to assess the sensitivity of patient-derived cancer

organoids to chemotherapeutic drugs. These assays can be completed within a short culture period, and the resulting organoids cultured exhibit drug response profiles consistent with the biological behavior of patient tumor tissues.[138] Organoid cell viability is commonly evaluated using adenosine triphosphate (ATP) release assays, which serve as an endpoint validation method consistent with label-free biomass monitoring results.[139] When drug concentrations reach specific thresholds, ATP levels decrease significantly, enabling the determination of drug sensitivity.[140] For each drug, concentration gradients are established, and therapeutic efficacy is compared by calculating half-maximal inhibitory concentration values.[141]

In addition, by bioprinting heterogeneous models that incorporate tumor cells, immune cells, and vascular networks, the cytotoxic effects of drugs on different cell subpopulations can be evaluated simultaneously, providing a rational basis for designing combination therapies.[132,142] In these mixed models, the proportions of cellular subpopulations can be adjusted according to the genetic and morphological characteristics of clinical samples, thereby preserving the intrinsic heterogeneity of original tumors. Through selective labeling of tumor and immune cells, drug-induced cytotoxic effects can be clearly distinguished. For example, in one study evaluating the combined use of colony-stimulating factor 1 receptor inhibitors and signal transducer and activator of transcription 6 inhibitors, a synergistic effect was observed in regulating macrophage polarization and suppressing tumor cell growth, with combination therapy demonstrating significantly greater efficacy than monotherapy.[142]

This high-throughput platform offers several advantages. It minimizes tissue requirements, typically requiring only 5–10 mm of tumor tissue, which is particularly important given the limited availability of clinical samples.[143] It also shortens experimental timelines: PDOs can be established within hydrogels in 1–2 weeks, and drug screening assays can be completed within a few days, enabling rapid feedback for clinical treatment decisions[144,145] (Figure 6A). Additionally, the platform supports multi-parameter analysis, allowing simultaneous assessment of cell viability, immune cell activation, and cytokine secretion to comprehensively evaluate drug efficacy.[146] Beyond viability measurements, immunofluorescence can be used to detect tumor-infiltrating T cells[147] (Figure 6B), while quantitative reverse transcription polymerase chain

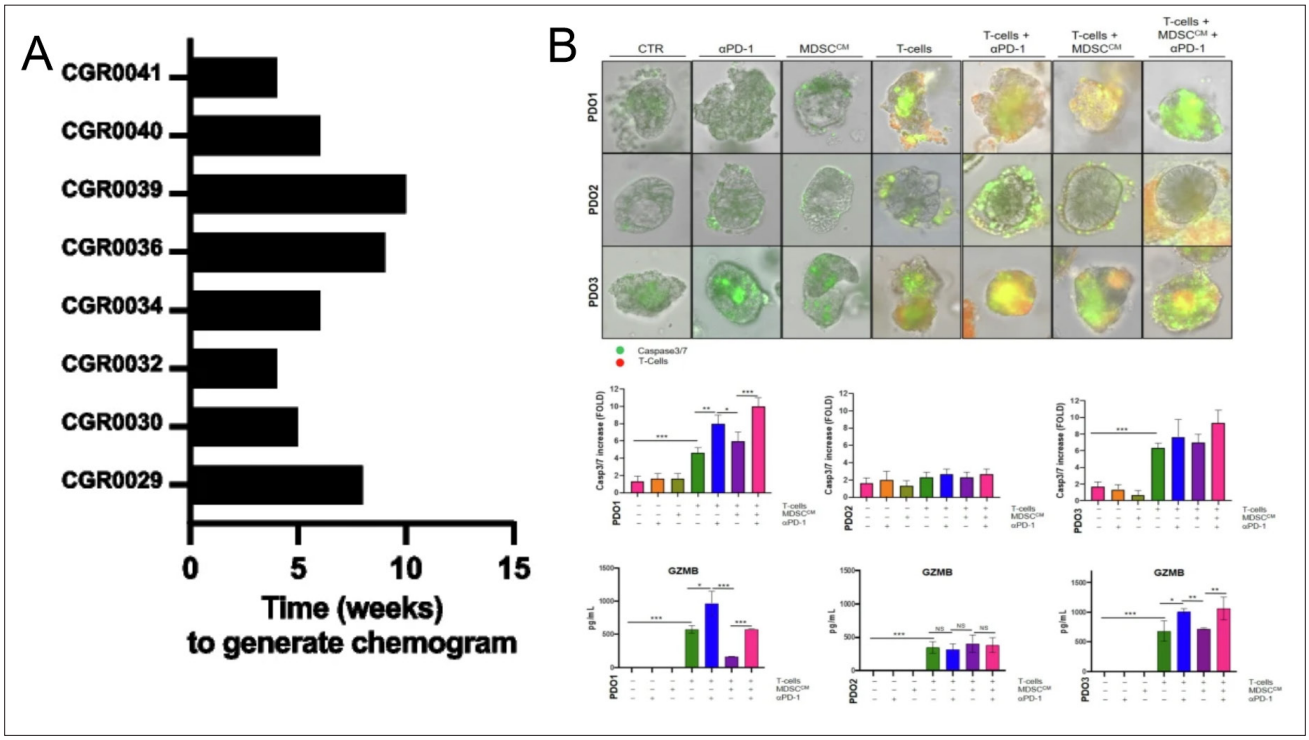

Figure 6. Integrated evidence supporting the high-throughput patient-derived organoid (PDO) platform. (A) Core needle biopsy-derived PDOs enable completion of chemograms within 6 weeks. Reprinted from Cartry *et al.*[145] (B) A patient-derived immuno-organoid platform simultaneously evaluates apoptosis (caspase 3/7), T-cell activation (granzyme B), and responses to immunotherapy. Reprinted from Esposito *et al.*[147] Together, these results demonstrate that PDO-based platforms require minimal tissue input, operate on clinically relevant timelines, and enable the multi-parametric evaluation of both tumor cell fate and immune engagement, highlighting their potential for precision oncology and studies of resistance mechanisms.

reaction enables analysis of tumor-killing factor expression, providing deeper insights into drug-induced modulation of tumor–immune interactions[142,147] (Figure 6B).

Finally, this platform serves as a powerful tool for investigating mechanisms of drug resistance. Following drug treatment, organoids can be subjected to gene sequencing and proteomic analyses to identify genes and proteins associated with resistance. These findings facilitate the discovery of novel therapeutic targets for overcoming resistance phenotypes.[142]

4.4. Validation of bioprinted organoids in clinical samples

To validate 3D bioprinted tumor organoids against clinical samples, multidimensional, multimodal approaches have been implemented across different cancer types to ensure that these models faithfully recapitulate the biological characteristics of patient-derived tumors.

In breast cancer studies, bioprinted organoids generated using patient-derived TNBC cells and GelMA scaffolds are validated by comparing their gene expression profiles with those of clinical TNBC tissue samples and PDX models using RNA sequencing. Key cancer hallmarks, such as cell proliferation, invasion, and drug response, are assessed in bioprinted organoids and correlated with clinical treatment outcomes.[148] For instance, organoid resistance to doxorubicin shows high consistency with both PDX models and patient response data. Furthermore, one study confirms that bioprinted scaffolds encapsulating *Citrus limon* L.-derived extracellular vesicles retain the anticancer efficacy observed in clinical TNBC samples, as evidenced by inhibited phosphorylation of phosphoinositide 3-kinase/protein kinase B and mitogen-activated protein kinase/extracellular signal-regulated kinase pathway proteins, consistent with molecular changes detected in patient tumors.[148]

In liver cancer research, bioprinted organoids constructed from patient-derived HCC cells, hepatic stellate cells, and dECM are validated through the assessment of liver-specific functions, including albumin secretion, urea synthesis, and cytochrome P450 enzyme activity, and are compared with primary human hepatocytes obtained from clinical samples.[77] Moreover, gene expression of HCC-related markers in the organoids aligns with RNA sequencing data from clinical HCC tissues. Their responses to hepatotoxic drugs, such as acetaminophen, also correspond to clinical cases of drug-induced liver injury, confirming their predictive accuracy. Patient-derived liver organoids have additionally been shown to replicate genetic defects associated with hereditary liver diseases, such as α-1 antitrypsin deficiency, further demonstrating their ability to model *in vivo* pathological conditions.[77]

For gastric cancer, bioprinted GelMA-based organoids incorporating patient-derived gastric cancer cells are validated through histopathological staining and immunofluorescence analyses, which demonstrate close resemblance to the morphological and phenotypic features of clinical gastric cancer tissues.[110] Transcriptomic analysis reveals that these organoids retain gastric cancer-specific genetic alterations, such as *TP53* mutations and *MET* amplification, as well as gene expression signatures such as activation of the transforming growth factor-β signaling pathway. Furthermore, their sensitivity to chemotherapeutic agents, including oxaliplatin and 5-fluorouracil, is consistent with responses observed in PDX models and patient clinical records.[110] These organoids also exhibit metabolic features characteristic of clinical gastric cancer tissues, such as the Warburg effect, as confirmed by extracellular acidification rate measurements, further validating their physiological relevance.[110]

In pancreatic ductal adenocarcinoma (PDAC) studies, bioprinted organoids that simulate the dense fibrotic TME using patient-derived PDAC cells and cancer-associated fibroblasts are validated by comparing the expression of stromal markers, such as α-smooth muscle actin and collagen I, with those observed in clinical PDAC tissue sections.[111] Drug responses to gemcitabine and nab-paclitaxel were evaluated and correlated with patient treatment outcomes. Notably, the high drug resistance observed in these organoids, attributed to stromal barrier effects, closely mirrors the poor clinical response of PDAC patients to standard chemotherapy.[111] Proteomic analysis further revealed similar expression patterns of drug resistance-related proteins, such as ATP-binding cassette transporters, in both bioprinted organoids and clinical PDAC samples. Additionally, patient-derived PDAC organoids recapitulate the immunosuppressive TME of clinical tumors, including high infiltration of M2-type macrophages, which aligns with immune profiling data from patient biopsies.[111]

In summary, the validation of bioprinted organoids across multiple cancer types has been achieved through comprehensive, multi-faceted approaches, including gene expression profiling, functional assays, histopathological assessments, and drug response evaluations against clinical samples and PDX models. Collectively, these validation strategies demonstrate the biological fidelity and clinical relevance of bioprinted organoids, supporting their use as robust preclinical platforms for cancer research and personalized medicine.

4.5. Clinical translation, regulatory frameworks, and Good Manufacturing Practice compliance of tumor organoids

Tumor organoids, especially patient-derived tumor organoids (PDTOs), have emerged as a crucial link between preclinical research and clinical practice. In rectal cancer, a biobank of 142 rectal cancer organoids has been established with an optimized success rate of 90.2%, accurately predicting clinical responses to neoadjuvant chemoradiotherapy, with predictive accuracies of 92.5% and 93.75% in discovery and validation cohorts, respectively.[39] These organoids have also been used to identify synergistic and antagonistic interactions among therapeutic agents. In gastrointestinal cancers, PDTOs have been used in clinical trials to guide personalized treatments.[149] In pancreatic cancer, PDTO-guided therapies improved the 6-month disease control rate by 23%. Similarly, in colorectal cancer, PDTOs predicted the efficacy of cetuximab and regorafenib with 78% consistency.[149] PDTOs have also enhanced the efficacy of adoptive cell therapies, and in breast cancer, they have supported preclinical evaluation of chimeric antigen receptor–macrophage therapies, demonstrating significant anti-tumor activity.[150]

Regulatory pathways governing the clinical translation of tumor organoids are progressively being standardized, with laboratory-developed tests serving as the primary regulatory framework for early clinical applications. As outlined in organoid-based clinical laboratory development testing, PDTO-based drug sensitivity assays must undergo rigorous analytical validation and clinical validation prior to clinical implementation.[151] In the United States, the FDA has recognized the potential of organoid models to reduce reliance on animal testing and has collaborated with biotechnology companies to validate gastrointestinal organoid-on-a-chip platforms for evaluating drug safety. In the European Union, PDTOs have been integrated into Innovative Medicines Initiative projects aimed at harmonizing technical standards for their application in clinical trials.[152]

Compliance with Good Manufacturing Practice (GMP) standards constitutes a core prerequisite for the large-scale clinical translation of tumor organoids. GMP regulations encompass the entire production workflow. Raw materials such as Matrigel and culture media must have traceable batch records and meet sterility and stability requirements,[149] while tissue sample processing requires standardized operating procedures. For example, digestion of rectal cancer tissue is strictly controlled to 15–20 min with intermittent agitation to maintain cell viability and minimize genetic damage.[39] During manufacturing, automated systems are increasingly employed to minimize batch-to-batch variation. A microfluidic-based PDTO culture system has been developed to enable automated drug administration and real-time imaging, reducing manual operation errors by 35%.[152] Quality control represents another critical GMP component, requiring microbial testing, short tandem repeat authentication to verify donor identity, and genetic validation.[151] In the establishment of a gastric cancer PDTO biobank, GMP-compliant cryopreservation protocols ensured that thawed PDTOs retained >50% viability and stable histological features, thereby supporting long-term storage and multi-center clinical collaboration.[149] Collectively, these GMP-compliant practices provide a solid foundation for the reliable application of tumor organoids in precision oncology.

5. Advantages and current challenges

3D bioprinting-based tumor organoids offer precise spatial control,[6,30,153] personalized modeling capabilities,[154] improved standardization,[155] and high-throughput potential.[156,157] These advantages address key limitations of traditional organoid systems, like random architecture and uneven cell distribution,[158] while enabling more accurate studies of TME interactions.[156]

Despite significant progress, 3D bioprinting of tumor organoids still faces several challenges that limit its broader application:

Limited printing resolution: Extrusion-based systems typically achieve resolutions of 100–300 μm, which is insufficient for accurately recreating microvessels or tumor–stroma interfaces.[159] Although stereolithography-based approaches can reach resolutions of 10–50 μm and have been used to fabricate perfusable microvessels in GBM organoids, construct height is often restricted to <2 mm due to limitations in light penetration and potential photoinitiator cytotoxicity.[68] In bioprinted gastric cancer models, the inability of extrusion-based methods to generate microvessels has been shown to weaken the therapeutic effects of trastuzumab/ramucirumab, leading to discrepancies with clinical monoclonal antibody responses.[160]

Bioink instability: Bioinks exhibit multiple forms of instability arising from their chemical and physical properties. Natural hydrogels like Matrigel possess undefined chemical compositions that result in batch-to-batch variability, while weak crosslinking leads to mechanical instability.[161] Synthetic hydrogels may undergo uncontrolled degradation over extended culture periods, compromising structural integrity, and some bioinks exhibit photoinstability, with batch-to-batch optical drift reducing light transmittance.[162] Collectively, these issues can lead to structural collapse, cell death, or failure to

meet clinical performance standards. To address these limitations, composite hydrogels, e.g., GelMA blended with dECM, have been formulated, achieving storage moduli of 4–10 kPa while maintaining cell viability >90% for up to 14 days.[76,77] However, Yoon *et al.*[163] reported that batch-to-batch optical drift in electron beam-sterilized collagen-derived dECM bioinks significantly reduced light transmittance, falling below graft transparency thresholds and ultimately hindering clinical translation of bioprinted corneal patches.

Long-term culture viability: Sustaining long-term viability remains a major bottleneck in 3D bioprinted constructs, primarily due to inadequate nutrient and oxygen diffusion. Under static culture conditions, this limitation becomes more pronounced as constructs exceed the oxygen diffusion limit of approximately 100–200 μm in diameter. Consequently, cells within the core experience nutrient deprivation and impaired metabolic waste removal, leading to necrotic core formation, typically within 2–3 weeks.[158] The integration of microfluidic perfusion systems, operating at a flow rate of 20–50 μL/min, has been shown to double viable tissue thickness and preserve drug response fidelity for up to 6 weeks.[132]

Standardization gaps: Variability in bioink composition, printer parameters, and analytical readouts continues to hinder interlaboratory reproducibility.[154] For example, natural bioinks, such as Matrigel, exhibit undefined compositions and batch-to-batch differences, whereas synthetic hydrogels often lack standardized formulations tailored to specific cancer models, resulting in inconsistent cell behavior across studies. Printer parameters, such as extrusion pressure and crosslinking conditions, further affect reproducibility, as demonstrated in breast cancer spheroid studies, where variations in printing conditions altered cell viability and sensitivity to paclitaxel. In addition, inconsistent readout metrics complicate data comparison across laboratories.[154] To address these challenges, a recently developed modular bioprinting platform standardized GelMA/dECM ratios, nozzle height, and post-print crosslinking dose, yielding intra-batch coefficients of variation <5% while preserving patient-specific drug responses.[77] Furthermore, a newly reported aspiration-assisted bioprinting protocol provides unified guidelines for hardware assembly, software parameter configuration, and post-bioprinting validation. This approach enables consistent fabrication of tumor organoids with positional errors <50 μm across experimental batches, effectively mitigating interlaboratory reproducibility issues.[164] Yang *et al.*[61] similarly emphasize that only rigorously standardized formulations, protocols, and artificial intelligence (AI)-monitored perfusion platforms can maintain both precision and predictive power in routine bioprinting workflows.

6. Future perspectives

To realize the full potential of 3D bioprinted tumor organoids, deeper integration with AI, big data analytics, and microfluidics is rapidly evolving from a conceptual vision to a quantitative reality.

Artificial intelligence can be employed to optimize bioprinting parameters by learning from large datasets of printing outcomes, thereby improving reproducibility and structural fidelity. For instance, machine learning algorithms have been used to predict post-printing cell viability based on real-time imaging and bioink composition, significantly reducing trial-and-error during protocol development.[165] More specifically, AI models trained on live microscopy images can automatically adjust printing speed and extrusion pressure in real time to maintain uniform strand diameters and minimize shear-induced cell death.[166,167] Additionally, AI has been applied to the design and performance prediction of tissue engineering materials, optimizing mechanical properties and enhancing structural design.[168] In the context of bioprinting, AI-driven approaches such as GRACE (generative, adaptive, context-aware 3D printing) enable the creation of complex, adaptive geometries that respond to the spatial distribution of cells or organoids, facilitating the fabrication of biomimetic scaffolds with highly controlled architectures.[169] Such levels of automation and adaptability would be impractical with manual design, substantially streamlining fabrication workflows while expanding the complexity and functionality of achievable structures.

Beyond optimizing printing parameters, material design, and performance prediction, AI is increasingly integrated into post-printing analysis and the clinical translation of bioprinted tumor models. Recently, Han *et al.*[77] developed an embedded bioprinting-enabled arrayed PDO platform that combines 3D bioprinting with supervised learning-based morphometric analysis. This platform achieved 98–99% accuracy in classifying colorectal cancer patients into high- and low-carcinoembryonic antigen-related cell adhesion molecule 5 expression subgroups using a majority voting algorithm and demonstrated significantly higher gene expression similarity to primary tumor tissues compared with conventional PDO models. Importantly, it successfully replicated individual patient responses to 5-fluorouracil chemotherapy, highlighting the potential of AI-enhanced platforms for personalized treatment planning.[77]

Big data, particularly multi-omics datasets from patient tumors, can further guide the design of personalized bioinks and organoid architectures that more accurately recapitulate individual tumor phenotypes. This approach has been demonstrated in integrated platforms where

genomic and proteomic information informs the selection of matrix stiffness and growth factor composition in bioprinted models.[170]

Microfluidics complements bioprinting by enabling dynamic culture environments that simulate physiological gradients of oxygen, nutrients, and drugs. For example, perfusable microfluidic chips integrated with bioprinted vascularized tumor organoids have been used to investigate hypoxia-driven drug resistance in non-small cell lung cancer, offering a more clinically relevant model than static culture systems.[106] Notably, microfluidic chips embedded with real-time oxygen sensors can generate hypoxic niches within printed tumors, allowing precise investigation of hypoxia-inducible factor 1 alpha-mediated drug resistance under defined oxygen gradients.[171,172] Although challenges remain in terms of throughput and long-term perfusion stability, addressing these engineering limitations is expected to unlock the full potential of chip-integrated bioprinted tumors for high-content, patient-specific drug screening.[173,174]

Together, these technologies form a feedback-augmented loop in which omics data train AI algorithms, AI governs bioprinting processes, and microfluidic readout feedback to refine both the biological models and computational frameworks. This closed-loop system enables the transformation of multi-modal biological information into quantitative precision oncology assays.

7. Conclusion

The integration of 3D bioprinting with tumor organoid technology marks a pivotal advancement in cancer research. By combining the histological and genetic fidelity of organoids with the spatial precision and structural controllability of 3D bioprinting, these hybrid models effectively overcome many limitations of traditional *in vitro* systems. They accurately recapitulate key features of the TME, including cellular heterogeneity, vascular networks, and tumor–immune crosstalk. Consequently, bioprinted tumor organoids show strong potential for modeling tumor progression, engineering vascularized tissues, enabling high-throughput drug screening, and investigating immunotherapy mechanisms. Despite these advances, several challenges remain, including limited printing resolution for fine microstructures, bioink instability, difficulties in maintaining long-term cultures, and ethical and regulatory considerations. Looking ahead, continued convergence with microfluidics, AI, and big data analytics, alongside the development of universally standardized platforms, is expected to drive further innovation. These advances will strengthen the translational bridge between basic research and clinical application, ultimately accelerating the development of personalized cancer therapies and improving patient outcomes.

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Conflict of interest

The authors declare that they have no competing interests.

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