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REVIEW ARTICLE

3D bioprinting–microfluidics technology: Pioneering advances in tumor microenvironment modeling, cancer treatment optimization, and diagnostic biomarker discovery

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Abstract

Conventional tumor models have historically failed to fully recapitulate the intricate pathophysiological complexity and dynamic microenvironment of human malignancies, significantly limiting their translational potential. The recent convergence of microfluidic technology and 3D bioprinting has ushered in a paradigm shift in oncology research, enabling more physiologically relevant models. This review provides a comprehensive analysis of the limitations inherent in traditional tumor modeling platforms and elaborates on the fundamental principles underlying microfluidics and additive manufacturing. It systematically explores the integrated applications of 3D-bioprinting–microfluidics systems across three core domains: engineering pathomimetic tumor models, advancing therapeutic screening platforms, and developing high-sensitivity diagnostic tools. This interdisciplinary synergy allows for unprecedented spatiotemporal control over the tumor microenvironment, precise biochemical gradient formation, and seamless integration of functional biosensors. The review further discusses persistent challenges—such as material biocompatibility, fabrication scalability, and the need for standardized validation—and proposes future directions—including the development of multiorgan-on-chip systems, stimuli-responsive biomaterials, and artificial intelligence-enhanced analytical frameworks. The continued integration of 3D bioprinting and microfluidics holds transformative potential for accelerating precision oncology and improving clinical outcomes.

Keywords: 3D printing; Cancer treatment optimization; Diagnostic biomarker discovery; Microfluidic technology; Tumor microenvironment model

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1. Introduction

Contemporary oncology research has undergone transformative advancement through increasingly sophisticated tumor modeling platforms. While conventional two-dimensional (2D) monolayer cultures facilitate high-throughput compound screening, they fundamentally lack the multidimensional complexity of native tissue ecosystems—failing to recapitulate critical physiological features, such as oxygen/nutrient gradients, metabolic crosstalk, and spatially organized cell–cell communication[1–3] (Figure 1). Three-dimensional (3D) models address critical limitations by preserving key architectural features, such as extracellular matrix (ECM)–cell crosstalk and the niche architecture of human malignancies.[4] Nevertheless, standard 3D systems remain inadequate for capturing dynamic tumor microenvironment (TME) remodeling or essential mechanical stimuli (e.g., interstitial fluid pressure, vascular shear stress) that drive tumor progression[5] (Figure 1).

Genetically engineered mouse models (GEMMs) and patient-derived xenografts (PDXs) partially overcome these shortcomings. GEMMs recapitulate tumor initiation and progression within native microenvironments, closely modeling molecular and systemic interactions across tumorigenesis, metastasis, and therapeutic response while preserving human cancer characteristics.[6] On the other hand, PDX models, established by engrafting patient-derived tumor fragments into immunodeficient mice, retain TME architecture and replicate the pathological, histological, and genomic profiles of original tumors while maintaining drug response fidelity.[7,8] Nevertheless, both models face significant scalability, time, and cost constraints, hindering clinical translation.[9] Furthermore, these static systems particularly fail to recapitulate hemodynamic parameters (e.g., blood/interstitial flow) essential for studying metastasis, immune recruitment, and drug pharmacokinetics[10] (Figure 1), highlighting the unmet need for dynamically perfusable platforms.

Conventional tumor models, while valuable for foundational insights, are increasingly superseded by microfluidic-integrated tumor-on-a-chip platforms that overcome their limitations through interdisciplinary innovation (Figure 1):

(i) Organoid-on-a-chip: Organoids are generated by isolating normal or cancer stem cells from human epithelial tissues and culturing them within an ECM gel.[11] Under optimized conditions, these cells self-assemble into 3D organoid microstructures, which recapitulate key architectural and functional elements of the human TME.[12–14] The integration of organoid culture with microfluidic technology has created a transformative "organoid-on-a-chip" platform that significantly advances *in vitro* modeling.[15] By enabling precise spatiotemporal control over the cellular microenvironment through dynamic perfusion and regulation of oxygen, nutrients, and mechanical forces, this combined approach overcomes limitations of traditional 3D cultures while better replicating *in vivo* complexity.[16,17] Microfluidic systems facilitate high-throughput parallel culturing of organoids with automated monitoring, revolutionizing drug screening and personalized medicine applications through patient-derived models. The technology allows reconstruction of sophisticated tissue interfaces by incorporating vascular networks, immune cells, and stromal components, thereby closely mimicking TMEs and tissue barriers.[18–20] With capabilities for gradient-based drug testing and enhanced physiological relevance, this next-generation platform shows tremendous potential for cancer research, regenerative medicine, and toxicology. Future developments in 3D bioprinting, vascularization, and artificial intelligence (AI)-assisted analysis promise to further refine these models, potentially reducing the need for animal

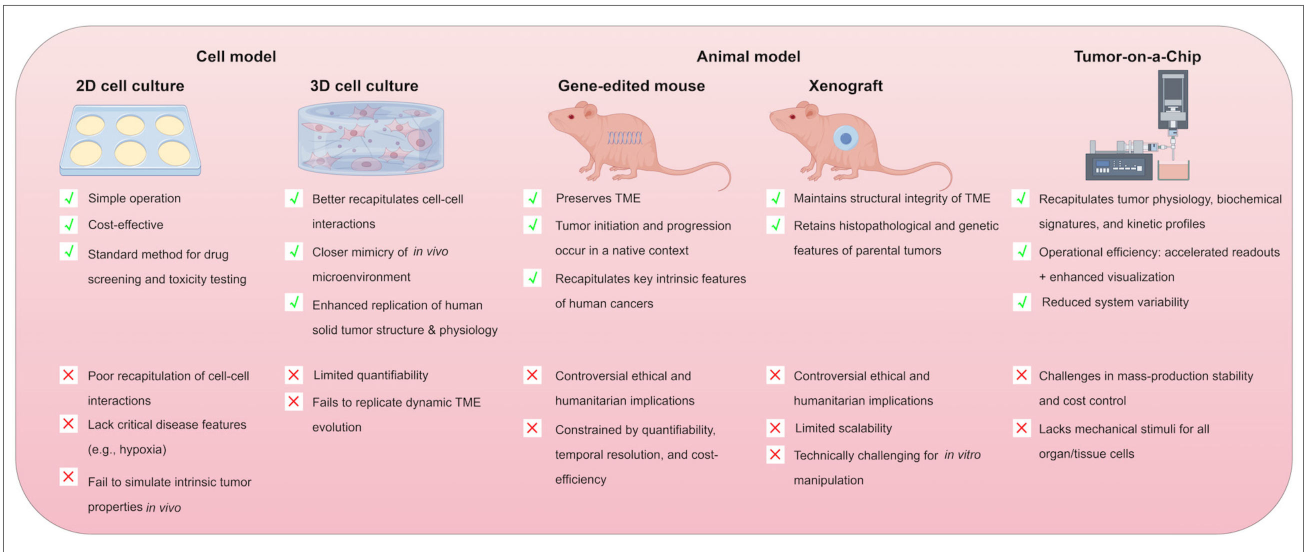

Figure 1. Advantages and limitations of different tumor models.

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testing while accelerating translational research with unprecedented precision.

(ii) Organ-on-a-chip: Micro-engineered systems that replicate human organ functions and disease states under controlled conditions. This platform enables precise regulation of cellular microenvironment, closely mimicking *in vivo* tissue architectures and dynamic physiological processes.[21,22] Compared to traditional animal models, organ-on-a-chip platforms provide superior experimental simplicity and cost-efficiency—requiring significantly less infrastructure, reducing ethical constraints, and enabling parallelized experimentation—while achieving unprecedented fidelity in recapitulating human TME dynamics.[23] These micro-engineered systems facilitate precise modeling of multistep carcinogenesis: from initial tumor–stroma interactions and angiogenesis to metastatic intravasation/extravasation, all under physiologically relevant mechanical stimuli. Simultaneously, they support high-throughput drug efficacy screening with real-time resolution of pharmacokinetic/pharmacodynamic relationships.[24] Despite these advantages over animal models, organ-on-a-chip technology faces persistent challenges, including fabrication scalability limitations, material biocompatibility constraints, and the need for advanced sensor integration—all requiring further optimization to achieve clinical predictive validity.[25,26]

(iii) Tumor spheroids: Uniform, high-throughput multicellular spheroids permit drug screening under physiologically relevant conditions. The development of tumor spheroids can be precisely controlled in terms of size and composition through continuous perfusion using microfluidic technology.[27–29] Additionally, integrating 3D-printed scaffolds with microfluidic control enables the precise generation of tumor stem-like spheroids with enhanced physiological relevance.[30] These spheroids simulate a solid tumor 3D structure while retaining cancer stem cell characteristics. They exhibit *in vivo*-like cellular complexity, critical cell–cell interactions, ECM deposition, and chemical gradients that restrict drug diffusion to levels comparable to human tissues.[27,31] Spheroids also serve as models for evaluating drug sensitivity and resistance, typically displaying heightened resistance to chemotherapy and radiotherapy versus 2D monolayers.[32] They enable analysis of growth constraints, such as oxygen tension, nutrient deficiency, radiation effects, and angiogenesis.[33,34] Therefore, convergent advances in 3D bioprinting and tumor-on-a-chip technologies are yielding biomimetic tumor models with unprecedented clinical relevance.[35] 3D printing facilitates personalized manufacturing, complex structure construction, and cost reduction.[36,37] Microfluidics dynamically simulates the TME, enables high-throughput drug screening, and precisely controls physicochemical conditions.[10] Integrating these technologies enhances tumor model humanization and clinical translation, evidenced by successes in tumor-on-a-chip and drug delivery applications[38] (summarized in Table 1).

Existing reviews typically examine microfluidics or 3D printing in isolation, lacking a systematic analysis of their synergistic convergence for oncology applications. Therefore, this review comprehensively elucidates advancements and prospects in integrated microfluidics–3D printing technologies through mechanistic analysis

Table 1. Summary of advanced tumor modeling platforms

Platforms	Principle	Applications	Advantages	Limits
Organoid-on-a-chip	Patient-derived stem cells self-organized in an extracellular matrix gel integrated with microfluidic perfusion control	Personalized therapy development through patient-specific mutation modeling	Reconstructs the human tumor microenvironment's complexity through vascular/immune integration in dynamic microenvironments	Limited replication of native mechanical forces despite perfusion control
Organ-on-a-chip	Micro-engineered systems replicating human organ-level physiology and disease dynamics	High-throughput drug efficacy studies with real-time pharmacokinetic/pharmacodynamic analysis	Models multistep metastasis processes, including intravasation or extravasation, under mechanical stimuli	Faces fabrication scalability challenges and material biocompatibility constraints
Tumor spheroids	Self-assembled 3D cell aggregates enabling high-throughput drug screening under physiological gradients	Initial assessment of chemoradiation resistance and drug toxicity profiles	Recapitulates solid tumor architecture with hypoxic cores and drug diffusion gradients	Lacks vascularization and stromal components, resulting in poor clinical correlation

of core principles and developmental trajectories. Distinctively, this review adopts a deliberately clinical lens—evaluating how these platforms directly address oncology practice gaps, including generating patient-specific tumor models for personalized therapy and liquid biopsy diagnostics at clinically actionable thresholds. Moreover, it uniquely focuses on 3D-printed microfluidic systems engineered explicitly for tumor pathophysiology, dissecting cancer-tailored architectures, such as perfusable vascular networks and metastasis models with tumor-derived bioinks. The review specifically maps applications across physiologically relevant tumor modeling, therapeutic screening, and high-sensitivity biomarker diagnostics using 3D-printed microfluidic platforms. Finally, it provides a forward-looking perspective addressing current technical constraints and emerging frontiers, including transformative four-dimensional/five-dimensional (4D/5D) bioprinting paradigms.

Fundamental principles of microfluidic and 3D bioprinting technologies

2.1. Characteristics of microfluidic technology

Microfluidics manipulates minute fluid volumes (10^{-9} to 10^{-18} L) through microscale channels (tens to hundreds of micrometers).[39] Commercial devices primarily utilize glass, polydimethylsiloxane (PDMS), and thermoplastics for their mechanical stability, chemical inertness, and biocompatibility.[40,41] Three principal fabrication techniques are summarized as follows, and in Table 2[42]:

(i) Micromolding: The most accessible method, requiring minimal equipment, ideal for phase-transition biomaterials such as hydrogels.[43]

(ii) Photolithography: Employing photochemical processes (e.g., photo-degradation/polymerization) to achieve geometrically complex quasi-planar networks with superior design flexibility.[44]

(iii) 3D printing: Offering unparalleled material diversity and spatial freedom through unrestricted nozzle movement, enabling biomimetic vascular networks and organ-on-a-chip architectures unattainable via conventional techniques.[45]

As previously established, microfluidics involves studying and manipulating fluids at submillimeter scales. Unlike macroscale systems, where gravity dominates fluid behavior, surface tension and capillary forces govern fluid dynamics at the microfluidic level. In fluid dynamics, the Reynolds number (R_e) quantifies the ratio of inertial to viscous forces within a fluid, defining its flow regime.[46] It is expressed as:

$$R_e = \frac{ul}{v} \quad \text{(I)}$$

where u is fluid velocity, L is characteristic length (e.g., channel hydraulic diameter), and v is kinematic viscosity. Submillimeter microfluidic channels yield low Reynolds numbers ($R_e << 2300$), indicating laminar flow. Under these conditions, flow properties (e.g., velocity and pressure) remain temporally stable and exhibit gradual spatial transitions. These force-dominated characteristics enable key passive functions: microchannel fluid pumping, analyte filtration, selective capture, and droplet generation without external energy.[41] Microfluidic devices are classified by actuation:

Table 2. Summary of preparation methods for microfluidic devices

Microfluidic fabrication techniques	Principle	Materials	Advantages	Limits
Micro-molding	Microfluidic channels are formed by molding biomaterials onto pre-patterned templates, followed by template removal	Biomaterials (e.g., hydrogels) undergoing liquid-to-solid phase transition	Low technical requirements Excellent for prototyping elementary microphysiological scaffolds Requires only basic laboratory equipment	Geometric limitations Mold cost constraints
Photolithography	Light-directed microchannel fabrication via photodegradation or photopolymerization patterning	Optically transparent materials (e.g., hydrogels)	Enables fabrication of arbitrary, complex microfluidic architectures Pattern formation is no longer restricted to material surfaces	Requires a trade-off between patterning resolution and fabrication speed
3D printing	Direct fabrication of microfluidic channels via 3D printing	Universal, suitable for a wide range of materials	Spatial multifunctionality (enabled by unconstrained 3D nozzle mobility)	3D printing technologies exhibit inferior resolution compared to micro-molding or photolithography techniques

(i) Passive devices: Utilize engineered geometries to harness intrinsic forces (e.g., interfacial effects, diffusion, and secondary flows) for fluid mixing and particle control.

(ii) Active devices: Employ external energy sources (e.g., magnetic/acoustic fields) to enhance fluid manipulation.[47]

2.2. Characteristics of 3D printing technology

Additive manufacturing, or 3D printing, constructs 3D objects from computer-aided design/digital models by depositing, connecting, or curing materials layer-by-layer under computer control (e.g., fusing plastic, liquids, or powders).[48,49] Recent advances in 3D printing have revolutionized the fabrication of microfluidic devices, offering unparalleled flexibility in design, material selection, and functional integration. Among the most widely adopted techniques are inkjet 3D printing (i3DP), stereolithography (SLA), two-photon polymerization (2PP), and fused deposition modeling (FDM), each with distinct operational principles, strengths, and limitations.[50]

The I3DP technique excels in multimaterial deposition, enabling the fabrication of heterogeneous tissue constructs with spatially controlled biochemical cues.[51] This capability is particularly valuable for tumor models requiring graded stiffness or embedded vasculature. However, i3DP is limited by moderate resolution (~50–100 μm) and challenges in maintaining droplet uniformity with viscous bioink.[52] SLA leverages photopolymerization to achieve high resolution (<20 μm),[51] critical for replicating intricate microchannel architectures that mimic *in vivo* vascular networks. Despite its precision, SLA is constrained by material brittleness and limited compatibility with cell-laden hydrogels unless modified for biocompatibility. Meanwhile, 2PP stands out for its sub-200 nm feature resolution, enabled by nonlinear optical absorption. This technique is ideal for creating nanoscale topographies that influence cell migration or drug diffusion in cancer models.[51] However, 2PP's slow throughput and high operational costs restrict its use to specialized applications. FDM remains the most cost-effective (<$0.50/cm^3$) option for rapid prototyping, using thermoplastics such as polyactic acid or acrylonitrile butadiene styrene. While FDM is accessible and scalable, its resolution (~100–300 μm) and surface roughness often necessitate post-processing for microfluidic applications.

The selection of an optimal 3D printing modality for tumor modeling demands a systematic evaluation of three critical, interdependent parameters: spatial resolution, which governs the precision of microchannel geometries and cellular-scale features; biomaterial compatibility, determining suitability for cell encapsulation, ECM mimicry, and long-term culture viability[53]; and economic feasibility, ensuring a balance among fabrication precision, functional performance, and budget constraints—particularly crucial for large-scale studies. A comprehensive comparative analysis of these factors (summarized in Table 3) is vital to align fabrication strategies with specific research objectives, such as replicating hypoxia gradients, vascular networks, or metastatic microenvironments. Emerging innovations, including hybrid printing techniques (e.g., integrating SLA with i3DP) and advanced bioresins,[54] hold promises for bridging current limitations in resolution, biocompatibility, and cost efficiency, thereby expanding the potential of 3D-printed tumor models.

Table 3. Summary of comparative advantages and limitations of different 3D printing types

3D printing type	Principle	Advantages	Limitations
Inkjet 3D printing	Droplet-by-droplet deposition of liquid materials	Multi-material capability High-speed printing Commercial viability Complex device fabrication	Limited material selection Challenges in droplet optimization Resolution-speed tradeoff
Stereolithography	UV laser sequentially solidifies the photopolymer resin layer-by-layer through point-by-point scanning	Balanced resolution, cost, and performance Superior surface finish Rapid innovation in materials and equipment	Resolution constrained by optical hardware and resin properties Challenges in uncured resin removal Single-material limitation per print
Two-photon polymerization	Nonlinear absorption of femtosecond near-infrared laser enables 3D voxel patterning	Sub-micron resolution (<1 μm) True 3D structuring capability Diverse material options	Extremely slow process High equipment costs Cleanroom requirements Difficult for enclosed microfluidics
Fused deposition modeling	Molten thermoplastic extrusion through a heated nozzle with XY motion control	Low-cost and user-friendly Wide material compatibility Minimal post-processing	Poor surface finish Poor Z-axis mechanical strength Low dimensional accuracy (200–500 μm features) Slow printing speed

Compared to traditional manufacturing, 3D printing provides exceptional design freedom and enables personalized, decentralized production. It produces complex geometries with high precision; its additive nature minimizes material waste, reducing costs and environmental impact.[37,55] However, there are still numerous challenges 3D printing technology faces in the biomedical manufacturing field (Table 3): adhesive selection, suboptimal product mechanical properties, limited dimensional accuracy, powder agglomeration, nozzle/distribution size constraints, material limitations, texture/color variations, material longevity, fit/design customization, layer height issues, and construction failures. Product management areas, such as employee training, pricing, cybersecurity, and intellectual property, also require attention.[56]

Cost-effectiveness and personalization drive 3D printing's adoption in traditional manufacturing, healthcare, and biological research. Particularly, SLA—characterized by layer-by-layer UV patterning in photopolymer resins—has gained considerable traction for its cost-effectiveness, high resolution, and ease of use.[57,58] For example, Shafique *et al*.[59] demonstrated that low-cost liquid crystal display 3D printing achieves 50 μm resolution at faster build rates than conventional methods, enabling scalable production of organ-on-a-chip devices. In summary, the integration of 3D printing with tumor microfluidics substantially reduces manufacturing costs and compresses design-to-validation cycles from months to weeks while reducing material waste by 40–60% compared to traditional fabrication.[36] Despite these advances, limitations remain: current 3D printing struggles with microstructures <100 μm, and restricted manufacturing precision[60,61] and insufficient transparency[62] impede sample visualization[63] (Figure 2).

3. Advanced tumor modeling: integrating 3D bioprinting and microfluidic technologies

Conventional static culture systems often inadequately capture the intricate complexity and dynamic nature of TME.[5] Although microfluidic technology has emerged as a promising solution through the development of tumor-on-chip platforms that overcome these limitations, challenges remain regarding cost-effectiveness and scalable production. The integration of 3D printing with microfluidics has consequently become crucial for engineering biomimetic tumor models.[64,65] This synergistic approach offers distinct advantages: microfluidic systems enable continuous perfusion of nutrients and therapeutic agents[5] while recapitulating both 3D tissue architecture and pathophysiological conditions.[66,67]

A notable example is the work by Ayuso *et al*.,[68] who engineered a tumor-lymphatic microfluidic model that closely mimics the 3D organization and functional characteristics, including endothelial barrier properties and lymphangiogenic potential, of *in vivo* lymphatic vessels. This innovative platform was employed to study breast cancer-associated lymphatic dysfunction through the analysis of altered gene expression patterns in lymphatic endothelial cells. As next-generation experimental tools, these tumor-on-chip systems provide physiologically relevant platforms that are transforming cancer research paradigms.

The application of microfluidics has yielded significant insights into fundamental cancer mechanisms, such as collective cancer cell migration/invasion processes (Figure 3A)[69] and hypoxia-inducible factor pathways,[70] substantially advancing both diagnostic and therapeutic development. For example, Ao *et al*.[71] demonstrated the clinical potential of this technology by developing a "mini tumor chip" through tumor cell injection into microwell arrays, enabling prediction of immunotherapy responses in just 24 hours—a dramatic reduction compared to conventional *in vitro* culture durations. Similarly, Ruzycka *et al*.[72] employed microfluidic platforms to model TMEs for metastatic lung cancer investigation, establishing that these systems provide more physiologically accurate assessment of nanomaterial toxicity and therapeutic efficacy while reducing reliance on animal models (Figure 3B).

The integration of 3D printing with microfluidic technology has significantly accelerated the development of tumor-on-chip systems with enhanced physiological relevance. For example, Behroodi *et al*.[73] demonstrated this synergy by combining projected micro-SLA 3D printing with computer-numerical-control micromachining to fabricate large-scale microfluidic molds, streamlining the production of high-resolution devices (Figure 3C). Meanwhile, Steinberg *et al*.[60] engineered fully 3D-printed microfluidic platforms capable of maintaining patient-derived multicellular spheroids for prolonged high-throughput drug screening, thereby facilitating personalized medicine and improving the prediction of optimal therapeutic regimens.

Beyond conventional applications, this combined approach has unlocked unique capabilities in specialized tumor research. For example, in microgravity biology, 3D-printed microfluidic systems overcome critical limitations of traditional culture flasks and plates—such as medium leakage and bubble formation—while offering superior biocompatibility and experimental control. Leveraging this advantage, Silvani *et al*.[74] employed such a platform to subject glioblastoma and endothelial cells to simulated microgravity for 24 hours, enabling

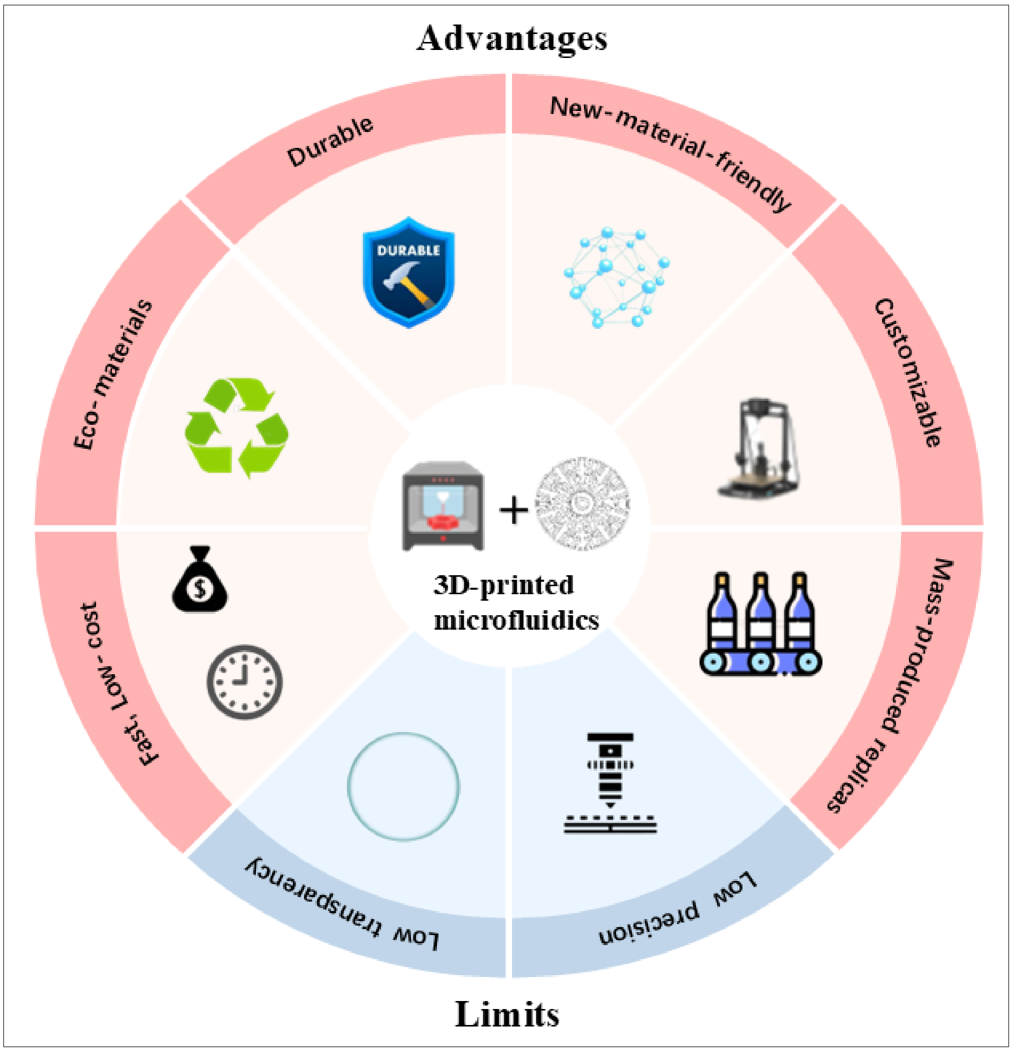

Figure 2. Advantages and limitations of 3D printing combined with microfluidic technology.

mechanistic investigation of cellular adaptation and mechanical signaling pathways in response to altered gravitational forces.

4. Applications in drug screening and therapeutic development

Preclinical development of cancer therapies typically involves testing potential anticancer drugs in tumor cell cultures.[1] The lack of suitable models hinders the development of therapeutic strategies. Researchers have developed diverse *in vitro* cancer models—transwell-based 2D platforms, 3D organoids, hybrid platforms, and microfluidic systems—to simulate tumor tissue and serve as drug research platforms.[75–78] As understanding of cancer biology deepens and microtechnologies advance, microfluidic technology is increasingly utilized in the detection, diagnosis, and treatment of cancer. Its inherent advantages—e.g., minimal sample volume, high sensitivity, and rapid processing—overcome limitations of traditional tumor cultures, such as the lack of dynamic conditions, tissue–tissue interfaces, organ-level structures, and fluid flow.[79,80] These advancements overcome critical limitations of conventional models while positioning microfluidics at the forefront of cancer diagnostics and therapeutic development.

The comparative advantages and disadvantages of leading technologies—PDMS microfluidics versus 3D bioprinting—for tumor therapy applications are systematically evaluated in Table 4, highlighting their distinct application strategies, drug testing scenarios,

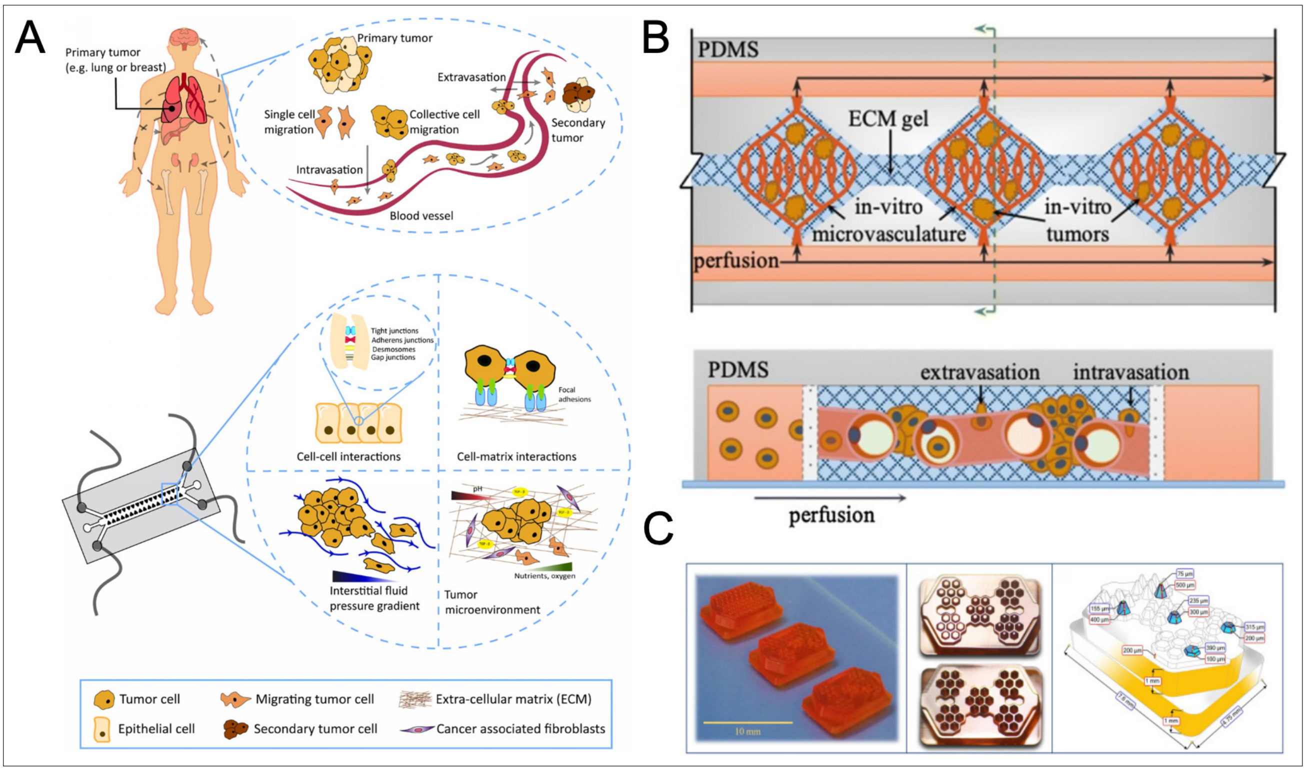

Figure 3. Different tumor models based on 3D printing microfluidic technology. (A) (top) Schematic diagram of the migration behavior of aggressive cancer cells. (Bottom) Application of microfluidics technology in the study of tumor invasion, including real-time imaging of cell–ECM interactions and biophysical changes, controlled application of promigratory factors, pathomimetic tumor microenvironment construction, and organ-specific metastasis modeling. Adapted from Mehta *et al.*[69] (B) Biomimetic microvascular network-on-chip for studying lung cancer metastasis. A diamond-shaped central chamber harbors co-cultured endothelial cells, fibroblasts, and smooth muscle cells within ECM gel, forming a perfusable 3D vasculature. Flanking perfusion channels deliver nutrients or cancer cells. Adapted from Ruzycka *et al.*[72] (C) Different types of 3D-printed molds for the production of 3D tumor spheroid models demonstrate the flexibility of 3D printing to produce microfluidic devices. Adapted from Behroodi *et al.*[73] Abbreviations: PDMS, polydimethylsiloxane; ECM, extracellular matrix.

advantages, and limitations. PDMS microfluidics excel in constructing TME components,[81] enabling spatiotemporal drug delivery control, and culturing spheroids with concentration gradient generation[82] to simulate nutrient and drug transport. However, it faces challenges in scalability. Tumor slice cultivation systems integrate biosensors and mechanical stimuli, but require complex operations,[83] while tumor cell invasion models facilitate high-throughput screening with minimal sample volumes,[84] albeit with contamination risks. PDMS-based *in vitro* cell extravasation models incorporate microvascular networks for real-time imaging, but involve laborious chip fabrication.[85] In addition, Sano *et al.*[86] also mentioned the high absorptivity of hydrophobic small-molecule drugs, leading to significant alterations in effective drug concentration and pharmacokinetic profiles. Key strengths of PDMS include high biomimicry for precise microenvironment replication, excellent reproducibility,[71] and multifunctional integration for real-time monitoring. Its limitations include restricted utility for large tissue specimens, time-consuming fabrication processes (e.g., photolithography), and contamination susceptibility during open operations.[84]

3D printing technologies offer innovative solutions for tumor modeling. For example, Gallegos-Martínez *et al.*[36] and Rahimifard *et al.*[87] developed devices for multicellular and hydrogel-embedded cultures, streamlining prototyping and reducing costs, though SLA resin limitations (e.g., biocompatibility and optical transparency) were noted. Similarly, Ong *et al.*[61] highlighted the user- and eco-friendly nature of 3D printing for spheroid formation assessment. However, resolution limitations at the cellular level, as well as limited optical transparency and biocompatibility concerns with certain materials, remain critical challenges. Li *et al.*[88] employed 3D bioprinting to generate liver cancer cell clusters, reducing preparation workload but facing precision issues that require bioink perfusion. Additionally, Moroni *et al.*[89] stated that most extruded bioprints have

Table 4. Summary of polydimethylsiloxane (PDMS) microfluidics and 3D printing technology in tumor therapy research

Fabrication	Strategy	Drugs	Advantages	Disadvantages	Reference
PDMS	Constructing tumor microenvironment components	Nanoparticles	Precise spatial and temporal control for microenvironment Precise simulation of the in vivo microenvironment Good transport and concentration gradients of nutrients and drugs by fluid flow Ability to integrate with various biosensors and mechanical stimuli Possible for high-throughput screening (low sample volume requirement) Adaptable for real-time imaging and measurements High experimental reproducibility Processing and routine analysis of multicellular spheroids Cost-effective Visualization of in vitro complex phenomena with high resolution Short in vitro culture time	Difficult to scale up No standardized protocol More complex in operation Require external equipment High chance of contamination Laborious process of chip fabrication Closed-system design of CoC devices restricted the utility for culturing larger tissue specimens High absorptivity of hydrophobic small-molecule drugs, leading to significant alterations in effective drug concentration and pharmacokinetic profiles	Oh *et al.*[81]
	Culturing spheroids integrated with a concentration gradient generation	Irinotecan			Lim and Park[82]
	Establishing a tumor slice cultivation system	Cisplatin			Komar *et al.*[83]
	Establishing a tumor cell invasion model	Paclitaxel			Du *et al.*[84]
	Creating an in vitro cell extravasation model with a microvascular network	Engineered T-cells			Pavesi *et al.*[85]
	Uniformly infusing dissociated tumor cells	Anti-PD1 therapeutic agents			Ao *et al.*[71]
	Simulate liver function and evaluate drug metabolism and toxicity	Coumarin			Sano *et al.*[86]
		Midazolam			
		Bufuralol			
3D printing	Using 3D bioprinting to fabricate liver cancer cell clusters and microfluidic chips for cellular maintenance	Metuzumab	Reduced model preparation workload Higher cell proliferation efficiency Streamlining the cycle of designing, prototyping, and testing new microfluidic devices Significant reduction in post-processing time and cost User-friendly Environmentally friendly (compared to polymethyl methacrylate)	Insufficient precision in 3D cell printing (still requires bioink-assisted perfusion), limiting the physiological accuracy of vascularized models Inadequate biocompatibility (SLA resin) Limited optical transparency (SLA resin)	Li *et al.*[88]
	Developing a device to sustain patient-derived multicellular spheroids	Different chemotherapies			Steinberg *et al.*[60]
	Using a device to culture cancer cells or spheroids embedded in hydrogels	Doxorubicin			Gallegos-Martínez *et al.*[36]
	Assessing tumor spheroid formation ability	New pyrazino (1,2-a) benzimidazole derivatives			Rahimifard *et al.*[87]
	Fabricating a perfusion cell culture device	Various drug			Ong *et al.*[61]

Abbreviations: PD1, programmed cell death protein 1; SLA, stereolithography.

a resolution of 50–200 μm, while human capillaries are only 5–10 μm in diameter. This directly leads to the fact that the bioprinted vascular network cannot truly mimic physiological capillaries at scale, and can only produce large "vasculatures" rather than real "capillaries."

In summary, PDMS excels in precision and reproducibility but struggles with scalability, while 3D printing offers rapid prototyping and reduced costs but faces material-related limitations. In clinical practice and scientific research, this review recommends:

(i) For studying nanoparticle transport and vascular permeability: PDMS-based devices are recommended, as they currently offer superior optical clarity for real-time imaging, despite inherent drug absorption issues.

(ii) For generating patient-specific avascular tumor models for high-throughput drug screening:

3D-bioprinted spheroid/organoid arrays present an ideal solution.

(iii) For constructing integrated multitissue models with perfusable vasculature: A hybrid approach utilizing high-resolution 3D-printed molds for PDMS device fabrication currently represents the most viable strategy.

4.1 Precision drug screening using microfluidic device arrays

3D tumor models overcome monolayer limitations by facilitating spatially relevant cell–cell and cell–matrix interactions.[90] Tumor spheroids, well-characterized early-stage cancer models, are widely used in research and drug development due to their simplicity and structural similarity to *in vivo* conditions. Constructed from cancer cells alone or co-cultured with stromal cells (scaffolded or scaffold-free), microfluidics simulates tumor tissue/organs via *in vitro* models and spheroid culture systems, establishing drug screening platforms.[91]

4.1.1. Microfluidic modeling of the tumor microenvironment

Researchers have developed microfluidic 3D tumor models with organ-specific characteristics or varying tumor progression by controlling ECM properties and cellular compositions for applications such as drug screening (Figure 4A).[92] Furthermore, microfluidic devices have been demonstrated to effectively deliver nutrients and/or drugs to tumor tissues through microchannels, thereby sustaining their physiological activities (Figure 4B).[93] The TME comprises heterogeneous ECM components, neighboring cells (e.g., fibroblasts, pericytes, and astrocytes), immune cells, adipocytes, stem cells, vasculature, lymphatics, and physical conditions governing convection/diffusion.[94,95]

Introducing TME components into microfluidic devices allows for the development of tumor models that replicate the *in vivo* environment, while enabling real-time high-resolution imaging for drug testing.[96] For example, Xiong *et al.*[97] developed a customized bladder-shaped microfluidic device with biomimetic guides and triangular markers. This platform enabled long-term culture (more than 4 weeks) of patient-derived spheroids while preserving histopathological and genetic signatures. By optimizing drug diffusion kinetics (uniform distribution within 6 s) and hydrodynamic conditions, the system achieved high-fidelity drug susceptibility testing across eight patients. Each microfluidic device was seeded with 5–15 tumor spheroids, serving as a preclinical model to predict drug response by assessing the effects of different chemotherapeutic agents and their concentrations on the spheroids. Results correlated closely with PDX models and clinical responses, demonstrating their capacity to recapitulate the *in vivo* TME. Crucially, the device overcame limitations of traditional methods (e.g., spheroid loss in ultra-low attachment plates), providing a clinically translatable tool for rapid personalized chemotherapy screening (Figure 4C & D).

Meanwhile, Skubal *et al.*[98] utilized a microfluidic platform to capture the vascularization process during the development of renal cell carcinoma spheroids in real time. They successfully obtained high-resolution images of the tumor-on-a-chip model before and after treatment with bevacizumab to evaluate the efficacy of the vascular-targeting therapy (Figure 4E). Similarly, Oh *et al.*[81] constructed a TME with spatiotemporal control by integrating components (e.g., ECM, vasculature, stromal cells, and interstitial fluid) on-chip, monitoring nanoparticle accumulation/uptake in target cells to address nanomedicine translation challenges. Du *et al.*[84] developed a microfluidic platform simulating/controlling multiple TME factors, evaluating 3D tumor invasion into stroma, and investigating paclitaxel (PTX) effects on cancer cell migration, survival, and morphology. Likewise, Pavesi *et al.*[85] designed a platform reconstructing human umbilical vein endothelial cell microvascular networks to observe cancer cell extravasation and capture T cell movement spatiotemporal data/cytotoxic efficiency, validating T cell therapy.

4.1.2. High-throughput drug screening enabled by the hybrid 3D printing–microfluidics system

Recent advances leverage 3D printing to fabricate microfluidic devices, offering a cost-effective and physiologically relevant strategy that streamlines production, reduces costs, and enables customization. Building on this concept, Steinberg *et al.*[60] engineered a fully 3D-printed microphysiological system capable of maintaining patient-derived tumor spheroids under long-term culture conditions, enabling comprehensive evaluation of drug combination therapies (Figure 4F). Gallegos-Martínez *et al.*[36] introduced a user-friendly, flexible 3D-printed device for culturing cancer cells or organoid spheroids in hydrogels within controlled environments.

Beyond device fabrication, 3D printing can directly generate tumor cell clusters; bioprinting potentially reduces culture cycles and simplifies experimental preparation. For example, Li *et al.*[88] prepared liver cancer cell clusters via 3D cell printing, with the microfluidic chip providing a biomimetic microenvironment, validating the feasibility as a novel *in vitro* drug screening model. What's more, Rahimifard *et al.*[87] used a 3D-printed microfluidic device to evaluate glioblastoma spheroid formation after exposure to novel pyrazinyl [1,2-a] benzimidazole derivatives, assessing

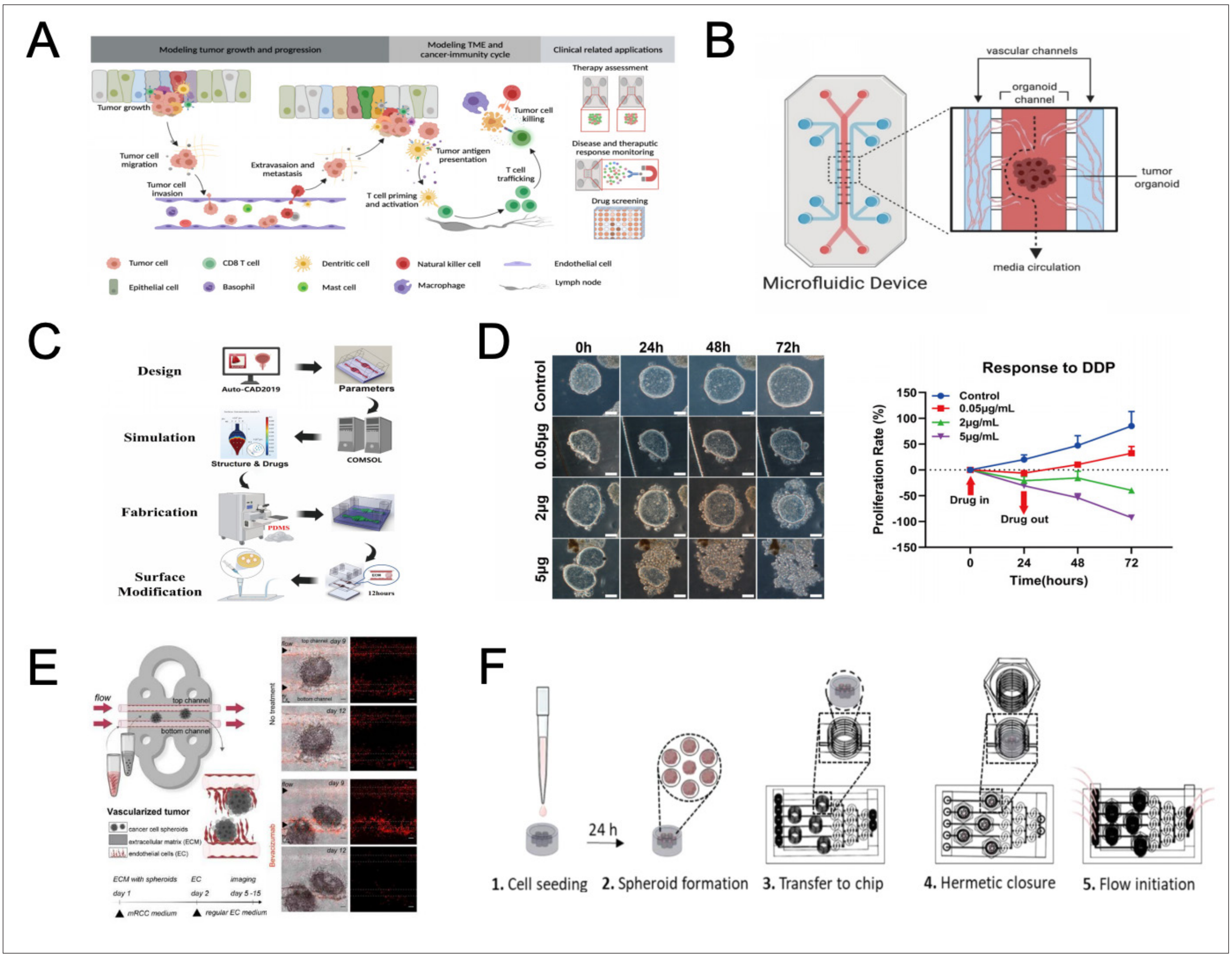

Figure 4. Advanced 3D-printed microfluidic platforms for anticancer drug development. (A) By integrating microfluidic technology with 3D culture systems, it is possible to precisely control matrix structure, cellular composition and ratios, flow velocity, and other characteristics to meet the demands of cancer modeling—such as tumor growth, cancer cell extravasation, and angiogenesis—clinically applied for efficacy evaluation, treatment response monitoring, and drug screening. Adapted from Xie *et al.*[92] (B) A schematic diagram of a tumor-on-a-chip model. Tumor organoids are cultured within the organoid channel, and nutrients and/or drugs are efficiently delivered to the tumor tissue through the vascular channels, enabling the long-term maintenance of the physiological activity of the tumor-like organoids and establishing a media circulation. Adapted from Gunti *et al.*[93] (C) Schematic diagram of the workflow for designing and constructing a microfluidic device for culturing tumor spheroids and drug susceptibility assays. Adapted from Xiong *et al.*[97] (D) Morphological changes of tumor spheroids following treatment with varying concentrations of cisplatin (DDP) (scale bar: 50 µm). Adapted from Xiong *et al.*[97] (E) Schematic illustration of the microfluidic chip setup with an integrated vascularized tumor model and confocal microscopy images of the chips before (Day 9) and after (Day 12) bevacizumab treatment. Left panels show mRCC spheroids (non-fluorescent) co-cultured with endothelial cells (red fluorescence). The right panels show endothelial cells only (red fluorescence). Adapted from Skubal *et al.*[98] (F) Illustration of the operating steps for using the 3D-printed versatile tumor-on-a-chip for spheroid culture. Adapted from Steinberg *et al.*[60]

their inhibitory effects. The application of 3D-printed microfluidics in the establishment of drug screening platforms has streamlined the cycle of designing, fabricating, and testing new devices, reducing the workload for model construction and improving reproducibility. Moreover, these devices demonstrate enhanced biocompatibility in many applications and are more environmentally friendly compared to traditional materials.

4.2 Enhancing cancer therapy using microfluidic drug delivery systems and biomimetic scaffolds

Recent advances in microfluidic technologies have enabled precise control over nanoparticle fabrication, offering significant advantages in reproducibility, scalability, and tunability compared to conventional bulk methods. Current microfluidic device designs, including droplet-based, continuous-flow, and hybrid systems, have been engineered to optimize nanoparticle synthesis through fine-tuned manipulation of fluid dynamics, mixing efficiency, and interfacial phenomena. These platforms have facilitated the development of diverse nanodelivery systems, such as lipid nanoparticles, polymeric nanocarriers, and inorganic hybrids, tailored for applications in drug delivery, diagnostics, and theranostics (Figure 5A).[99] However, challenges persist in achieving large-scale production, ensuring long-term stability, and addressing biocompatibility concerns. Furthermore, the integration of smart materials, AI-driven optimization,

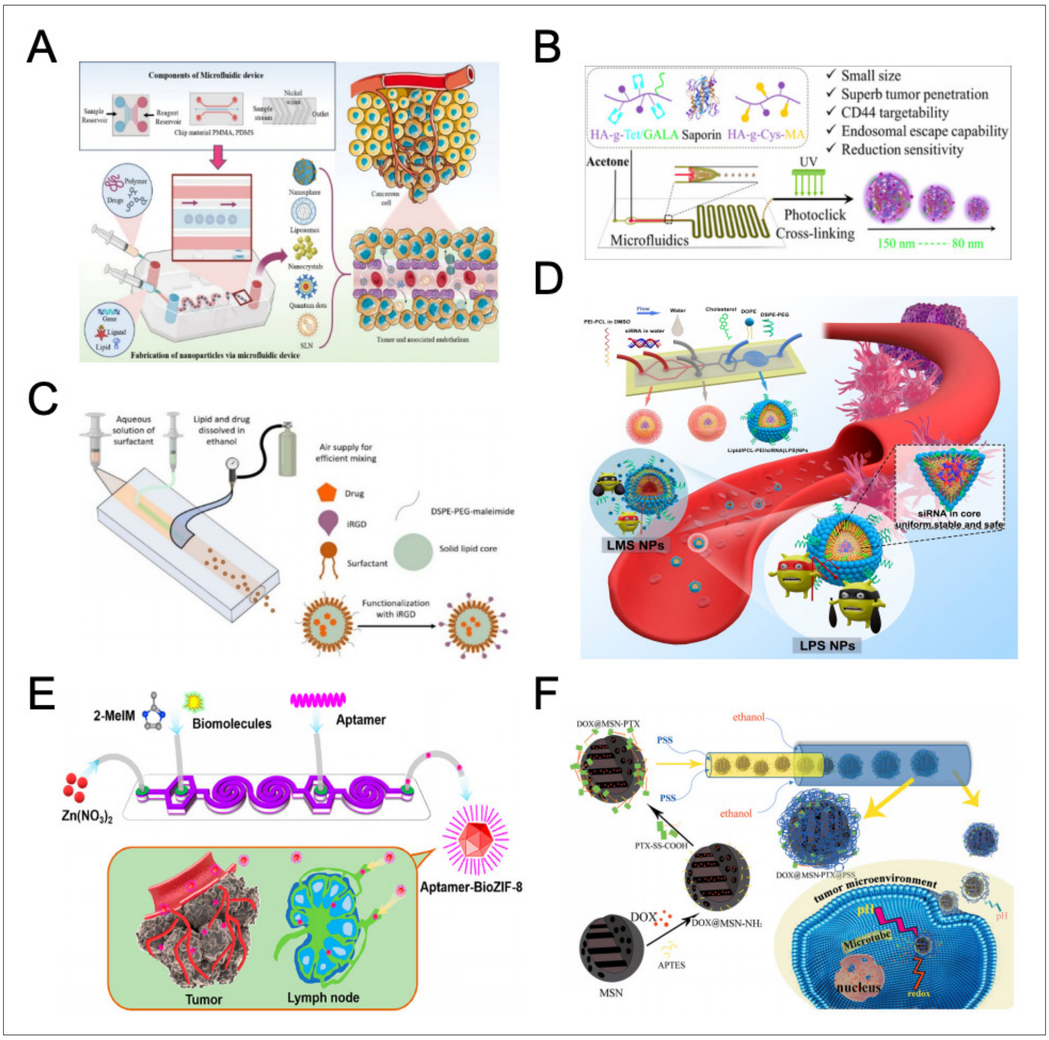

Figure 5. Therapeutic applications of 3D-printed microfluidics in oncology. (A) The fabrication of microfluidic devices and their application in formulating various nanodelivery systems, such as nanospheres, liposomes, nanocrystals, quantum dots, and solid lipid nanoparticles, enhances the efficacy and homogeneity of nanomedicines for tumor therapy. Adapted with permission from G *et al.*[99] Copyright © 2025 American Chemical Society. (B) Microfluidics-fabricated small-sized (with a kinetic diameter of 80–150 nm), tumor-penetrable, endosome-escaping, and bioresponsive hyaluronic acid nanogels (HA-NGs) were developed for cluster of differentiation (CD)44-targeted intracellular delivery of therapeutic proteins. Adapted with permission from Huang *et al.*[103] Copyright © 2019 American Chemical Society. (C) Paclitaxel (PTX)-solid lipid nanoparticles were produced using microfluidic technology by mixing an aqueous surfactant solution with lipids and drug dissolved in ethanol, followed by functionalization with iRGD peptide. Adapted with permission from G *et al.*[99] Copyright © 2025 American Chemical Society. (D) A microfluidic platform facilitates the precise fabrication of core–shell lipid–polymer nanoparticles (LPS NPs) for efficient small interfering RNA (siRNA) delivery. The process initiates with siRNA complexation using low-molecular-weight poly(ethylene imine) (LMW PEI) within reverse micelles, followed by interfacial assembly of a lipid membrane (DOPE/cholesterol/DSPE-PEG) to form monodisperse, anionic nanoparticles. Adapted with permission from Wei *et al.*[105] Copyright © 2020 American Chemical Society. (E) The microfluidic chip enables rapid, one-step synthesis of aptamer-modified biozeolitic imidazolate framework (BioZIF-8) nanoparticles for targeted delivery to lymph nodes and tumors. In the first stage, ZIF-8 nanoparticles encapsulating biomolecules are formed; in the second stage, their surfaces are functionalized with aptamers. Adapted with permission from Balachandran *et al.*[106] Copyright © 2021 American Chemical Society. (F) Schematic illustration of DOX@MSN-PTX synthesis and microfluidic preparation of DOX@MSN-PTX@PSS. The PSS layer swells in the acidic tumor microenvironment, conferring a positive surface charge on the DOX@MSN-PTX@PSS nanoparticles. This facilitates their internalization into tumor cells via endocytosis. Subsequently, the pH and redox conditions within tumor cells trigger the release of doxorubicin and PTX. Adapted from Yan *et al.*[107]

and modular microfluidic architecture presents promising avenues to overcome these limitations and advance next-generation nanomedicine.

Bevacizumab, a humanized anti-vascular endothelial growth factor monoclonal antibody, inhibits malignant cell proliferation/metastasis by suppressing tumor vasculature formation and is clinically approved for angiogenesis inhibition and cancer treatment.[100] However, poor biodistribution and pharmacokinetics limit its bioavailability at tumor sites, reducing efficacy.[101] Microfluidics addresses this by generating size-uniform microgels encapsulating proteins as effective drug carriers. For example, Chen *et al.*[102] developed microgels with tumor adhesive properties and pH-dependent degradability via microfluidics and photopolymerization for sustained local delivery of bevacizumab/docetaxel. This approach normalized tumor vasculature, enabling uniform drug distribution and deeper penetration, thereby improving chemotherapy outcomes and offering a platform for treating recurrent/metastatic cancers. Similarly, Huang *et al.*[103] prepared small, traceable, endosome-disrupting, and bioresponsive nanogels via microfluidics for the cluster of differentiation (CD) 44-targeted cytoplasmic delivery of therapeutic proteins ([Figure 5B]). Arduino *et al.*[104] developed a biomimetic liposome system based on microfluidic technology and 3D printing, overcoming blood–brain barrier penetration and immune clearance challenges in glioblastoma treatment, enhancing PTX/carboplatin targeting and efficacy. This system offers precise control over nanoparticle synthesis, enabling the development of tailored nanocarriers for targeted central nervous system malignancies therapeutics. Building on this, they developed internalizing Arg-Gly-Asp motif (iRGD)-functionalized PTX-loaded solid lipid nanoparticles via microfluidic technology, enhancing their anticancer potential through selective drug delivery to cancer cells and increased accumulation and penetration at tumor sites. The study also demonstrated that iRGD functionalization improved cellular uptake and cytotoxicity (Figure 5C). Meanwhile, Wei *et al.*[105] utilized microfluidics to synthesize a novel core–shell hybrid nanoparticle with a small interfering RNA (siRNA) core, exhibiting strong siRNA protection and loading capacity, improved *in vivo* stability and biosafety, as well as effective antitumor efficacy (Figure 5D). Balachandran *et al.*[106] developed an integrated microfluidic chip to synthesize aptamer-modified biozeolitic imidazolate frameworks for targeting lymph nodes and tumors (Figure 5E). Yan *et al.*[107] fabricated a pH/redox-triggered mesoporous silica nanoparticle nanoplatform for the co-delivery of doxorubicin/PTX (Figure 5F).

Microfluidics also shows significant potential in tumor immunotherapy. Han *et al.*[108] used commercial devices (Dolomite) for fluoroconjugation of epigallocatechin gallate–ligand–siRNA anti-TOX nanoparticles to regulate tumor cells and exhausted T cells synergistically. Moreover, 3D-printed microfluidic devices show significant promise for advancing drug delivery across the blood–brain barrier. Therefore, by integrating advanced microfluidic platforms for precision nanoparticle synthesis with conventional antitumor drugs, the resulting hybrid drug delivery systems can significantly enhance therapeutic efficacy and targeting specificity. This synergistic approach leverages the tunability of nanocarriers and the proven mechanisms of chemotherapeutic agents, offering a promising strategy to improve cancer treatment outcomes.

Similarly, the integration of 3D printing and microfluidic technologies has led to the development of microfluidic 3D-printed scaffolds, a groundbreaking strategy for addressing post-operative tumor recurrence and facilitating tissue regeneration. A notable example is the work by Zhang *et al.*,[109] who designed a light-responsive platinum (IV) (Pt[IV]) prodrug-loaded scaffold using a microfluidic-assisted 3D printing approach. The scaffold, composed of polymerized gelatin methacryloyl, exploits the photoconversion of low-toxicity Pt(IV) to highly cytotoxic Pt(II) species upon irradiation, enabling localized and controlled drug release. This system demonstrated remarkable tumor-suppressive efficacy, anti-metastatic activity, and pro-regenerative properties in post-surgical applications. Building on this concept, Li *et al.*[110] developed a fish gelatin/berberine composite scaffold for post-operative gastric cancer therapy, exhibiting potent tumor growth inhibition and excellent biocompatibility, further validating the potential of hybrid 3D-printed microfluidic scaffolds in precision oncology and regenerative medicine. These advances highlight the transformative role of convergent biofabrication technologies in developing multifunctional implants for cancer therapy and tissue repair.

While integrated 3D-microfluidic bioprinting platforms have revolutionized preclinical drug development and therapeutic delivery, their clinical impact extends beyond treatment to transformative diagnostic capabilities. The same engineering principles enabling physiologically relevant tumor models and targeted nanotherapeutics—precision fluidic control, biomimetic spatial design, and patient-specific customization—now empower the development of next-generation liquid biopsy platforms. By transitioning focus from therapeutic intervention to early detection and real-time monitoring, these technologies address a critical unmet need in oncology: minimally invasive, longitudinal tracking of tumor dynamics to guide precision therapy adjustments.

5. 3D-printed microfluidic platforms for cancer diagnostics and biomarker detection

While tissue biopsy remains the clinical gold standard for tumor characterization, its invasiveness limits serial disease monitoring, especially when sample volumes are insufficient for diverse molecular testing.[111] Liquid biopsy approaches analyzing circulating tumor-derived components—including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, and proteomic signatures—provide minimally invasive access to real-time tumor dynamics.[112,113] State-of-the-art 3D-printed microfluidic systems have achieved unprecedented precision in tumor biomarker isolation through bioinspired architectures that recapitulate key features of *in vivo* capture microenvironments.[114,115] As comprehensively documented in Table 5, these engineered platforms demonstrate remarkable specificity and efficiency in isolating three critical classes of oncological biomarkers: CTCs, exosomes, and protein markers. Contemporary technological advances have yielded microfluidic designs that incorporate (i) biomimetic surface topographies for enhanced cellular interactions, (ii) tunable fluidic parameters for size-based exosome sorting, and (iii) nanostructured interfaces for ultrasensitive protein detection—collectively representing a paradigm shift in liquid biopsy technologies.[116]

5.1. Microfluidic isolation and detection of circulating tumor cells

CTCs are found in circulation, shed from malignancies to enter the bloodstream and migrate to distant organs, forming metastases.[117] Dissemination from primary tumors initiates metastasis and is a major cause of cancer mortality. CTCs are extremely rare compared to blood cells, making their separation from circulation—or of tumor cells from

Table 5. Summary of studies on 3D-printed microfluidic systems to capture tumor markers

Biomarkers	Microfluidic technology	Results	Advantages	Reference
CTCs	RUBYchip™	The average capture efficiency of renal cell carcinoma is 74.9%, significantly higher than other methods.	Efficiently captures CTCs and remains unaffected by epithelial–mesenchymal transition.	Leitão *et al.*[125]
	Sorting by interfacial tension (SIFT)	Effectively isolates cancer cell subpopulations with high glycolytic activity.	Adjusts the pH selection threshold by altering fluid conditions.	Zielke *et al.*[162]
	A 3D-nanostructured substrate coated with anti-EpCAM	The synergistic effect enhances CTC capture performance observed in blood samples.	The micro-mixer function enhances the interaction between cells and the substrate's local topography.	Wang *et al.*[122,123]
	The Parsortix® PR1 system	Using various downstream analyses to enrich breast cancer cells in a small volume of blood from healthy donors.	Various labeled tumor cell lines can be captured from the blood without being restricted by surface antigens.	Cohen *et al.*[124]
	Hyaluronic acid-functionalized electrospun chitosan nanofiber-integrated microfluidic platform	Specifically captured cluster of differentiation 44-overexpressing cancer cells with an efficiency of up to 91% and achieved 90% complete cell release through treatment with glutathione.	Enables non-destructive release of the captured cells, facilitating subsequent cell analysis.	Wang *et al.*[163]
	V-BioChip	Detecting CTCs in patients with cervical cancer and endometrial cancer; the expression of HER2 and GATA3 in CTCs is highly associated with early recurrence.	Allows for genomic amplification and mutation analysis of individual CTCs, offering potential for personalized treatment.	Law *et al.*[126]
Exosomes	Microfluidic chip	Efficiently and specifically isolates exosomes with high purity and superior yield.	Isolates exosomes from small sample volumes, with the isolated exosomes exhibiting high purity and integrity.	Dorayappan *et al.*[141]
Protein markers	A microfluidic microarray made using 3D printing	Successfully detected the metastatic marker desmoglein 3 and other accompanying biomarkers for head and neck squamous cell carcinoma.	The first microfluidic device with sub-fg level sensitivity for single-cell protein measurements and cancer metastasis.	Sharafeldin *et al.*[144]

Abbreviations: CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; GATA3, GATA binding protein 3; HER2, human epidermal growth factor receptor 2.

body fluids—significant for diagnosis, disease monitoring, and metastasis understanding. In metastatic patients, the peripheral blood CTC concentration is typically extremely low (<10 cells/mL). Efficient recovery of highly pure, viable single CTC or clusters from large blood volumes (>5 mL) poses a core technological challenge. Early strategies relied on CTC-specific surface markers, such as epithelial cell adhesion molecule (EpCAM), exemplified by the antibody-based "CTC-chip". However, affinity-based capture applicability is limited for non-epithelial tumors (e.g., melanoma) and CTCs undergoing epithelial–mesenchymal transition with downregulated EpCAM.[118] Sorting technologies based on physical properties (e.g., size, density, deformability, electrical impedance, and acoustics) have advanced rapidly to address this. Notably, Fachin *et al*.[119] developed a microfluidic device called CTC-iChip to separate CTCs based on cell size and EpCAM expression heterogeneity, overcoming CTC heterogeneity challenges (Figure 6A).

5.2. Integrated microfluidic systems for circulating tumor cells capture and molecular profiling

Leveraging the capabilities of microfluidic devices for microchannel fluid pumping, analyte filtration, and selective capture, alongside the high design flexibility and material versatility offered by 3D printing technology in the fabrication of such devices, researchers have developed diverse and efficient strategies for capturing CTCs.

5.2.1. Optimized microfluidic geometries for improved separation purity and throughput

Leveraging additive manufacturing, Chen *et al*.[62] employed 3D printing to fabricate microfluidic devices featuring tailored microchannel geometries and specialized surface modifications. This innovative approach significantly enhanced CTC capture efficiency by optimizing cell-surface interactions. Stiefel *et al*.[120] integrated immunomagnetic bead enrichment, microfluidic fluorescence activation sorting, and single-cell droplet distribution modules, and designed a multifunctional microfluidic chip including sample storage area, hydrodynamic focusing channel, optical detection area, and cell dispensing nozzle to achieve efficient separation of rare cells, and successfully isolated CTC subsets of head and neck squamous cell carcinoma patients, showing good clinical translation potential and providing real-time monitoring tools for personalized treatment (Figure 6B).

Building on geometric innovation, Tan *et al*.[121] engineered microchannels integrated with crescent-shaped isolation well arrays. These structures incorporated precisely calibrated 5-μm gaps that selectively expelled smaller blood components while retaining target cells, achieving both high-purity capture and efficient background depletion. Further advancing structural design, Wang *et al*.[122,123] systematically compared 3D anti-EpCAM antibody-coated micropillar arrays against conventional planar substrates. Their results confirmed that the 3D-printed nanostructured substrates dramatically increased capture efficiency, attributed to their expanded surface area for ligand binding and optimized local hydrodynamics (Figure 6C & D). For broader clinical applicability, Cohen *et al*.[124] utilized the commercial Parsortix® PR1 system, which exploits serpentine microchannels to perform label-free, size-based CTC separation and enrichment. This method demonstrated robust broad-spectrum capture across diverse tumor lines, irrespective of variable antigen expression profiles.

5.2.2. From lab to clinic: validating 3D-printed microfluidic systems for ultra-sensitive diagnostic applications

Advancing clinical translation, Leitão *et al*.[125] engineered the RUBYchip™, a 3D-printed microfluidic platform that demonstrates superior capture efficiency for renal cell carcinoma compared to conventional methods. Critically, their work established a robust association between CTC enumeration and patient prognosis, highlighting its clinical utility for disease monitoring. Complementing this, Law *et al*.[126] developed a vertically structured 3D-printed biosensor (V-BioChip) for high-efficiency enrichment and detection of CTCs in gynecological malignancies (e.g., cervical/endometrial cancers). Their analysis revealed novel prognostic relationships: expression levels of transcription factors human epidermal growth factor receptor 2/GATA binding protein 3 and surface markers CD13 in captured CTCs showed significant correlations with early cancer recurrence. Collectively, these 3D-printed innovations—through optimized capture performance and biomarker discovery—substantially accelerate the integration of CTC diagnostics into clinical practice (Figure 6E).

5.3. Detection of circulating nucleic acids, exosomes, and protein biomarkers

Over the past decade, microfluidic devices for molecular analysis have made remarkable progress, evolving from proof-of-concept demonstrations to sophisticated platforms capable of performing complex analytical workflows. These miniaturized systems now offer distinct advantages over conventional techniques, including reduced sample/reagent consumption, faster analysis times, improved sensitivity through enhanced mass transport, and the ability to integrate multiple processing steps on a single chip. With demonstrated success in applications ranging from point-of-care diagnostics to high-throughput omics analysis, microfluidics has reached a technological

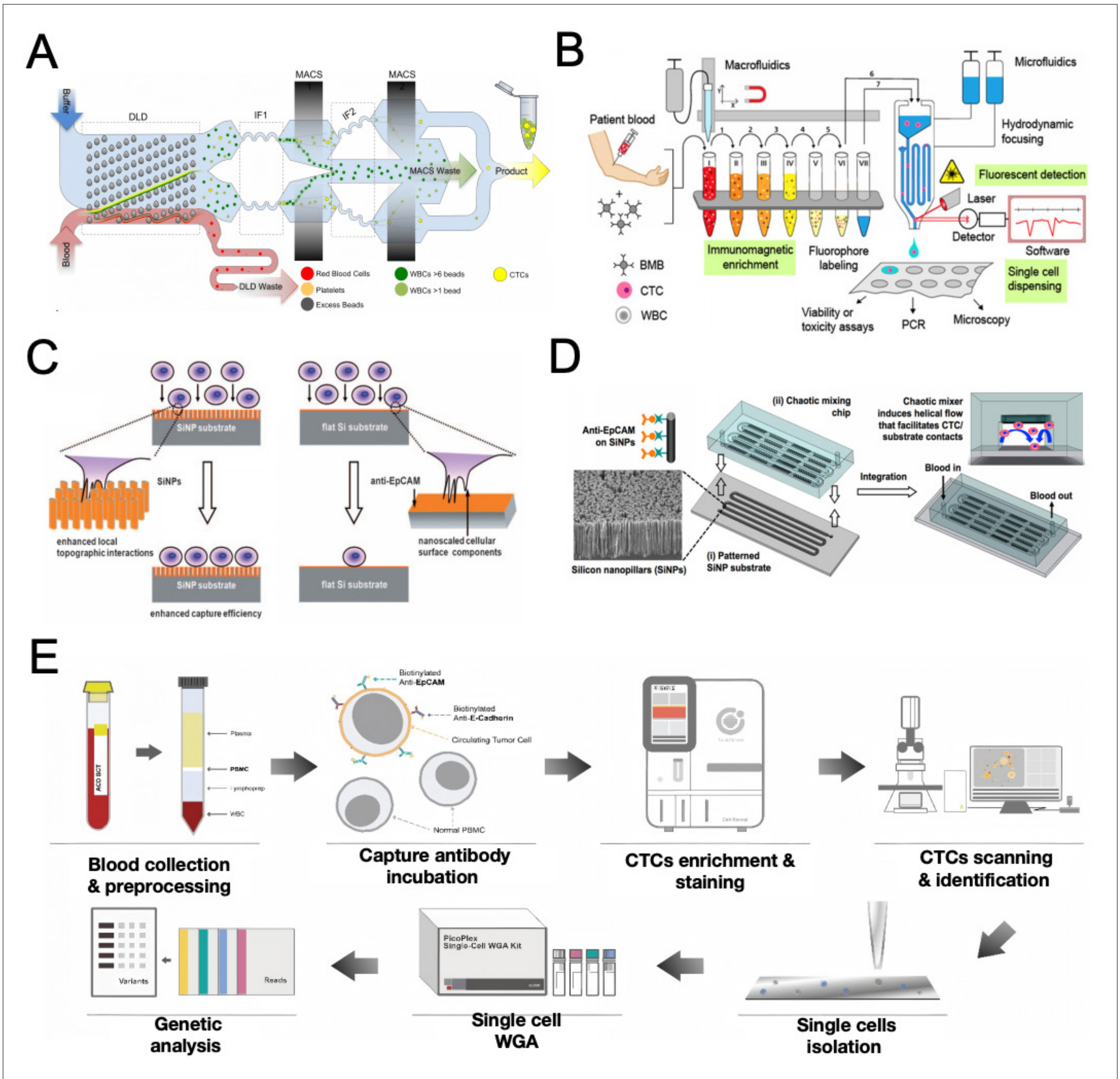

Figure 6. Advanced circulating tumor cell (CTC) isolation platforms using 3D-printed microfluidics. (A) Schematic of a rapid CTC processing chip featuring deterministic lateral displacement for size-based enrichment, coupled with integrated cell handling modules for downstream molecular analysis. Adapted from Fachin *et al.*[119] (B) CTCelect automated workflow for single-cell isolation. Positive selection of tumor cells and subsequent white blood cell (WBC) consumption and fluorophore-conjugated labeling were performed in the enrichment module of the device. The enriched sample is then transferred to the cell sorting subunit into a microfluidic chip for cell sorting and dispensing of single cells in microliter droplets. Adapted from Stiefel *et al.*[120] (C) A new CTC capture platform consisting of patterned SiNP substrates with high CTC affinity and serpentine chaotic mixing channels with anti-EpCAM coatings. The platform enables efficient CTC capture through the synergistic effect of high CTC/substrate affinity and increased contact frequency. Adapted with permission from Wang *et al.*[122] Copyright © 2011 WILEY-VCH Verlag GmbH & Co. KGaA. (D) 3D SiNP architecture coated with anti-EpCAM antibodies demonstrating significantly enhanced capture efficiency (>90%) for cancer cells in whole blood, attributed to increased surface area and biomimetic nanotopography. Adapted with permission from Wang *et al.*[123] Copyright © 2009 WILEY-VCH Verlag GmbH & Co. KGaA. (E) Schematic of the 3D-printed V-BioChip biosensor for gynecological cancers (cervical/endometrial), featuring CTC enrichment via size-based filtration, immunoaffinity-based capture, and on-chip HER2/GATA3 biomarker detection for recurrence risk stratification. Adapted from Law *et al.*[126]

maturity where it can legitimately compete with, and in some cases surpass, traditional analytical methods, including enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and mass spectrometry. Particularly in scenarios requiring portability, automation, or multiplexed detection, microfluidic platforms are increasingly becoming the preferred choice, suggesting they are ready to transition from research laboratories to widespread clinical and industrial adoption. Microfluidic models for molecular diagnostics have emerged as powerful platforms for comprehensive biomarker analysis, particularly through innovative device designs enabling back-to-back processing of CTCs, cell-free DNA (cfDNA), tumor-derived exosomes, and protein biomarker isolation from minimally processed whole blood (Figure 7A).[127]

5.3.1. Circulating nucleic acid separation

Circulating cfDNA, primarily released by apoptotic or necrotic cells, includes tumor-derived mutant fragments termed ctDNA.[128,129] CtDNA is a highly tumor-specific biomarker (Figure 7B).[130–132] However, elevated cfDNA levels also occur in benign conditions or tissue injury, overlapping with early-stage cancer concentrations.[133] Detection systems must therefore discriminate and quantify mutant allele frequencies.[134] Compared to CTC isolation, integrated microfluidic devices for direct quantitative cfDNA isolation from whole blood remain scarce, largely due to extraction challenges from minute volumes and complex preprocessing. Recent advances include nanostructured microelectrode electrochemical sensors enabling amplification-free, direct detection of mutant ctDNA in serum.[135–137]

5.3.2. Tumor-derived exosome enrichment

Exosomes are extracellular vesicles actively secreted by cells, including tumor cells.[138] They carry molecular cargo (e.g., DNA, RNA, proteins, and lipids), harboring rich tumor information. Highly abundant in body fluids and more stable than ctDNA, exosomes enable detection in early-stage cancers, making them promising biomarkers (Figure 7C).[139] Traditional isolation techniques (e.g., ultracentrifugation, precipitation, and filtration) are laborious and time-consuming, as well as yield suboptimal purity. Microfluidic techniques primarily use immunoaffinity capture. The ^{EV}HB-chip, with a Y-configuration, processes milliliters of serum; its nanostructured surface captures vesicles across size ranges, outperforming ultracentrifugation/magnetic bead methods.[140] Dorayappan *et al.*[141] developed a microfluidic device combining anti-CD63 and anti-EpCAM antibodies for high-purity, high-yield specific exosome isolation from culture media/patient serum.

6. Low-abundance protein biomarker isolation

Detection of proteins and their metabolites in microfluidics is among the most mature and commercialized microfluidics applications.[142] Breakthroughs exist in integration, multiplexing, sensitivity, and throughput. Integrated devices perform plasma separation from a blood drop while quantifying multiple proteins, matching conventional ELISA efficiency. Emde *et al.*[143] used 3D printing to develop a chip that detects acute promyelocytic leukemia-specific biomarkers (promyelocytic leukemia-retinoic acid receptor α [PML::RARA] fusion protein), significantly shortening development cycles for rapid prototyping/personalized diagnostics (Figure 7D). Sharafeldin *et al.*[144] designed and 3D-printed a low-cost microfluidic immunoarray for on-chip cell lysis and quantitative protein analysis. Antibodies captured in chambers coated with swellable 3D chitosan hydrogel films enabled sandwich immunosorbent assays, successfully detecting metastatic head and neck squamous cell carcinoma markers, such as desmoglein 3, and co-biomarkers.

Overall, these integrated systems utilize continuous perfusion of whole blood through cascaded microchambers and microchannels that sequentially apply multiple microscale separation principles, including deterministic lateral displacement, inertial focusing, affinity capture, and size-exclusion filtration, to achieve high-purity isolation of diverse blood components, such as leukocytes, CTCs, ctDNA, proteins, and exosomes. Following separation, the captured cells and biomolecules can either be extracted for downstream genomic/proteomic analysis (e.g., single-cell RNA sequencing or digital PCR) or directly analyzed on-chip through integrated detection modules (e.g., impedance cytometry for cellular characterization or surface-enhanced Raman spectroscopy for protein profiling). This "sample-in-answer-out" capability positions microfluidic systems as transformative tools for liquid biopsy applications, offering advantages in automation, multiplexing, and analytical sensitivity compared to conventional centrifugation-based and immunoaffinity-based workflows.

7. Outlook

3D microfluidic tumor models surpass traditional 2D cultures in biological fidelity by closely replicating essential *in vivo* features, such as (i) 3D tissue architecture, (ii) physiologically accurate cell–cell and cell–matrix interactions, and (iii) dynamic nutrient and oxygen gradients. The advent of 3D printing has further revolutionized these systems, enabling rapid prototyping of intricate microstructures while drastically reducing costs and operational complexity compared to animal models.

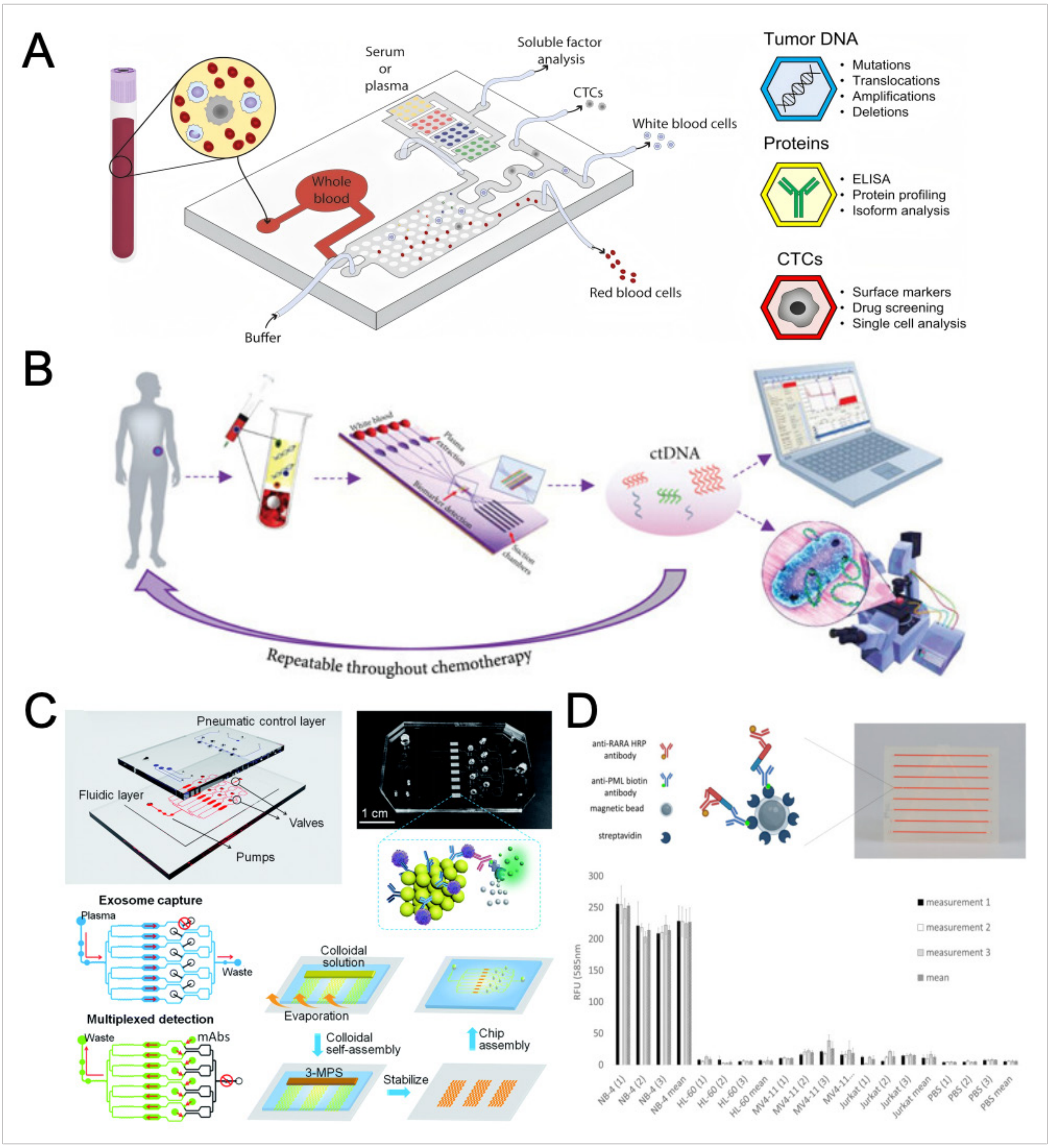

Figure 7. Separation of circulating tumor biomarkers using the 3D printing–microfluidic technology. (A) Theoretical device design for the analysis of circulating tumor cells (CTCs), circulating proteins, and DNA from whole blood. Whole blood is perfused through a series of microchambers and microchannels that leverage several microscale-based separation techniques to isolate multiple components such as immune cells, CTCs, circulating tumor DNA (ctDNA), proteins, and exosomes. Adapted from Ayuso *et al.*[127] (B) Clinical microfluidic devices have revolutionized noninvasive liquid biopsy for ctDNA detection and analysis by enabling rapid, cost-effective, and simple workflows that allow repeatable molecular analysis anytime and anywhere. Adapted from Gauri and Ahmad.[132] (C) An integrated multichannel platform for multiplexed, sensitive, and high-throughput immunophenotyping of circulating exosomes. Adapted from Wang *et al.*[139] (D) 3D-printed microfluidic enzyme-linked immunosorbent assay implementing magnetic bead-based sandwich immunoassay for the ultrasensitive detection of promyelocytic leukemia-retinoic acid receptor α oncoproteins in acute promyelocytic leukemia. Adapted from Emde *et al.*[143]

Beyond tumor modeling, the convergence of microfluidics and 3D printing has driven significant innovations in therapeutic delivery,[145,146] including (i) precision-engineered drug-loaded microcarriers with programmable release kinetics, (ii) patient-specific implantable scaffolds for targeted therapy, and (iii) microphysiological systems for personalized drug screening.

It must be acknowledged that although 3D-printed microfluidics holds considerable promise, it still faces multiple challenges. There exists a fundamental trade-off between resolution and printing speed, where high-precision printing is often time-consuming and requires costly equipment, thereby limiting the fabrication of complex structures and large-scale implementation.[147] Furthermore, existing 3D-printing materials, such as photopolymer resins, exhibit insufficient biocompatibility, optical transparency, and mechanical properties,[148] along with difficulties in achieving multimaterial printing. These limitations make it challenging to meet the stringent requirements of applications such as biological detection and chemical synthesis. Additionally, the lack of standardization in 3D-printed microfluidic devices and competition with conventional microfabrication techniques further hinder their translation from laboratory research to commercial and clinical adoption. Beyond current applications, this review explores how integration with novel biosensing modalities and computational analytics could revolutionize personalized cancer diagnostics and treatment development.

3D-printed microfluidics demonstrates transformative potential in tumor research. Building upon 3D printing, 4D printing introduces a temporal dimension through stimulus–response systems activated by pressure, light, or heat, enabling dynamic modulation of flow dynamics. The reversible shape-changing behavior of soft materials enhances the flexibility and complexity of the microfluidic actuator.[149–151] Utilizing shape-memory polymers' phase-change capability to dynamically tune microchannel geometries via thermal/optical stimuli,[152] next-generation tumor-on-chip models will precisely mimic patient-specific biology and enable real-time drug delivery optimization, advancing precision therapy.

However, 4D printing still faces considerable technical challenges, such as the biocompatibility and long-term stability of shape-memory polymers, precise control over stimulus application, and reproducible dynamic responses under physiological conditions.[153] Moreover, clinical translation is hampered by significant hurdles, including chip-to-human physiological discrepancies, a lack of standardization in fabrication and operational protocols, and the inherent complexity of integrating dynamic materials into biomedical devices.[154,155]

Building on 3D/4D printing, Lai and Wang[156] conceptualized 5D printing: embedded information (e.g., growth factors, nanoparticles, genetic/cellular data) as the fifth dimension. 5D printing yields structures with shape-changing and information-embedding capabilities that actively interact with their environment, unlike passive 3D/4D objects. 5D-printed scaffolds represent a transformative advancement in biofabrication, integrating spatially encoded structural information with dynamic, stimuli-responsive properties that actively interface with native biological microenvironments. These sophisticated constructs achieve two paradigm-shifting capabilities: (i) spatiotemporal control of therapeutic agent release through shape-morphing architectures that respond to physiological cues (e.g., pH, enzymes, and temperature),[34] significantly enhancing treatment precision and efficacy; and (ii) fabrication of organ-level constructs with unprecedented biological fidelity—incorporating vascular networks, heterogeneous cell distributions, and ECM gradients that more accurately recapitulate *in vivo* conditions than conventional 3D models.[157]

Despite these promising advances, 5D printing encounters substantial barriers to clinical adoption. Challenges include scalability, biocompatibility of embedded synthetic components, full recapitulation of human tissue complexity, and regulatory obstacles associated with multicomponent implantable devices. A critical and balanced assessment of these limitations—including safety, manufacturing reproducibility, and validation under clinically relevant conditions—is essential to translate this technology into viable clinical solutions.[156]

The convergence of microfluidics with 5D printing technology is particularly promising for oncology applications,[158] enabling: (a) tumor-on-a-chip platforms with physiologically relevant drug response profiles, (b) biomimetic drug delivery systems that adapt their release kinetics to TME dynamics, and (c) patient-specific tumor models that evolve post-implantation to match disease progression.[159] Recent studies demonstrate 5D-printed scaffolds achieving 92% shape fidelity upon environmental triggering and sustaining three-week drug release profiles with 89% bioactivity retention.[156] This technological synergy addresses critical limitations in conventional drug screening by providing dynamic, vascularized tumor models that better predict clinical outcomes while reducing animal testing requirements (Figure 8).

While 3D-printed microfluidics currently represents an emerging technology still in its developmental phase, it has already demonstrated transformative potential in revolutionizing our understanding of tumor biology. These advanced systems enable researchers to recreate

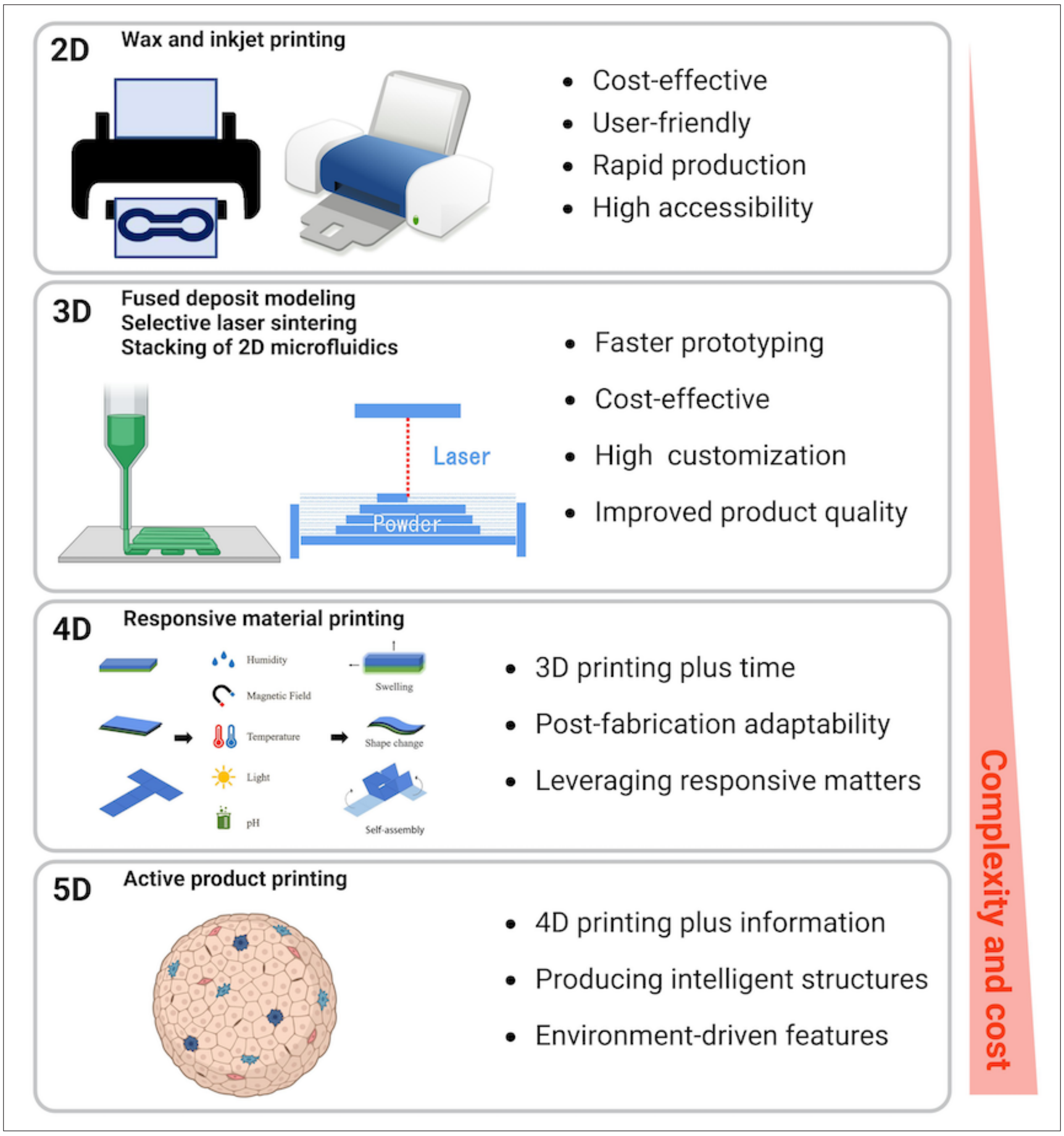

Figure 8. Comparison of 2D, 3D, 4D, and 5D printing technologies. Created in BioRender. Zhicheng, Z. (2025) https://BioRender.com/yq3p4vc.

complex TMEs with unprecedented spatial and temporal precision,[157] facilitating investigations into critical oncological processes such as angiogenesis, metastasis, and drug resistance mechanisms.[160] Technology's unique capability to incorporate multiple cell types, ECM components, and vascular networks within precisely controlled microarchitectures[161] offers a powerful platform for studying tumor–stroma interactions and cellular crosstalk under physiologically relevant conditions.[79]

8. Conclusion

This review provides a systematic examination of the transformative role of conventional and 3D-printed microfluidic technologies in three pivotal areas of cancer research: tumor modeling, therapeutic development, and clinical diagnostics. By comparing these platforms with traditional methodologies, the review underscores their ability to overcome key limitations of conventional approaches.

Looking toward the future, the integration of microfluidics with next-generation 4D and 5D printing technologies promises to usher in a new era of intelligent, adaptive systems for precision oncology. These sophisticated platforms will feature dynamic, stimuli-responsive materials capable of morphological or functional changes in response to specific biological cues, effectively blurring the boundaries between engineered systems and living tissues. Such biologically integrated engineering solutions are poised to create a seamless continuum from tumor modeling to therapeutic intervention, enabling real-time monitoring of treatment responses, autonomous adjustment of drug release profiles, and continuous optimization of therapeutic strategies based on evolving tumor dynamics. This convergence of technologies represents a paradigm shift in cancer research and treatment, potentially enabling truly personalized medicine approaches that adapt to each patient's unique disease progression.

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Conflict of interest

The authors declare no conflict of interest.

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