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Matrix-integrated microfluidic tumor models for evaluating drug delivery systems and pre-clinical testing

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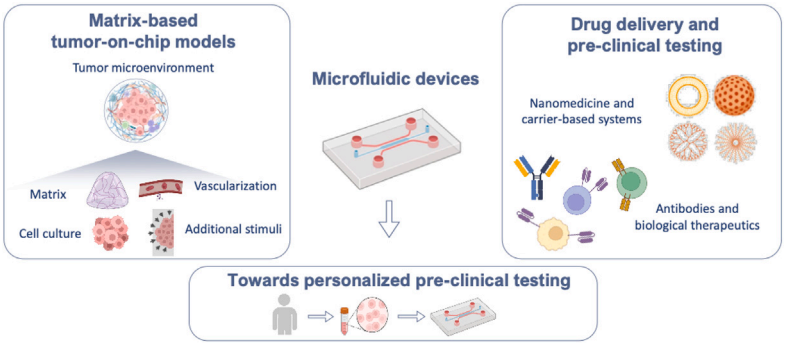
GRAPHICAL ABSTRACT



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ABSTRACT

Drug delivery research strongly depends on experimental models that faithfully mimic the tumor microenvironment (TME) and its barriers to evaluate therapeutic efficacy. Conventional systems provide valuable insights but suffer from some limitations in physiological relevance, reproducibility, scalability or translational predictability. In this context, microfluidic 'tumor-on-chip' platforms have emerged as innovative tools that integrate engineering technology to model biological complexity, offering controlled microenvironments to investigate drug penetration, transport dynamics, and therapeutic response. A distinctive aspect of these microsystems is the possibility of incorporating matrices that mimic the extracellular matrix (ECM) of different tissues. These matrices enhance the ability of the *in vitro* models to replicate the structural, biochemical, and mechanical features of solid tumors. In this review, we focus on the application of microfluidic matrix-integrated tumor-on-chip platforms for drug delivery evaluation. We first outline key microenvironmental features that regulate therapeutic efficacy and discuss how they can be engineered within microfluidic models. We then examine how transport dynamics and delivery mechanisms are modeled under physiologically relevant conditions and review the use of these platforms to assess a broad range of therapeutic strategies, including nanocarriers, biologics, and gene- and cell-based therapies. Finally, we highlight emerging computational and data-driven approaches, together with current translational and regulatory perspectives, that position matrix-integrated tumor-on-chip technologies as powerful preclinical

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tools. These models aim to bridge the gap between simplified *in vitro* assays and more complex *in vivo* studies, ultimately accelerating the translation of drug delivery systems into clinical practice and paving the way for more personalized therapeutic strategies.

List of acronyms

ACT: Adoptive cell transfer,
ADC: Antibody drug conjugate,
ADME-Tox: Absorption, Distribution, Metabolism, Excretion, or toxicity,
AI: Artificial Intelligence,
AKT: Protein Kinase B,
ASTM: American Society for Testing and Materials
BBB: Blood-brain barrier,
Birc2: Baculoviral IAP repeat-containing protein 2,
BME: Basement Membrane Extract,
BSA: Bovine Serum Albumin,
BsAb: Bispecific antibodies,
CAFs: Cancer-associated fibroblasts,
CAR: Chimeric antigen receptor,
CAIX: Carbonic Anhydrase IX,
CD: Cluster of Differentiation,
CDER: Centre for Drug Evaluation and Research,
CSF1R: Colony Stimulating Factor 1 Receptor,
DDS: Drug Delivery System,
DDTs: Drug Development Tools,
DTs: Digital twins,
dECM: Decellularized Extracellular Matrix,
ECM: Extracellular Matrix,
EGFR: Epidermal growth factor receptor,
EMT: Epithelial-mesenchymal transition,
EPR: Enhanced permeability and retention effect,
FAK: Focal Adhesion Kinase,
FDA: Food and Drug Administration,
GAG: Glycosaminoglycan,
GBM: Glioblastoma,
GelMA: Gelatin Methacryloyl,
HA: Hyaluronic acid,
HCC: Hepatocellular Carcinoma,
HIF: Hypoxia-inducible factor,
HUVEC: Human umbilical vein endothelial cells,
IC50: Inhibitory Concentration 50%,
IFP: Interstitial fluid pressure,
IL-6: Interleukin-6,
IL-10: Interleukin-10,
IND: Investigational New Drug,
ISO: International Organization for Standardization,
ISTAND: Innovative Science and Technology Approaches for New Drugs,
LOX: Lysyl Oxidase,
MCT4: Monocarboxylate Transporter 4,
MDSCs: Myeloid-derived suppressor cells,
MHC: Major Histocompatibility Complex,
MMP: Matrix metalloproteinase,
MPS: Microphysiological Systems,
MSN: Mesoporous Silica Nanoparticles,
NAMs: New Approach Methods,
NDDS: Nanodrug Delivery System,
NPs: Nanoparticles,
NK: Natural Killer,
PBS: Phosphate Buffered Saline,
PD-1: Programmed Cell Death Protein 1,
PDMS: Poly-dimethyl-siloxane,
PDO: Patient-derived organoids,
PDX: Patient-derived xenografts,
PEG: Polyethylene Glycol,
PEGPH20: Pegvorhyaluronidase alfa,
PHEMA: poly(2-hydroxyethyl methacrylate),
PI3K: Phosphoinositide 3-kinase,
PMMA: Polymethyl Methacrylate,
PVA: Poly(vinyl alcohol),
Redox: Reduction-oxidation,
ROS: Reactive oxygen species,
SCPN: Single-Chain Polymeric NPs,
SERS: Surface-Enhanced Raman Spectroscopy,
STP: Solid tissue pressure,
TAMs: Tumor-associated macrophages,
TAZ: Transcriptional coactivator with PDZ-binding motif,
TCR: T-cell receptor,
T-DM1: Trastuzumab-emtansine,
TEER: Trans-Epithelial Electrical Resistance Assay,
TGF-β: Transforming growth factor β,
TIMP-1: Tissue inhibitor of metalloproteinase-1,
TME: Tumor Microenvironment,
mTOR: Mammalian Target of Rapamycin,
Tregs: Regulatory T cells,
VDR: Vitamin D Receptor,
VEGF: Vascular endothelial growth factor.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, with millions of new cases and deaths reported annually [1]. Despite continuous advances in diagnosis and therapy, treatment success is still limited by the complex biology of the disease. A major challenge arises from the tumor microenvironment (TME), which is not only central to tumor initiation and progression but also shapes heterogeneity within tumors [2,3]. Distinct subpopulations of cancer cells interact with stromal and immune cells, embedded in a dynamic extracellular matrix (ECM) that regulates biochemical and mechanical cues. This heterogeneity underlies intrinsic resistance to therapy, posing a significant barrier to effective treatment and contributing to poor clinical translation of preclinical findings [4,5].

Drug delivery systems (DDSs) have long been developed to improve the efficacy and safety of cancer treatments by enhancing bioavailability, controlling release profiles, and reducing off-target effects [6]. From common release oral and transdermal systems to more advanced nanocarriers, antibody–drug conjugates, and microneedles, controlled delivery technologies have greatly expanded the therapeutic arsenal. Nevertheless, many DDSs face persistent challenges: limited biodistribution, rapid clearance, degradation across biological barriers, and insufficient penetration into tumor tissues. As a result, higher drug doses are often required, increasing systemic toxicity and reducing patient compliance [7]. Furthermore, preclinical evaluation relies heavily on conventional 2D cultures, which fail to reproduce the complexity of the human TME, and on animal models, which, although more

representative of *in vivo* conditions, offer limited control over specific components influencing drug transport and response. These platforms frequently overestimate efficacy and cannot reliably predict clinical outcomes. Notably, several studies have shown that 3D tumor models often require different drug doses than their 2D counterparts, underscoring the impact of the microenvironment on treatment response and the limitations of traditional systems in translational research [8,9].

To address these limitations, microfluidic platforms for tissue culture have emerged as powerful tools for modeling microphysiological systems (MPS) [10], particularly in cancer-related drug delivery research, where interest is rapidly growing. Originating from microfabrication and analytical chemistry, microfluidics enables precise control over fluid flow, mass transport, and cell–matrix interactions at the microscale [11,12]. Its miniaturized architecture allows integration of multiple steps, such as cell culture, carrier synthesis, drug release, and real-time monitoring, within a single platform. Microfluidic devices consume minimal reagents, generate reproducible results, and offer the possibility of high-throughput experimentation. Importantly, they provide a means to mimic key features of the TME, including oxygen and nutrient gradients, dynamic perfusion, coculture of different cell lines, and mechanical stresses, which are difficult to reproduce in static systems [13–15].

Among the different strategies for tumor modeling, microfluidic-based devices supporting three-dimensional cultures within ECM-like matrices have attracted particular attention. Unlike flat monolayers, these matrix-integrated systems provide a physiologically relevant microenvironment where cancer and stromal cells are surrounded by biomimetic hydrogels that recapitulate native tissue mechanics and biochemistry [16,17]. This configuration not only enhances cell–cell and cell–matrix interactions, but also permits the formation of realistic barriers for therapeutic agents. As such, they serve as valuable models to investigate the penetration, transport, and efficacy of drug delivery systems under conditions that more closely approximate *in vivo* tumors. By enabling the fine regulation of dosage and release kinetics while minimizing artifacts of oversimplified cultures, matrix-integrated microfluidic tumor models improve the predictive power of preclinical testing and reduce reliance on animal studies [18,19].

In this review, we therefore focus on the application of these MPS for drug delivery evaluation, with a particular emphasis on those platforms that incorporate three-dimensional (3D) matrices to recapitulate key features of the whole TME. We first present a comprehensive and descriptive overview of the main components of the TME that critically influence drug delivery and therapeutic efficacy, illustrating how these elements have been targeted or modulated in preclinical and clinical studies, either as standalone therapeutic strategies or to enhance the performance of existing and emerging treatments. We then introduce the design principles of microfluidic systems and discuss their relevance for reproducing these key TME features. We highlight how these models have been employed to test a variety of delivery mechanisms and strategies, ranging from nanoparticles (NPs) and biologics to gene- and cell-based therapies. In addition, we discuss the emerging contribution of computational modeling and data-driven approaches, including artificial intelligence, to support the interpretation, scalability, and predictive power of microfluidic tumor models. Finally, we address current perspectives on the regulatory and translational positioning of matrix-integrated microfluidic systems, outlining their evolving role as complementary tools within preclinical development pipelines and their potential to contribute to the progressive reduction of animal testing.

2. Key tumor microenvironment properties relevant to drug delivery

The TME in solid tumors is a dynamic and interconnected ecosystem composed of cellular, physical, and biochemical components that collectively regulate how therapeutic agents are transported, distributed, and ultimately exert their effects. Rather than acting as isolated obstacles, the ECM, vasculature, stromal and immune cells, and the physical and metabolic conditions of the tumor tissue form a tightly connected network where alterations in one component trigger changes in others. Abnormal matrix remodeling alters tissue mechanical properties and compresses blood vessels, leading to hypoxia and metabolic gradients that further modify the behavior of cancer, stromal, and immune cells (Fig. 1) [20]. These feedback processes increase tumor heterogeneity, resulting in nonuniform drug distribution, variable treatment responses, and limited predictive accuracy of conventional *in vitro* models. The following sections describe the main features of the TME and how each contributes to the barriers that limit drug delivery and treatment effectiveness in solid tumors.

2.1. Extracellular matrix

The ECM is a dynamic and heterogeneous network of macromolecules that provides both structural and biochemical support to cells. Within the TME, the ECM represents a major obstacle to effective drug delivery, as its composition, organization, and mechanical properties collectively determine how therapeutic agents penetrate, diffuse, and interact with target cells. Alterations in these properties reduce drug availability in the tumor and promote therapeutic resistance [21,22].

Tumor ECMs are rich in fibrillar collagens, proteoglycans, glycosaminoglycans (GAGs), fibronectin, and laminins. Excessive deposition of fibrillar collagens, mainly types I and III, forms a dense, aligned, and highly cross-linked network that physically restricts molecular diffusion, particularly for large molecules such as antibodies or NP-based drugs, creating tortuous pathways that trap drugs near perivascular regions and prevent uniform distribution [23,24]. This dense collagen network reduces pore size and overall porosity [25], lowering hydraulic permeability and diffusivity within the ECM [14,26,27]. As a result, both convective transport and molecular diffusion are limited, producing a slow and heterogeneous drug movement toward tumor cells. Another parameter less commonly recognized, reduced by increased collagen and alignment, is the mesh size, which refers to the average distance between molecular cross-links in the ECM network, capturing the scale of the network at the nanometric scale and directly influencing the mobility of molecules and cells within the ECM. Importantly, mesh size is not equivalent to porosity: whereas porosity reflects the overall void volume fraction within the matrix, mesh size defines the specific spatial constraints at the nanoscale. In tumor matrices, a reduced mesh size can restrict the diffusion of nutrients, oxygen, and therapeutic agents, limiting their bioavailability and contributing to the metabolic adaptation of cancer cells and immune cell exclusion [28,29]. This environment enhances cell survival under drug stress and reduces drug sensitivity, directly linking ECM organization to molecular resistance mechanisms [30]. In addition to collagen, proteoglycans and GAGs such as hyaluronic acid (HA) form a hydrated, gel-like network that retains water and increases interstitial fluid pressure (IFP), compressing vessels and reducing perfusion [31,32]. Aberrant fibronectin and laminin expression also enhance cell–matrix adhesion and activate integrin-mediated survival signaling, reinforcing the biochemical and mechanical constraints of the ECM [33,34].

These compositional and structural alterations modify the mechanical properties of the ECM, establishing a high-stress state in tumor tissue that critically influences drug transport and therapeutic efficacy. Matrix stiffening arises from the deposition and alignment of fibrillar collagens, enzymatic cross-linking of matrix fibers, cellular contractility, continuous ECM remodeling, and the accumulation of solid stresses [21]. As the tumor mass grows within the confined space of host tissue, it accumulates solid stresses, transforming solid tumors into complex mechanical systems governed by both solid and fluid mechanics [35]. The stresses originating from a tumor's solid phase largely determine both fluid stresses and cancer progression. At the tissue level, solid stresses can be categorized into external, swelling, and residual. These solid stresses can affect cancer cell growth both directly, by compressing cancer cells, and indirectly, by compressing

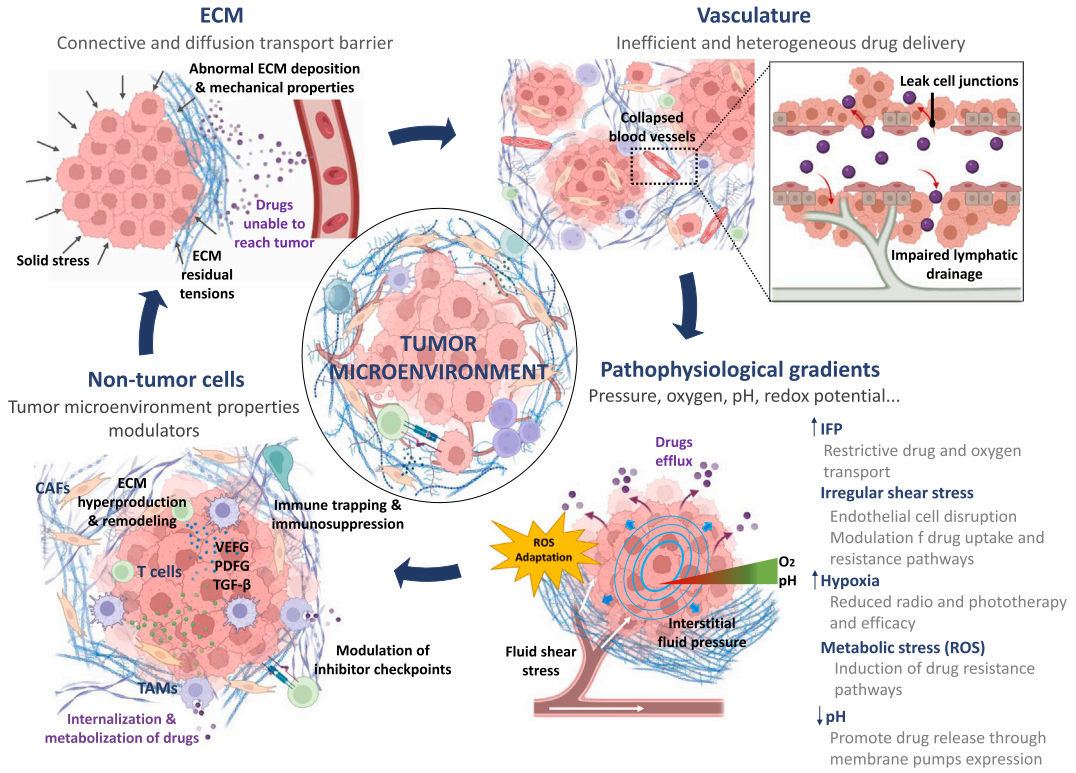

Fig. 1. Overview of tumor microenvironmental determinants to effective drug delivery and treatment outcome. Structural, cellular, and biochemical components of the TME act synergistically to restrict drug transport and efficacy. Abnormal ECM remodeling, vascular deficiencies, and mechanical forces create physical barriers to drug perfusion and diffusion, while hypoxia, acidosis, and reduction-oxidation (redox) imbalance generate biochemical resistance niches. Stromal and immune cells actively sustain these conditions through matrix remodeling, cytokine secretion, and drug metabolization, ultimately leading to heterogeneous drug distribution and reduced therapeutic response in solid tumors.

blood and lymphatic vessels. Swelling stress can result from the high negative charge density of HA, which causes electrostatic repulsive forces among closely spaced chains of HA. Alternatively, it can result from the swelling of cancer cells that modulate their tonicity in order to survive under compressive solid stresses [36,37]. Residual stresses, in turn, remain trapped within the tumor owing to persistent collagen cross-linking and cellular contraction. Collectively, these stresses reduce perfusion and impair convective drug transport into tumor core regions. Moreover, ECM stiffening activates mechanotransduction pathways, including integrin-Focal Adhesion Kinase (FAK) and Yes-Associated Protein (YAP)/ Transcriptional coactivator with PDZ-binding motif (TAZ) signaling, in both cancer and stromal cells, leading to gene expression changes that promote epithelial–mesenchymal transition (EMT), cell survival, and therapy resistance [38,39]. Mechanical stress further activates cancer associated fibroblasts (CAFs), which secrete additional ECM components and growth factors such as transforming growth factor β (TGF-β), reinforcing a self-sustaining cycle of matrix stiffening, solid stress, and therapeutic resistance [40,41].

Addressing these structural obstacles is critical for restoring intratumoral transport. Clinical-stage research has focused on disrupting the Hyaluronic acid (HA) network using enzymatic depletion (e.g., pegvorhyaluronidase alfa - PEGPH20), which aims to reduce swelling stress and IFP to improve the convection of co-administered drugs [42]. Complementarily, preclinical *in vivo* studies have demonstrated that inhibiting lysyl oxidase (LOX) prevents the cross-linking of fibrillar collagens, effectively increasing the ECM pore size and allowing a more uniform distribution of NPs within the tumor core [43]. These strategies underscore that modulating the mechanical and biochemical state of the ECM is a prerequisite for overcoming the physical exclusion of therapeutic agents.

2.2. Tumor vasculature

In healthy tissues, the vasculature forms a well-organized, hierarchical network ensuring uniform perfusion and tightly regulated exchange between blood and interstitial compartments. Tumor angiogenesis, in contrast, produces a disorganized and heterogeneous vascular network that shows variable diameters, discontinuous endothelium, and fragmented or thickened basement membranes [44,45]. These abnormalities generate the enhanced permeability and retention (EPR) effect, which can facilitate NP extravasation but also lead to inefficient and heterogeneous drug delivery [46,47]. Although these transient leaks may enhance drug transport, their stochastic nature leads to erratic blood perfusion and highly variable local delivery. The spatial heterogeneity of tumor vasculature further compounds this problem: peripheral regions are often highly vascularized, whereas the tumor core can be nearly avascular. As a result, systemically administered drugs tend to accumulate near the tumor periphery but fail to reach the interior regions of the tumor, where resistant cell populations typically reside [44,48]. Functionally, irregular vessel geometry and poor integrity create steep gradients of oxygen, nutrients, and drugs, while impaired lymphatic drainage and the dense, stiff ECM increase IFP, compress vessels, and exacerbate hypoperfusion, which limits convective transport and drug penetration into the tumor tissue [49,50]. Beyond drug delivery, the dysfunctional tumor vasculature acts as a critical bidirectional gateway for cellular transport. The dysfunctional endothelium and aberrant flow dynamics create an impediment to the effective recruitment and infiltration of anti-tumor immune cells, such as T-cells and natural killer (NK) cells, into the tumor core, a factor recognized as a major hurdle for the efficacy of adoptive cell therapies and immune checkpoint inhibitors [51,52]. Conversely, the leaky and fragile endothelium facilitates the intravasation of cancer cells into the systemic circulation, promoting

the process of metastasis [53,54]. This dual role underscores how vascular abnormalities not only restrict therapeutic access but also actively contribute to immune exclusion and systemic disease progression.

Vascular normalization strategies that reduce stromal components, such as enzymatic HA degradation via PEGPH20, have demonstrated vessel decompression, perfusion restoration, and improvement of drug delivery in preclinical models and early-phase clinical trials, particularly in desmoplastic tumors like pancreatic cancer [42,55,56]. Similarly, the use of angiotensin II receptor blockers (e.g., losartan) has been shown in preclinical studies and clinical trials to alleviate solid stress and enhance the delivery of chemotherapy by reducing collagen and hyaluronan production [57,58]. Other approaches, such as low-dose antiangiogenic therapy targeting vascular endothelial growth factor receptors, such as VEGFR-2 or the activation of angiopoietin-1 receptor (Tie2) signaling, have also demonstrated the ability to stabilize the tumor vasculature and improve drug distribution in experimental preclinical studies [59,60].

In addition to its structural abnormalities, tumor vasculature exerts biochemical control over tumor behavior and therapy response. Endothelial cells' paracrine signaling to cancer and stromal cells, for instance, via interleukin-6 (IL-6) and tissue inhibitor of metalloproteinase-1 (TIMP-1) secretion, promotes survival under cytotoxic stress [20,61]. Similarly, perivascular regions often harbor tumor-initiating cells with stem-like, drug-resistant properties, supported by endothelial paracrine cues that facilitate recurrence [62,63]. Overall, the aberrant structure, function, and signaling of tumor vasculature create a paradoxical microenvironment: it allows initial drug entry but restricts deep penetration while fostering cellular resistance.

2.3. Interstitial fluid pressure and shear stress

The stiff, cross-linked ECM, leaky yet compressed vasculature, and deficient lymphatic drainage profoundly alter fluid transport in solid tumors, resulting in elevated IFP and aberrant shear stresses, key biophysical features that impair drug delivery and treatment efficacy. Tumor IFPs can reach up to 60 mmHg in the tumor core, compared with –1 to 5 mmHg in normal tissues. This elevated pressure remains relatively uniform inside the tumor but drops sharply toward the periphery, disrupting fluid dynamics and reducing molecular exchange between vascular and interstitial compartments [64,65]. Nevertheless, IFP values also vary significantly depending on tumor type, experimental model, and measurement approach. For example, while IFP in healthy pancreatic tissue remains close to atmospheric pressure, values of approximately 20 mmHg have been reported in pancreatic ductal adenocarcinoma patients [66]. In murine models, IFP values range from 13 to 45 mmHg in tumors derived from established cell lines [67], whereas substantially higher pressures (75-130 mmHg) have been observed in genetically engineered mouse models of the disease [68]. As discussed previously, the main drivers of this imbalance are excessive plasma leakage from immature, hyperpermeable vessels and the lack of functional lymphatic drainage. The resulting collapse of the normal filtration–drainage equilibrium eliminates convection across vessels, forcing drug and NP transport to rely primarily on diffusion, a slow and inefficient process for large macromolecules and nanocarriers. Consequently, drugs tend to accumulate in perivascular and peripheral regions, often failing to reach the tumor core, a phenomenon known as the "rim effect" [49,69]. Moreover, the combined action of IFP and solid stresses compresses intratumoral blood vessels, severely restricting delivery of macromolecular drugs and NPs, including monoclonal antibodies and liposomal formulations, which exhibit poor penetration in regions of elevated IFP [64].

Recent preclinical investigations have shown that mechanotherapeutic interventions can effectively lower this pressure. For instance, the administration of Tranilast, an anti-fibrotic agent, has been experimentally proven to reduce both solid stress and IFP by normalizing the extracellular matrix and decompressing tumor blood vessels, which significantly enhances the delivery of both small-molecule drugs and nanomedicines [70]. Similarly, the inhibition of TGF-β signaling leads to a pronounced reduction in ECM components and an increase in the intratumoral vessel diameter and pericyte coverage. These modifications result in a significant increase in tumor perfusion and oxygenation, as well as a reduction in IFP, thereby enhancing the delivery and efficacy of co-administered treatments [71].

Fluid shear stress also significantly influences both transport and cellular behavior. Physiologically, endothelial cells experience stable laminar shear forces that preserve vascular integrity and permeability, but tumor vessels' irregular geometry and intermittent flow create heterogeneous shear profiles, disrupting junctions, altering transvascular exchange, and modulating drug uptake and tumor resistance. Moreover, local shear variations affect NPs' margination and adhesion dynamics, further contributing to the nonuniform distribution of therapeutic agents across the tumor tissue [72,73]. Even low-magnitude interstitial shear stresses activate oncogenic signaling pathways, upregulate TGF-β, and promote fibroblast contractility, stiffening the ECM and further impeding drug transport [74–76]. Interstitial flow can also guide angiogenic sprouting, exacerbate vascular disorganization, and activate mechanotransduction pathways that enhance cancer cell dissemination, enrich stem-like populations, and increase chemoresistance [76,77].

2.4. Hypoxia, acidosis and reduction-oxidation alterations

Solid tumors exhibit pronounced biochemical and metabolic heterogeneity that strongly influences drug transport and efficacy. These gradients arise from abnormal ECM, dysfunctional vasculature, and mechanical stresses described previously. Among the resulting alterations, oxygen deprivation or hypoxia is a critical determinant of treatment response. Hypoxia arises when the oxygen consumption of rapidly proliferating cancer and stromal cells exceeds the limited supply delivered by the abnormal tumor vasculature. Unlike healthy tissues, tumors exhibit normoxic regions near vessels and progressively hypoxic or necrotic zones toward the tumor core [78]. Oxygen tension can drop from physiological levels at the periphery to below 1 mmHg at the core, establishing functional zonation in which peripheral cells remain oxidative and proliferative, while central cells adopt glycolytic, quiescent, or stress-resistant phenotypes [79,80]. Hypoxia-inducible factors (HIF-1, HIF-2, HIF-3) orchestrate these adaptations, promoting angiogenesis via vascular endothelial growth factor (VEGF). Nevertheless, HIF-driven vessels remain disorganized and leaky, perpetuating hypoxia and resistance to therapy. Hypoxia can also modulate metabolic reprogramming toward glycolysis, survival, invasion, vasculogenic mimicry and DNA repair, and facilitate tumor regrowth [81–83].

From a therapeutic perspective, the consequences of hypoxia are multifaceted. These altered oxygen dynamics profoundly reshape reduction-oxidation (redox) homeostasis, another hallmark of the TME. Mitochondrial dysfunction and enhanced glycolysis generate abnormal reactive oxygen species (ROS), which at moderate levels stabilize HIFs, drive angiogenesis, and support metabolic adaptation through glycolysis and autophagy [84]. Tumor cells upregulate antioxidant systems, including glutathione, thioredoxin, and NADPH- generating enzymes and creating a new equilibrium ("ROS addiction") that enables survival under oxidative stress [85]. This redox imbalance extends to the immune microenvironment, impairing cytotoxic T cell function, enhancing regulatory T cell activity, and reducing responsiveness to immune checkpoint inhibitors. Moreover, the resulting hypoxic and oxidative landscape limits the efficacy of ROS-dependent therapies, such as radiotherapy and photodynamic therapy, and promotes multidrug resistance through altered transporter expression [81]. Interestingly, preclinical experimental studies have sought to overcome these issues through metabolic reprogramming. For instance, to counteract oxidative stress, Chimeric Antigen Receptor T (CAR-T) cells have been engineered to co-express catalase, which reduces intracellular ROS and preserves their anti-tumor activity even under high-stress conditions [86,87]. Similarly, NPs have been developed to both alleviate and exploit these conditions. The use of NPs

that deliver oxygen or oxygen-generating agents directly and those that selectively target hypoxic regions using hypoxia-activated prodrugs or ligands has been shown in experimental models to alleviate hypoxia, thereby sensitizing tumors to radiotherapy and ensuring precise drug release within resistant tumor cores [88–90].

The heterogeneous perfusion further generates gradients in glucose and metabolic by-products such as adenosine and lactate, producing extracellular acidosis that compounds hypoxia-driven effects. Limited oxygen and nutrient delivery forces cancer cells to rely on glycolysis, even under normoxic conditions, producing excess lactate and protons. Extracellular pH drops to 6.5–6.8, while intracellular pH remains near 7.4, creating pH gradients overlapping hypoxic regions [91]. Acidic conditions reduce the uptake of weakly basic chemotherapeutics, activate efflux pumps (e.g., P-glycoprotein), and contribute to multidrug resistance [92,93]. Acidosis also reshapes the tumor immune landscape, inhibiting cytotoxic T cells and NK cells while promoting M2 macrophages and pro-tumorigenic neutrophils and dendritic cells, impairing tumor surveillance and reducing immunotherapy efficacy, including immune checkpoint inhibitors [94]. To counteract this, current clinical trials are evaluating carbonic anhydrase IX (CAIX) inhibitors, such as SLC-0111, to neutralize TME acidity and restore the efficacy of both chemotherapy and immune checkpoint inhibitors [95]. Finally, acidosis enhances tumor aggressiveness by activating matrix metalloproteinases (MMP-2, MMP-9), degrading ECM, reducing E-cadherin-mediated adhesion, and promoting invasion and metastasis. Intracellular signaling pathways such as Phosphoinositide 3-kinase (PI3K)/Protein Kinase B (AKT)/Mammalian Target of Rapamycin (mTOR) are upregulated in acidic niches, reinforcing proliferation, survival, and glycolytic metabolism [96]. Preclinical studies targeting metabolic transporters such as Monocarboxylate Transporter 4 (MCT4) have shown that inhibiting acid export can reverse these aggressive traits and enhance therapeutic sensitivity [97].

2.5. Stromal and immune cells' contributions

Stromal and immune cells play active roles in shaping the TME and establishing the physical and biochemical constraints that limit drug delivery and treatment efficacy. Among stromal cells, activated CAFs secrete abundant ECM components, including collagens, fibronectin, and laminins, together with cross-linking enzymes such as LOX, which change the mechanical and structural properties of the tumor ECM detailed in Section 2.1. In parallel, CAFs release cytokines and growth factors including TGF-β and platelet-derived growth factor (PDGF) that sustain fibroblast activation, promote aberrant angiogenesis, and reprogram the metabolic and redox environment to favor tumor cell survival, proliferation, and therapy resistance [98,99]. These biochemical and structural alterations jointly impair drug transport: dense ECM deposition and cross-linking restrict diffusion, while CAFs-generated contractile forces compress intratumoral vessels, exacerbating perfusion deficits. Moreover, CAFs-derived soluble factors modulate endothelial permeability and influence drug distribution in the perivascular space, and some CAFs subsets can even metabolize or sequester therapeutic agents, reducing their bioavailability and spatial penetration effects. These effects extend to hindering NP extravasation and limiting the penetration of antibody–drug conjugates and liposomal formulations [40,100].

Immune cells act in concert with these stromal elements to further regulate drug delivery and efficacy. Tumor-associated macrophages (TAMs) secrete cytokines such as Interleukin-10 (IL-10) and VEGF that sustain angiogenesis and vascular dysfunction, reinforcing the same barriers established by CAFs [101]. They also internalize or metabolize therapeutic compounds, including NPs and antibody–drug conjugates, limiting their distribution and intratumoral accumulation [102]. Myeloid-derived suppressor cells (MDSCs) and regulatory T cells contribute to this process by releasing inhibitory cytokines that dampen cytotoxic lymphocyte activity while engaging in crosstalk with CAFs to influence ECM remodeling, vascular permeability, and IFP [103]. Additional myeloid populations, such as neutrophils and dendritic cells, release proteases and growth factors that further remodel the ECM and alter local vascular and chemical conditions, generating niches of therapeutic resistance [104]. Beyond physical and vascular alterations, stromal and immune cells modulate therapy response through redox and metabolic regulation. Cytokines derived from CAFs and TAMs activate pro-survival and anti-apoptotic signaling in tumor cells, while ROS generated by these same populations influence redox balance, affecting drug sensitivity and promoting adaptive resistance. Notably, these cell populations can generate microdomains with distinct metabolic profiles that alter local pH and redox conditions, which reduce chemotherapeutic efficacy [105,106]. Therapeutically, targeting these cellular components has shown promise in overcoming TME-imposed barriers. Strategies that reprogram or deplete CAFs can reduce ECM stiffness, decompress vessels, and improve the penetration of chemotherapeutics and NPs in desmoplastic tumors [107,108]. The use of vitamin D receptor (VDR) ligands is being evaluated in clinical trials, which explore how paricalcitol can reprogram the stroma to enhance the efficacy of chemotherapy delivery [109,110]. Additionally, preclinical *in vivo* studies have explored the inhibition of signaling pathways like Hedgehog to modulate CAF activity and decrease the dense collagenous matrix that hinders NP transport [111]. Similarly, modulation of TAM activity via colony stimulating factor 1 receptor (CSF1R) inhibition or polarization toward a pro-inflammatory phenotype normalizes vasculature, limits drug sequestration, and enhances both chemotherapy and immunotherapy efficacy [101,112]. This approach has reached clinical evaluation: for instance, the use of CSF1R inhibitors like Emactuzumab has demonstrated the depletion of immunosuppressive macrophages, restoring anti-tumor immunity in patients with advanced solid tumors [113,114]. These examples underscore the necessity of considering stromal and immune interactions in therapeutic design, as addressing their collective contribution is key to achieving more uniform and effective drug distribution.

3. Traditional strategies

Traditional *in vitro* cancer models have long relied on 2D cell cultures, in which tumor cells are grown as monolayers. These systems are simple, cost-effective, and highly reproducible, making them widely used for basic cancer biology studies and high-throughput drug screening. To partially improve cell adhesion and signaling, 2D cultures can be performed on surfaces coated with ECM components such as collagen, fibronectin, or laminin [115]. However, even with these modifications, 2D models fail to recapitulate the 3D architecture, organization, and complex cell–cell and cell–matrix interactions characteristic of native tumor tissues [15]. As a result, CAR-T cell therapies, which have shown remarkable activity in 2D tumor cultures, are well known to lose efficacy in the context of solid tumors, where physical barriers, ECM density, and immunosuppressive cues strongly limit their function [116]. While ECM coatings can enhance the physiological accuracy of 2D cultures, promoting cell–substrate interactions and influencing signaling pathways, they remain insufficient to provide the 3D spatial organization, the mechanical complexity, the dynamic microenvironment, and transport barriers found in real tumors. This highlights the fact that, while 2D systems may suggest therapeutic potential, their translational value remainslimited for predicting drug delivery and efficacy in solid tumors.

To address some of these limitations, 3D culture models have been developed, allowing tumor cells to grow as multicellular clusters or within ECM-like matrices. In this context, spheroids typically refer to simple 3D aggregates generally derived from immortalized cell lines, whereas organoids are self-organized multicellular structures derived from stem or primary cells that recapitulate key architectural and functional features of the tissue of origin, as recognized by regulatory agencies such as the FDA [117].

Table 1
Comparison of tumor models with emphasis on ECM representation and relevance for drug delivery evaluation.

	2D cultures	Traditional 3D tumor models	Tumor-on-chip models	Animal models
ECM presence	Absent; cells adhere to plastic or coated surfaces	Tunable; natural/synthetic hydrogels (collagen, Matrigel®, PEG, fibrin) can be incorporated	Tunable; natural/synthetic hydrogels (collagen, Matrigel®, PEG, fibrin) can be incorporated	Native ECM, heterogeneous and dynamic
Control over ECM properties	None	High; stiffness, porosity, degradability, and biochemical composition can be engineered	High; stiffness, porosity, degradability, and biochemical composition can be engineered	None; ECM not experimentally tunable
Drug penetration / transport relevance	Overestimates diffusion and efficacy	Gradients can be formed but lack control over the process	Enables systematic evaluation of ECM density and structure on drug transport	Physiologically relevant barriers, but complex to isolate effects
Physiological complexity	Very low	Intermediate to high	Intermediate to high; controllable complexity while maintaining experimental accessibility	High; full tissue–immune–stromal interactions
Scalability and reproducibility	High throughput and reproducible	Limited; large waste of material	Moderate; advances in microfabrication improving reproducibility but scalability remains limited	Low throughput, high variability, costly
Translational predictive value	Poor; weak correlation with clinical outcomes	Limited; better than 2D but still oversimplified ECM	Promising; predictive capacity strongly linked to ECM integration and design	Historically accepted, but poor correlation in some therapies (e.g., nanomedicine)

Scaffold-free 3D systems promote enhanced cell–cell interactions and the emergence of gradients in oxygen, nutrients, and metabolites, which better reflect *in vivo* tumor conditions. Alternatively, scaffold-based 3D cultures embed cells within matrices and are typically established in conventional culture formats, such as well plates or transwell inserts. These matrices provide structural support and biochemical cues that influence tumor growth, invasion, and drug response. While these approaches offer improved physiological relevance compared to 2D cultures, they are generally limited in their ability to precisely control matrix properties, spatial organization, and dynamic environmental parameters, such as fluid flow or gradient formation; limitations that motivate the transition toward miniaturized and microengineered culture systems. Microfluidic tumor-on-chip platforms provide a tool to overcome these issues by embedding spheroids [14] or organoids [118] within matrix-mimicking substrates and subjecting them to controlled biochemical and biophysical cues, such as perfusion and interstitial flow. In this way, tumor-on-chip platforms do not compete with traditional 3D cultures but rather integrate them, combining their biological fidelity with engineering-driven precision and reproducibility. This hybrid approach expands their utility for drug delivery evaluation, as it enables more predictive modeling of drug penetration, therapeutic efficacy, and resistance mechanisms within complex TME [119,120].

Animal models, including xenografts, genetically engineered mice, and also patient-derived xenografts (PDXs) [121], have long been considered the gold standard for preclinical drug testing due to their ability to integrate systemic physiology, immune responses, and multicellular interactions within a whole-organism context. These models provide insights into pharmacokinetics, pharmacodynamics, and toxicity that cannot be captured *in vitro*, making them indispensable for translational research [122,123]. However, they are also associated with major drawbacks: experiments are time-consuming, costly, and subject to significant ethical concerns, while interspecies differences frequently compromise the predictive value of therapeutic outcomes in humans. Furthermore, animal models often exhibit substantial biological variability, which complicates reproducibility and large-scale screening efforts. Microfluidic models, offer a controllable and reproducible alternative that retains key aspects of the TME while reducing reliance on animal experimentation. Although they cannot fully substitute systemic studies, tumor-on-chip platforms bridge the gap between reductionist *in vitro* assays and complex *in vivo* models, offering a more ethical, cost-effective, and human-relevant approach to drug delivery system evaluation [124,125].

Collectively, these traditional models have contributed important insights into tumor biology and therapeutic response, with each approach offering specific strengths but also clear limitations regarding physiological relevance, experimental control, and translational predictability. Table 1 provides a comparative overview of these models, highlighting key aspects such as ECM presence and tunability, relevance for drug penetration and transport, physiological complexity, scalability, reproducibility, and predictive value. This comparison underscores the need for experimental platforms that better balance biological realism with controlled and reproducible conditions, thereby motivating the transition to tumor-on-chip models discussed in the following sections.

4. Microfluidic platforms for tumor modeling

Traditional *in vitro* models have played a key role in elucidating tumor biology and therapeutic responses, while *in vivo* models capture many aspects of the complex TME. Microfluidic tumor-on-chip platforms have emerged as complementary systems that enable controlled integration of cellular and acellular components within a 3D microenvironment, facilitating the study of drug transport, distribution, and cellular responses under physiologically relevant conditions.

Translating these matrix-integrated tumor-on-chip concepts into functional platforms requires precise microfabrication strategies that allow spatial control over channels, matrices, and cell compartments. The fabrication of microfluidic devices typically relies on well-established soft lithography techniques using Poly-dimethyl-siloxane (PDMS) due to its transparency, biocompatibility, and ease of prototyping [13,126,127]. However, emerging methods such as 3D printing [128,129], laser ablation [130,131], and micromilling [132,133] are increasingly employed to achieve more complex geometries or to integrate multiple materials within a single chip. The choice of fabrication route is closely tied to the desired resolution, mechanical properties, and biological compatibility of the final device. Comprehensive discussions on fabrication methodologies, material selection, and design strategies for organ-on-chip applications can be found in recent bibliographic reviews that offer detailed technical insights into these processes [10,124,134].

Researchers seek to incorporate within the tumor-on-chip platforms many of the essential features of the TME, summarized in Section 2, to

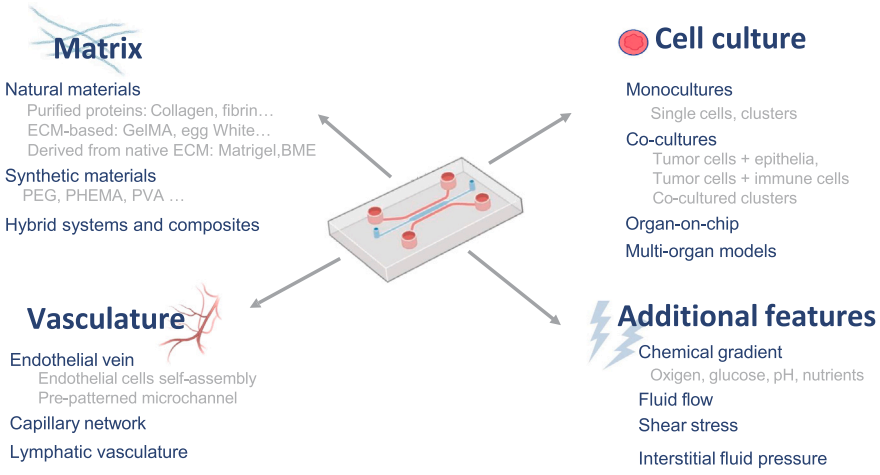

Fig. 2. Schematic representation of tumor-on-chip platforms highlighting the main design parameters: matrix biomaterial, cell culture configurations, the presence of vascular structures and additional biochemical or mechanical features. Together, these elements enable faithful modeling of the TME for drug delivery evaluation.

more accurately reproduce the complexity of solid tumors. These include: the ECM composition [16], vascular-like structures [26], flow perfusion [135], stromal and immune-tumor interactions [136], and biochemical or mechanical gradients [137]. One of the main goals is to provide versatile systems for the evaluation of specific biomolecules, from conventional chemotherapeutics to advanced nano-encapsulated drugs [138] and immunotherapies [139], with higher predictive accuracy and translational value than traditional models.

Consequently, different design strategies are pursued depending on the specific objectives of each particular work. Experimental variables are tailored not only to the tissue under investigation, but also to the specific biological process being modeled (such as metastasis, immune evasion, or tumor-pathogen interactions) and the intended application of the device [119]. This diversity of approaches underscores both the versatility and the complexity inherent to microfluidic tumor models. Thus, to establish reliable platforms for drug delivery systems evaluation, tumor-on-chip models are designed around four principal variables: i) the matrix, ii) the cell culture, iii) the presence of vasculature and iv) the additional biophysical or biochemical stimuli (Fig. 2). Building upon these core design elements, further aspects such as transport dynamics and mass transfer mechanisms play a critical role in determining model performance and translational relevance, and they are discussed below.

4.1. Extracellular matrix in tumor-on-chip systems

A critical component of tumor-on-chip systems is the cell structural support, which aims to emulate the native ECM described in Section 2. This matrix is commonly replicated *in vitro* using biomaterials that encapsulate cells and can be engineered from either natural or synthetic components. Natural matrices can also be further divided into i) purified ECM components, ii) other natural ECM-based hydrogels or iii) those directly derived from native ECM. Purified ECM components are specific proteins or polysaccharides extracted from natural sources which provide a biomimetic environment with well-defined biochemical cues; such as collagen [13], fibrinogen [140], chitosan [141], alginate [142], or HA [143]. Other natural ECM-based hydrogels include protein-derived materials such as gelatin methacryloyl (GelMA) [144], albumin [145], and egg white [146], which can offer additional structural or biochemical features. Natural biomaterials derived from native ECM can be obtained from commercial sources, such as Matrigel® [147] or basement membrane extract (BME) [148], which are rich in laminin, collagen IV, entactin, and growth factors. They provide an inherently bioactive environment that closely mimics the *in vivo* tumor niche, but their undefined composition and batch-to-batch variability limit reproducibility and quantitative analysis, which are critical for translational and regulatory applications. Alternatively, lab-generated matrices obtained through decellularization of native tissues [149] or cell-secreted matrices [150] offer more physiologically relevant scaffolds with retained biochemical complexity and tissue-specific cues. Decellularized ECM (dECM) preserves the native ultrastructure, stiffness, and signaling motifs of the original tissue, thereby promoting cell–matrix interactions and tumor-specific behavior. Importantly, dECM hydrogels can be tailored to replicate different TMEs, such as liver [151], lung [152], or breast [153], allowing organ-specific modeling of tumor growth and drug response. While their production is more complex and they are not yet widely used, dECM-based hydrogels offer a promising strategy to enhance the physiological relevance of tumor-on-chip systems, providing tissue-specific biochemical and mechanical cues that are difficult to achieve with conventional synthetic or protein-based hydrogels. [16,151,154]

Alternatively to natural hydrogels, synthetic materials can also frequently be employed for the fabrication of tumor-on-chip models, particularly when mechanical robustness, reproducibility, and tunable physicochemical properties are required [155]. Synthetic polymers such as poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(vinyl alcohol) (PVA) have been similarly used due to their hydrophilicity and biocompatibility [156]. Among them, polyethylene glycol (PEG) is generally considered the gold standard, offering excellent resistance to protein adsorption, attributed to its hydrophilicity, high chain mobility, and absence of hydrogen bond donor groups, along with easy chemical modification and the capacity to reproduce a wide range of tissue stiffness [157]. For instance, Clancy et al. developed a PEG-based hydrogel encapsulating U87 glioblastoma cells for drug screening applications [158].

In recent years, surface biofunctionalization strategies have been developed to address the inherent limitations of synthetic materials, such as their limited bioactivity and reduced capacity for supporting tissue-specific cell functions. The incorporation of biomolecules such as integrin-binding RGD peptide molecules, MMPs, growth factors, or specific biomolecules (like collagen, HA, fibronectin, or laminin fragments) [159,160] enhances cell adhesion, proliferation, and differentiation, bringing synthetic matrices closer to the biological performance of natural ECM [161]. Such hybrid systems designed for the direct modeling of the ECM have demonstrated significant utility in replicating hard tissues, including bone tumors, due to their capacity to offer enhanced mechanical stiffness and finely tunable bioactivity [162].

In practical applications, hybrid hydrogels are frequently employed in tumor-on-chip platforms, as they integrate the complementary biochemical and mechanical properties of each material. These systems typically integrate natural with synthetic materials, yielding tunable scaffolds with improved mechanical strength, stability, and bioactivity [163]. This combination enables independent control of stiffness and ligand density, while maintaining a physiological architecture suitable for cell invasion and migration studies. Beyond these hybrid systems, natural–natural composites [164,165] and multicomponent hydrogels incorporating additional physiological portions such as dECM fractions [166] or bioactive particles (silica, hydroxyapatite, or graphene oxide) have also emerged [167]. They offer the ability to fine-tune mechanical properties and biochemical gradients within microfluidic channels, which are especially valuable for tumor models that require tissue-specific stiffness.

For example, alginate–Matrigel® hydrogels tuned to the stiffness range of bone marrow (0.7–16 kPa) revealed that, in stiffer gels, breast cancer cell lines exhibited increased proliferation and elongation, whereas softer gels more closely resembled the behavior of healthy tissue [168]. Similarly, using adipose-derived dECM combined with silk fibroin, a temporally stiffening hydrogel (from 10–15 kPa to 25 kPa) induced malignant phenotypes in breast cancer cells, whereas at lower stiffness the cells maintained more benign, proliferative features [169]. Cavo and colleagues cultivated breast cancer cells in composite hydrogel. They demonstrated that a 50:50 Alginate–Matrigel® composite hydrogel supported malignant cell behaviors, including cytoskeletal remodeling, invadopodia formation, and migration toward a vascular-mimicking membrane, effectively mimicking the early stages of metastasis in a 3D *in vitro* model [170]. Notably, hydrogel stiffness was tuned by alginate concentration, with higher alginate content yielding stiffer matrices (66–76 kPa) and lower concentrations producing softer gels (24–26 kPa). Others created hybrid PEG-dECM hydrogels that can mimic healthy versus diseased lung stiffness, illustrating how composite design can emulate the pathological rigidity associated with diseased tissue [154]. In this approach, polyacrylamide hydrogels tuned to match healthy (1.8 ± 0.5 kPa) and fibrotic (23.7 ± 2.3 kPa) lung tissue stiffness were coated with dECM derived from healthy or idiopathic pulmonary fibrosis lungs. In addition, a hydrogel model combining egg white and gelatin was developed to evaluate early angiogenesis in pancreatic ductal adenocarcinoma [146]. This material has been previously shown to exhibit tunable mechanical properties, with stiffness values ranging from 113.80 ± 29.85 Pa to 407.10 ± 55.7 Pa [171]. Moreover, Baker et al. fabricated a tunable hydrogel composed of HA crosslinked with MMPs and incorporated either laminin or collagen. They showed that the hydrogel supports the growth and polarization of healthy mammary organoids and enables the expansion and proliferation of patient-derived cells both *in vitro* and *in vivo*. Importantly, tuning the crosslinking density allowed modulation of matrix stiffness, with increased crosslinker concentration yielding stiffer hydrogels (Young's modulus $\approx 2.1 \pm 0.4$ kPa) [172].

4.2. Cell culture in tumor-on-chip systems

Within microfluidic platforms, tumor models can range from single-cell-type systems to multicellular cocultures, depending on the level of biological interaction one aims to capture. Nowadays, monocultures remain the most straightforward approach for tumor modeling within microfluidic devices. Their simplicity facilitates experimental reproducibility and allows for controlled interrogation of tumor-intrinsic processes such as proliferation [126], migration [173], and response to chemotherapeutics [174]. By simplifying the experiment, monocultures enable the isolation of key cellular responses to drugs or environmental cues, providing fundamental insights into tumor cell behavior and intrinsic resistance mechanisms. Furthermore, monocultures provide a platform for high-throughput drug screening, where reducing biological variability is essential [175]. For instance, Pandya and colleagues engineered a microfluidic platform for rapid screening of chemotherapeutic drugs through a dynamic delivery of anti-cancer drugs to mouse melanoma, breast cancer and prostate cancer cells [176].

Nevertheless, the monoculture's main limitation lies in the absence of stromal and immune components, which are critical determinants of drug delivery and resistance *in vivo*. As such, their translational predictive power remains limited. In contrast, coculture systems in microfluidics introduce additional biological complexity by combining cancer cells with stromal fibroblasts, endothelial cells, or immune cell subsets within the same microenvironment [137,177,178]. This configuration enables the modeling of cellular interactions such as tumor–stroma crosstalk, angiogenesis, and immune evasion, which are central to drug delivery and therapeutic efficacy. Thus, coculture approaches can be implemented either with dispersed single-cell populations [179] or through multicellular clusters, such as spheroids or organoids [119], allowing for the evaluation of how heterotypic cell interactions influence drug penetration, metabolism, and resistance. Importantly, microfluidic coculture platforms can recapitulate spatial organization and dynamic gradients that are absent in static 3D models, thus improving their physiological relevance.

Notably, these microfluidic approaches do not replace established *in vitro* culture strategies, but rather incorporate them within a microfluidic framework to achieve enhanced spatial organization, dynamic regulation, and physiological relevance. Three-dimensional tumor architectures, including spheroid- or organoid-like assemblies, can be generated *in situ* by embedding individual cells within a hydrogel matrix (Fig. 3(a)), allowing cells to self-organize under defined biomechanical and biochemical constraints. This configuration enables the study of proliferation, migration, invasion, and tumor growth as emergent processes driven by cell–matrix interactions [14,180,181]. Alternatively, they can be cultured externally to form pre-assembled clusters (Fig. 3(b)), which can be fabricated by a wide range of techniques [182,183]. They can subsequently be introduced into the device, either directly or after partial dissociation, to reproduce native-like architecture and gradients [184,185]. These approaches are applicable to both monocultures and multi-cell-line cocultures, allowing fine-tuned control over spatial organization and cellular interactions.

When these cellular structures are cultured in such microenvironments, they benefit from controlled nutrient and oxygen delivery, dynamic fluid flow, and the possibility of incorporating vascular or immune compartments. This overcomes some limitations of conventional static cell clusters and enables more predictive drug screening and mechanistic studies. For example, Haque et al. reported organoids derived from pancreatic ductal adenocarcinoma cocultured with stromal cells (pancreatic stellate cells and macrophages) embedded in a Matrigel® substrate in a two-chamber microfluidic device [186]. They successfully tested the device by examining the enhancing effect of microenvironment modulating agents on the antitumor efficacy of chemotherapy. Shin and colleagues embedded lung cancer organoids derived from primary small-cell lung cancer tumors in Matrigel® to test them against cisplatin and etoposide [187]. They concluded that the centers of the organoids are able to survive chemotherapy-induced cell death. Kobayashi et al. used mammary tumor spheroids and intestinal organoids to investigate the effects of anti-cancer drug perfusion [133]. Maulana and colleagues embedded breast tumor organoids in microfluidic chips to model breast tumors within an endothelial barrier, enabling real-time study of CAR-T cell infiltration, cytokine activity, and patient-specific immune responses [188]. Chuaychob et al. developed a vascularized alveolar soft-part sarcoma-on-chip by coculturing tumor spheroids with pericytes and endothelial cells in fibrin-collagen type I matrix. This platform successfully replicated the pericyte-rich vasculature characteristic of the native tumor, providing a valuable tool to investigate tumor–vascular interactions preceding metastasis [177]. Additionally, Penarete-Acosta et al. demonstrated the utility of their microfluidic device by co-culturing colonocyte spheroids with colorectal microbiota to investigate the impact of a pathogen associated with

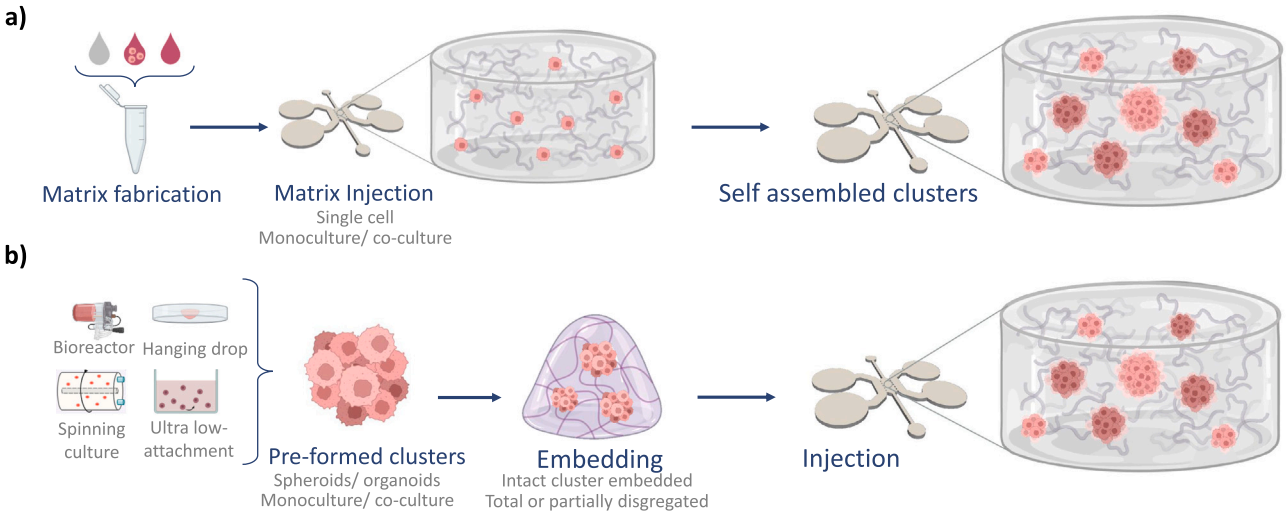

Fig. 3. Cell culture strategies for fabricating tumor-on-chip models. (a) The ECM is prepared including individual cells and the mixture is injected into the device, allowing cells to proliferate over time and self-assemble into multicellular clusters such as spheroids or organoids. (b) Pre-formed spheroids or organoids are generated using specialized techniques (e.g., bioreactors, spinning disks, hanging drops, or ultra-low attachment plates) and subsequently embedded, either intact or partially/fully dissociated, within a matrix before injected into the device.

colorectal cancer [189]. Another example, developed by Adjei-Sowah and colleagues, involved endothelial cells, astrocytes, and glioma stem cells to recapitulate the perivascular niche and study its impact on glioma stem cells [179]. Other authors created pancreatic ductal adenocarcinoma spheroids (composed of PANC-1 cells and pancreatic stellate cells) in Matrigel® and then fabricated a device that enables dynamic administration of drugs or immune cells through a layer of endothelial cells [119].

Taking complexity a step further, multi-organ models have also been fabricated as body-on-chip platforms. These are able to mimic absorption, distribution, metabolism, excretion, or toxicity (ADME-Tox) processes across interconnected tissues [190]. Such systems can bridge the gap between *in vitro* models and *in vivo* pharmacokinetics by capturing tissue–tissue crosstalk and matrix-mediated barriers to drug delivery. Combining tumor-on-chip devices with hepatic, renal, or immune compartments will enable the evaluation of therapeutic distribution and off-target effects in a dynamic, human-relevant setting [191]. For instance, Liu et al. combined patient-derived cholangiocarcinoma organoids with recellularized liver and kidney slices to evaluate anticancer drug efficacy and hepatorenal toxicity simultaneously for personalized therapy testing [185].

4.3. Vasculature modeling in tumor-on-chip systems

Unlike static or non-perfused 3D cultures, vascularized microfluidic models enable real-time analysis of perfusion and barrier integrity, providing a powerful platform to assess drug delivery efficiency and NP transport across endothelial interfaces [192]. A critical advantage of these systems over conventional 2D endothelial monolayers is their ability to recapitulate shear-stress–dependent endothelial maturation and tight barrier function, which are essential for physiologically relevant predictions of drug extravasation and immune cell trafficking. In contrast, 2D endothelial cultures generally exhibit elevated permeability and simplified junctional organization, limiting their ability to model vascular barrier function observed *in vivo* [193]. Two main strategies are commonly employed to incorporate blood vasculature into microfluidic tumor models: sprouting angiogenesis–based models and vasculogenesis-based self-assembly models. In sprouting angiogenesis models, endothelial cells first establish a perfused vessel or monolayer and then extend sprouts into an adjacent hydrogel matrix in response to angiogenic cues. These systems are particularly suited for investigating endothelial sprouting dynamics, directional migration, and tumor-induced angiogenic signaling [194]. In contrast, vasculogenesis-based models rely on the *de novo* self-assembly of endothelial cells—often human umbilical vein endothelial cells (HUVECs) or tissue-specific endothelial cells—cocultured with stromal cells within a 3D hydrogel matrix, where they spontaneously organize into interconnected capillary-like networks [195]. This approach closely mimics the dynamic processes of angiogenesis and vasculogenesis observed *in vivo*, allowing investigation of lumen formation, and tumor-induced vascular remodeling. The resulting microvasculature often exhibits physiological barrier properties, including selective permeability and shear-dependent maturation, making it highly suitable for studies on extravasation or immune cell infiltration [196,197].

Alternatively, patterned microchannel techniques rely on the fabrication of defined vascular geometries. These methods enable precise control over vessel dimensions, branching, and flow directionality, which are essential parameters for studying transport kinetics and drug gradients. While they may lack the biological spontaneity of self-assembled systems, their reproducibility and tunability facilitate standardized assays for high-throughput screening [11]. Recent advances increasingly combine both approaches, integrating self-assembled microvessels within engineered channel architectures to couple biological realism with structural precision [198,199]. Such hybrid systems are emerging as powerful tools to interrogate endothelial–tumor interactions, angiogenic signaling, and therapeutic delivery under controlled, yet biomimetic, conditions.

Further than blood vasculature, microfluidics also allows the fabrication of lymphatic network models. The lymphatic system plays a critical part in maintaining homeostasis, yet it has been historically underestimated in *in vitro* tumor models. This omission is particularly relevant in cancer, where lymphatic vessels actively regulate IFP, tumor-associated inflammation, immune surveillance, and lymph node metastasis, as well as the clearance and biodistribution of therapeutic agents. [200]. Recent lymphatic-on-a-chip platforms have transitioned from simple endothelial linings to the reconstitution of functional, perfusable microvasculature that replicates specialized lymphatic features, such as the characteristic 'oak-leaf' cell morphology and discontinuous basement membranes [201]. These models have elucidated how the mechanical environment, including interstitial pressure and luminal shear stress, regulates the integrity of lymphatic junctions and their permeability, and have highlighted the role of lymphatic vessels in

coordinating vascular, stromal, and immune interactions [202–204]. Furthermore, advanced integrative models have started to simulate 'lymphatic-interstitial-immune' coupling, providing a platform to study how lymphatic transport facilitates the trafficking of immune cells and the drainage of signaling molecules toward sentinel lymph nodes [205,206]. These MPS are now proving essential for quantifying drug clearance rates and understanding how lymphangiogenic signaling and trans-lymphatic transport actively contribute to tumor progression and therapy resistance [207,208]. In Section 5, specific examples of microfluidic models incorporating vascular components (both blood and lymphatic) are presented, following the design principles outlined here. These studies further demonstrate how vascularized tumor-on-chip systems are applied for anticancer drug evaluation and therapeutic screening.

4.4. Additional features in tumor-on-chip systems

Beyond the matrix composition, cellular components, and vascularization, the TME is shaped by a range of physical and chemical stimuli that critically influence therapeutic outcomes (Fig. 1). In microfluidic systems, many of these stimuli; such as fluid flow, shear stress, interstitial pressure, and chemical gradients, are closely linked to the presence of a vascular network, which naturally generates dynamic mechanical cues [209]. Nevertheless, in avascular configurations, similar forces can be artificially introduced through controlled perfusion or gradient formation, allowing independent investigation of their effects on tumor behavior and drug transport.

Microfluidic systems might succeed in reproducing the fluid dynamics of the TME, which is absent in static models. Laminar flow across endothelialized channels generates physiologically relevant shear stress [196]. Importantly, by tuning flow rates, researchers can mimic vascular versus interstitial conditions. For example, Zhao et al. developed a microphysiologically engineered vessel–tumor model in which controlled flow allowed the investigation of vascular transport dynamics of immune cells, thereby demonstrating the utility of flow modulation for evaluating therapeutic delivery strategies [210]. Baye et al. designed a microfluidic platform integrating colon cancer spheroids to investigate how fluid flow modulates fluorescent NPs transport. They demonstrated that local flow velocity gradients across the spheroid surface led to heterogeneous NPs accumulation and penetration depths, highlighting the crucial role of hemodynamic forces in drug delivery performance [211].

Shear stress is also crucial in shaping cancer cell physiology and therapeutic response.*In vivo*, tumor and endothelial cells are constantly exposed to shear forces arising from blood and interstitial flow, which regulate cytoskeletal organization, mechanotransduction pathways, and gene expression profiles associated with malignancy and treatment resistance [212,213]. Reproducing these dynamic cues *in vitro* has proven essential for understanding how biomechanical stress contributes to tumor progression, invasion, and drug delivery efficiency. Dash and colleagues developed a microfluidic device capable of applying physiological ranges of shear stress, demonstrating that mechanical cues modulate gene expression related to stemness and drug resistance in HeLa cells [214]. Moreover, shear forces also influence tumor cell behavior, promoting invasive phenotypes and modulating drug uptake [35]. Ayuso et al. demonstrated as well that shear stress modulates key tumor signaling pathways; such as PI3K/Akt and YAP/TAZ, and affects cytoskeletal organization, proliferation, invasiveness, and drug response, underscoring its critical role in reproducing physiologically relevant tumor behavior in microfluidic models [212].

Another hallmark of solid tumors is the presence of chemical gradients resulting from both irregular vascularization and perfusion, as mentioned. Microfluidic devices enable the control of these gradients: oxygen, glucose, pH and specific nutrients; which might recapitulate, for instance, regions of hypoxia or acidosis that are known to impair drug penetration and drive therapeutic resistance [9,215]. These systems are particularly useful for evaluating therapies targeting hypoxia pathways, as well as for studying metabolic heterogeneity in cancer cells. Moreover, the ability to maintain dynamic gradients over time provides a closer approximation to *in vivo* tumor physiology than conventional 3D cultures [91]. For instance, Oh et al. designed a microfluidic device that induces hypoxia and oxygen diffusion barriers in human breast cancer and prostate cancer cell cultures [216]. Ayuso et al. used a microfluidic tumor-on-chip platform to evaluate NK cells in a suppressive environment involving nutrient depletion, hypoxia and pH acidification [212]. Liu et al. developed a microfluidic platform combining a concentration gradient generator with a vascularized 3D coculture in dECM hydrogels, which facilitated automated, high-throughput assessment of nanomedicine doses [166]. In addition, Barisam and colleagues developed a microfluidic platform featuring U-shaped microchannel arrays that enabled controlled gradients of oxygen, glucose, and shear stress during the 3D culture of A549 lung adenocarcinoma spheroids. They demonstrated that fine-tuning these parameters modulated the enrichment of cancer stem-like cells and significantly altered chemoresistance to cisplatin, with oxygen concentration emerging as the dominant factor influencing therapeutic response [217].

4.5. Transport dynamics modeling in tumor-on-chip systems

The ECM, cellular organization, vascular architectures and further stimuli discussed above collectively govern the mechanisms of drug transport and distribution within microfluidic platforms. Tumor-on-chip models enable precise control over the physical and biochemical parameters of drug transport and release, including diffusion, convection, and barrier permeability. Accordingly, this section specifically focuses on the *in vitro* modeling of transport mechanisms relevant to drug delivery, and how these features influence delivery efficiency, spatial distribution, and therapeutic response, thereby reproducing the complex spatiotemporal exposure dynamics that occur *in vivo* (Fig. 4).

The most basic approach involves passive diffusion, where the therapeutic compound is introduced directly into the culture medium and reaches the cells following a concentration gradient, representing the most traditional configuration. In this scenario, a drug or biomolecule is placed in a channel adjacent to or overlaying the culture, and molecule transport occurs purely by concentration gradients. Such setups allow clean assessment of how matrix porosity, matrix composition, or diffusivity of the molecule affect penetration. For example, a recent study used Matrigel®-filled microfluidic channels and synchrotron X-ray scattering to map NP diffusion, showing that diffusion behavior varies strongly with matrix porosity and NP size/shape [218]. Additionally, Zhang et al. evaluated in collagen-I matrix the diffusive chemotactic effect of the CXCL12 cytokine, whose levels are increased in the TME, over T and engineered T cells [139].

A more advanced strategy is the advection, which relies on assisted diffusion or perfusion-based delivery, in which the transport of the drug is enhanced by applying a controlled fluid flow [219]. Microfluidic platforms often allow the application of fluid flow, as mentioned above, which not only mimics the TME, more faithfully but also enhances biomolecule transport. It can be established either transversely (directly across the cell-laden matrix) or longitudinally, along the channel axis, generating continuous mixing and more homogeneous drug distribution, similar to mechanical stirring in macroscopic systems [209]. Here, flow can generate convective flux, which helps to overcome mass transport limitations in dense matrices. For instance, Goyal and colleagues showed the *in vitro* formation of lymphoid follicles when culturing B and T lymphocytes in a Matrigel-type I collagen hydrogel microchip under fluid flow [165]. Aceves et al. reported perfusable 3D kidney-on-chip model and studied the impact of flow on drug transport and uptake in kidney organoids [220].

Finally, some microfluidic models integrate intermediate barriers or membranes between the drug compartment and the tumor tissue, either under static or flow conditions. These engineered barriers are distinct from the intrinsic fluid-ECM interface present in all compartmentalized

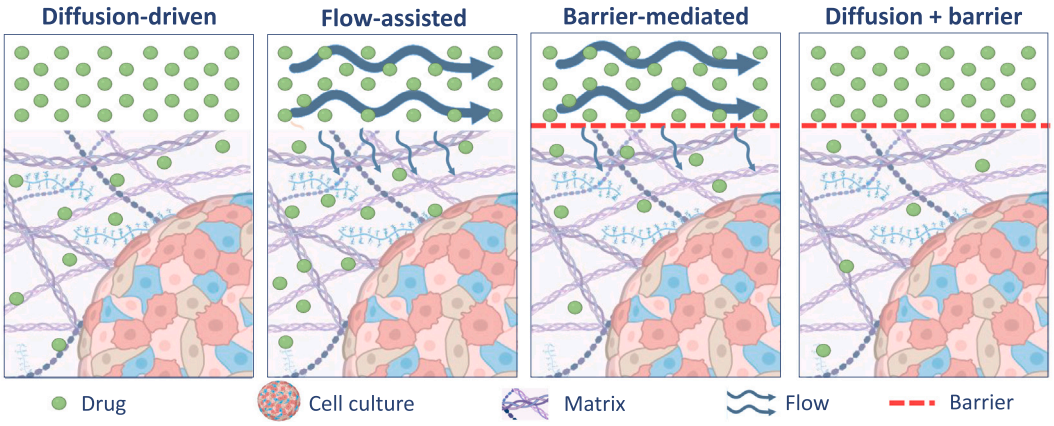

Fig. 4. Schematic representation of the main mechanisms for drug delivery in microfluidic tumor models. (i) Diffusion-driven delivery: passive transport of soluble compounds from adjacent channels or reservoirs into the tumor compartment via the liquid-ECM interface. (ii) Advection as flow-assisted diffusion: controlled perfusion of the drug-containing medium through microchannels, reproducing vascular or interstitial flow conditions. (iii) Transport through engineered barrier: delivery across semi-permeable membranes or endothelial barriers. (iv) Barrier-limited diffusion: passive diffusion of therapeutic agents across an engineered barrier.

tumor-on-chip models, as they represent additional physical obstacles, that actively impede cells and drug diffusion and more faithfully recapitulate pathological barriers encountered *in vivo* (Fig. 4). The engineered barrier may consist of synthetic or natural biomaterials engineered to mimic specific physiological barriers, such as the blood–brain [219,221], endothelial interfaces, or cellular monolayers cultured *in situ* to emulate biological interfaces [222]. This setup allows the investigation of selective transport, permeability, and differential penetration of therapeutic agents, providing a more physiologically relevant framework for evaluating *in vitro* drug delivery strategies [223]. For instance, one microfluidic cancer-on-chip platform used a porous membrane to separate a drug-flow channel from cells, enabling dynamic concentration profiles and demonstrating that oxaliplatin diffusion into the cell layer could be temporally controlled via membrane thickness and flow conditions [224]. Another device featured 3D tumor tissue on one side of a barrier and a 2D endothelial monolayer on the other (tumor-2D endothelium co-culture), allowing comparison of soluble versus vesicle-anchored TRAIL delivery across the barrier. This illustrated how anchoring or delivery format alters penetration efficacy when a barrier is present [225].

4.6. Advantages and limitations of microfluidics

One of the key advantages of using microfluidics to study tumor models compared to all conventional platforms lies in the intrinsic reduction of experimental scale. Working at the microscale substantially decreases the required volumes of media, reagents, and therapeutic compounds. This fact not only lowers costs but also minimizes waste generation, an increasingly relevant aspect of sustainable laboratory practices [226]. Moreover, the reduced sample requirements of these systems make them compatible with the use of patient-derived cells or tumor biopsies, facilitating the development of more personalized *in vitro* models.

Beyond these practical benefits, the microscale environment is biologically advantageous as it enables experimental conditions that operate at the natural cellular scale, facilitating direct observation of physiological phenomena. As a result, microfluidic systems enable more precise control over the cellular microenvironment, including local gradients of nutrients and drugs, mechanical cues such as shear stress, and direct cell–cell or cell–matrix interactions [227]. This level of control surpasses that achieved in macroscopic culture formats and enhances the physiological relevance of the experimental outcomes, thereby strengthening the predictive value of drug delivery studies. Moreover, the aforementioned ability to integrate and finely tune multiple stimuli; such as matrix stiffness, vascular perfusion, specific chemical gradients and interstitial fluid flow within a single micro-engineered platform, represents one of the major advantages of tumor-on-chip technologies over traditional models. Importantly, not all these features contribute equally to *in vivo* predictiveness, and their relevance strongly depends on the drug delivery mechanism under investigation. Among them, the incorporation of functional vascular barriers under flow has emerged as one of the strongest determinants of translational relevance, as it governs key processes such as drug extravasation, endothelial permeability, and shear-dependent transport, which are critical *in vivo* but absent in static models [121,219]. Likewise, the presence of a three-dimensional ECM with physiologically relevant composition, density, permeability and stiffness is essential to capture diffusion limitations, matrix-mediated retention, and drug–matrix interactions [153,156]. Additional features such as interstitial flow, oxygen and nutrient gradients, and the inclusion of stromal or immune cells further refine the predictive capacity of these platforms by recapitulating tumor-specific resistance mechanisms. Overall, the microfluidic feature that most strongly determines *in vivo* predictiveness is context-dependent and dictated by the therapeutic mechanism under investigation, whether it involves vascular extravasation, matrix-limited diffusion, intratumoral distribution, or therapy-induced resistance.

Ultimately, one advantage of microfluidic systems over other 3D tumor models is their exceptional compatibility with advanced microscopy, enabling 4D studies with real-time optical observation. The optical transparency of commonly used materials (i.e. PDMS), allows high-resolution imaging through the entire culture chamber, enabling precise visualization of cellular dynamics, flow behavior, and drug distribution within microchannels. The integration of live-imaging techniques such as bright-field, fluorescence, confocal, and light-sheet microscopy allows continuous monitoring of physiological processes, drug responses, and cell–matrix interactions without disturbing the experimental setup [228]. Additionally, at the end of the experiment, the samples can be fixed and easily evaluated under immunostaining. These approaches support resolution in the *in vitro* setting, allowing researchers to directly correlate microenvironmental conditions with biological outcomes. Furthermore, PDMS-based devices facilitate straightforward sample manipulation and optical access while maintaining biocompatibility and minimal autofluorescence [229], features essential for quantitative imaging. Beyond imaging advantages, matrix-integrated microfluidic platforms provide a level of quantitative control that differentiates them from other 3D tumor models. Compared to non-matrix organ-on-chip systems, the incorporation of defined ECM hydrogels enables direct tuning of mechanical and structural parameters such as stiffness, mesh size, and permeability, which critically regulate drug diffusion, penetration depth, and cellular response [26,28]. Relative to bulk

hydrogels and spheroids, microfluidic confinement and controlled perfusion improve the reproducibility and spatial resolution of transport-related measurements, allowing quantification of effective permeability coefficients, concentration gradients, and matrix-dependent Inhibitory Concentration 50% (IC50) shifts that better reflect heterogeneous *in vivo* drug exposure [230,231]. While organoids and PDX-derived models preserve higher biological complexity and tumor heterogeneity, their dense and poorly defined matrices often limit direct assessment of transport and barrier function. In contrast, matrix-based tumor-on-chip systems enable the integration of perfusable vascular barriers and real-time evaluation of extravasation-related parameters, including endothelial permeability and Trans-Epithelial Electrical Resistance Assay (TEER)-like readouts under flow, positioning these platforms as particularly powerful for the quantitative dissection of matrix- and transport-driven determinants of drug delivery [232,233].

However, despite their clear strengths, microfluidic tumor platforms still face significant challenges and limitations that hinder their translation into mainstream drug development workflows. Scalability remains a critical challenge: most tumor-on-chip devices are fabricated as individual prototypes rather than mass-produced platforms, while their single-unit designs also limit high-throughput drug screening capabilities. In addition to manufacturing and throughput constraints, scalability also poses challenges at the translational level, particularly in relating microscale *in vitro* observations to clinically relevant *in vivo* responses. The reduced volumes, simplified tissue architectures, and constrained cellular compositions inherent to tumor-on-chip systems complicate direct extrapolation of drug dose–response relationships, pharmacokinetics, and toxicity profiles [234,235]. Reproducibility is equally challenging, as variations in chip geometry, cell seeding protocols, or ECM composition can strongly influence outcomes, making it difficult to compare results across different laboratories [236]. Closely related is the problem of standardization: there is currently no consensus on design principles, culture conditions, or analytical readouts, which prevents the establishment of universal benchmarks for performance and validation. This lack of harmonization directly impacts regulatory acceptance and most regulatory agencies still rely predominantly on animal models for safety and efficacy assessment. Tumor-on-chip platforms are therefore positioned mainly as complementary tools rather than as regulatory-approved alternatives. Nevertheless, initiatives aimed at defining qualification pathways and best practices suggest that this landscape is actively evolving. These regulatory challenges and emerging efforts are discussed in detail in Section Section 5.6 [237]. In addition, practical issues frequently arise when polymerizing hydrogels inside PDMS-based devices. Small imperfections in surface treatment or channel geometry can lead to leakage, delamination, or incomplete gel confinement, ultimately compromising the integrity and reproducibility of the model. As a result, alternative fabrication strategies, such as 3D-printed [132] or thermoplastic chips with dedicated gel-loading architectures [238], are increasingly being explored to improve robustness and facilitate more standardized workflows.

Other limitations arise from the miniaturization that defines microfluidic tumor models. The downsized scale, while advantageous for mimicking the TME and reducing reagent consumption, inherently yields limited cell number, which poses a substantial issue for downstream molecular analyses such as quantitative PCR or RNA sequencing. Due to the limited biological material, researchers are often compelled to employ specialized equipment. Moreover, in microfluidic platforms utilizing ECM hydrogels herein reviewed, cell harvesting requires enzymatic or chemical digestion of the matrix. This process can disrupt delicate cell–matrix and cell–cell interactions, potentially altering cell phenotypes or inducing artifacts in gene expression profiles. Although these challenges continue to restrict the full adoption, continuous advances in microfabrication, automation, and analytical integration are progressively reducing many of these challenges. The combination of organ-on-chip procedures with, for instance: high-quality imaging, omics technology, and machine learning-based data analysis is expected to enhance reproducibility and scalability, paving the way for their gradual implementation in translational and regulatory frameworks [239].

5. Microfluidic tumor models in drug delivery and translational research

The capacity to reproduce key hallmarks of the TME has positioned microfluidic systems as transformative technologies for preclinical drug evaluation and translational research. In this context, microfluidic tumor models are increasingly employed to investigate drug transport, pharmacodynamic responses, and therapeutic efficacy under physiologically relevant conditions, while also enabling integration with computational approaches and emerging regulatory frameworks.

5.1. Nanomedicine and carrier-based delivery systems

Nanomedicine has emerged as a novel approach for cancer therapy, integrating nanotechnology with pharmacology to enhance drug delivery and therapeutic efficacy [240]. Nanodrug delivery systems (NDDSs), including liposomes, polymeric NPs, dendrimers, polymer micelles, exosomes and supramolecular aggregates, exploit their high surface-area-to-volume ratios and tunable physicochemical properties to improve drug loading, targeting, and release kinetics [7,241]. Several NPs-based products have already reached clinical use as imaging agents or drug vehicles; however, challenges such as non-scalable production, batch-to-batch variability and inconsistent payload delivery to target tissues are still limitations of this technology [242]. Microfluidic technologies offer a powerful solution to these hurdles. By manipulating fluids at the micrometer scale, microfluidic platforms enable highly controlled, reproducible and scalable fabrication of nanocarriers with precisely defined size, shape and composition, critical parameters for penetration, uptake and retention within tumor tissues. While microfluidics plays a pivotal role in the controlled fabrication of nanomedicines [243,244], matrix-integrated tumor-on-chip models have become particularly valuable for the preclinical evaluation of NDDSs, as they enable testing under physiologically relevant conditions that recapitulate key barriers of the TME governing NPs transport and therapeutic efficacy. These systems have been applied to study a wide range of nanomedicine formulations, revealing how physicochemical features of the carrier, together with the composition and architecture of the surrounding TME, shape their stability, penetration, and therapeutic outcome. Table 2 provides an overview of tumor-on-chip systems incorporating ECM components to study NDDSs, classified according to the mechanism of nanotherapy administration.

Metal-based and catalytic nanomedicines have demonstrated great potential as therapeutic platforms, whose tunable physicochemical properties enable controlled activation and catalytic activity under specific tumor microenvironmental conditions such as pH, matrix composition, or oxygen levels. For instance, Garcia-Peiro et al. developed PEGylated platinum nanodendrites capable of catalyzing bioorthogonal reactions for *in situ* pro-drug activation. Microfluidic platforms for 3D culture demonstrated that the dendritic morphology and Pt–S PEGylation synergistically enhanced the stability and diffusion through dense collagen type I matrix, allowing controlled prodrug uncaging in cancer cells [174]. Similarly, this group designed in another study copper-based nanostructures with distinct release profiles to investigate their impact on glioblastoma (GBM) progression and invasiveness. By employing a complex 3D microfluidic model mimicking the tumor ECM with a collagen type I hydrogel, they demonstrated how differences in copper release kinetics, together with NPs–matrix interactions, modulated cell proliferation and invasion, underscoring these parameters as key determinants of treatment efficacy [245]. Zhuang et al. developed a multiple tumor culture chip integrating 3D tumor spheroids embedded in Matrigel® and dynamic flow to study the penetration of

Table 2
Overview of tumor-on-chip systems incorporating ECM components to study NDDSs.

Mechanism of drug delivery	Diffusion-driven	Flow assisted
Barrier	• No barrier [164,166,174,245] • Endothelial barrier [11,225] • Basal Lamina [250] • BBB [251] • Lymphatic [208]	• No barrier [246,248,249] • Endothelial barrier [148,247,252,253]
NDDS type	• Metal-based NPs [245] • Dendrimers [174] • Polymeric micelles [164,208,250] • Liposomes [11,166,225,251]	• Mesoporous silica NPs [246] • Carbon dots [148,252] • Polymeric micelles [247–249] • Liposomes [253]
Properties studied	• Stability and diffusion through dense ECM [164,174,208,245] • Controlled delivery in TME [174,208,225,245] • NDDSs efficacy [166,174,245,251] • Cellular uptake [164,208] • NDDS-matrix interaction and tumor penetration [245,250] • NDDSs extravasation [11,208,225,251]	• Stability and diffusion through dense ECM [246–248,252] • NDDSs efficacy [246,248,249,252] • Cellular uptake [249] • NDDS-matrix interaction and tumor penetration [148,246] • NDDSs extravasation [148,247,252,253] • Oxidative stress (ROS) and citotoxicity [249] • Shear stress [249]

mesoporous silica NPs (MSN) under physiologically relevant conditions. They demonstrated that continuous perfusion markedly enhanced MSN diffusion compared to static administration, and that pretreatment with hyaluronidase improved the penetration of larger particles by modulating the ECM, emphasizing the relevance of dynamic microfluidic environments for evaluating NPs transport and therapeutic performance [246].

In parallel, polymeric and supramolecular nanocarriers have been extensively studied in microfluidic systems to assess their structural stability, degradation, and diffusion through 3D ECM. Deng et al. used a microfluidic tumor-on-chip with Matrigel®/collagen-HA as ECM to evaluate how single-chain polymeric NPs (SCPN) microstructure governs their diffusion, stability, and cellular uptake in 3D [164]. Likewise, Feiner-Gracia et al. combined spectral confocal imaging with a tumor blood vessel-on-chip to monitor in real time the disassembly, extravasation, and micelle stability of supramolecular nanocarriers as they cross ECM-like barriers to reach the tumor spheroids embedded in a collagen matrix [247]. In addition, several matrix-integrated microfluidic models have incorporated endothelial barriers and dynamic flow to better reproduce the physiological conditions governing polymeric nanocarrier transport. Virumbrales-Muñoz et al. developed a coculture device combining a 3D breast tumor model in a collagen hydrogel with a functional endothelium, demonstrating its relevance for high-throughput screening of vesicle-based formulations and highlighting the endothelial layer as a critical barrier for nanocarrier delivery [225]. Similarly, Chen et al. designed a breast tumor-on-chip comprising a microvessel wall, ECM simulated by BME hydrogel, and uniform spheroids to evaluate carbon dot-based nanocarriers under dynamic flow, enabling real-time monitoring of endothelial transport and intratumoral penetration [148]. Martins et al. further employed single- and double-channel microfluidic systems embedding glioblastoma cells in Matrigel® to assess the efficacy of polymeric NPs and molecular drugs, showing how ECM organization and perfusion geometry modulate NPs diffusion and treatment outcomes [248]. Interestingly, Kim et al. used a hepatocarcinoma-on-chip platform with collagen matrix integrating ROS sensors to study albumin–oleic acid NPs under hepatomimetic shear stress, revealing a correlation between oxidative stress, NPs uptake, and cytotoxicity [249]. Finally, Olea et al. employed a dual-ECM microfluidic chip containing tumor spheroids within a collagen-HA hydrogel to screen degradable polymeric micelles with different compositions, demonstrating that ECM type critically determines micelle–matrix interactions and tumor penetration [250]. Collectively, these studies emphasize how dynamic flow, endothelial barriers, and ECM composition jointly define the fate and therapeutic performance of polymeric nanocarriers within tumor-like environments.

Finally, vascularized and organ-specific tumor-on-chip models have been developed to recapitulate multicompartmental drug delivery scenarios and explore NP transport across physiological barriers. Liu et al. integrated a concentration-gradient generator with a vascularized 3D coculture system in dECM hydrogels, enabling high-throughput, automated testing of nanomedicine dose-response relationships in a biologically relevant setting [166]. Sharifi et al. employed a metastasis-on-chip bioreactor containing a tumor ECM compartment, composed of GelMA, and a bone-mimetic ECM niche, composed by GelMa and hydroxyapatite, separated by a vascular-like membrane to study hepatocellular carcinoma (HCC) cell migration and the prolonged inhibitory effect of encapsulated thymoquinone NPs [252]. Similarly, Lu et al. used a 3D lymphatics-on-chip to study size-dependent transport of PLGA–PEG NPs through the interstitial ECM and into engineered lymphatic vessels, revealing distinct endocytic and paracellular mechanisms depending on NP size [208]. In the field of vascularized models, Agarwal et al. engineered a hierarchical 3D vascularized tumor model by assembling avascular microtumors, encapsulated in collagen and alginate hydrogel shells, with endothelial and stromal cells, generating macroscale constructs that mimic *in vivo* tumor physiology [11]. The vascularized tumors exhibited markedly higher drug resistance than both avascular spheroids and 2D cultures, which could be effectively overcome through lipid-loaded NP delivery. Likewise, Tang et al. developed a biomimetic microfluidic TME comprising cocultured tumor in Matrigel® and endothelial cells under shear flow, allowing quantitative assessment of endothelial permeability and liposomal drug carrier extravasation. Their system revealed that metastatic cells or their secreted factors significantly disrupt endothelial junctions, enhancing permeability and recapitulating the EPR effect observed *in vivo* [253]. Finally, Straehla et al. established a vascularized glioblastoma-on-chip model incorporating endothelial cells, astrocytes, and pericytes in fibrin hydrogel to reproduce the blood-brain barrier (BBB) and investigate NP delivery across this highly selective interface. Using functionalized NPs with GBM-targeting motifs, they demonstrated improved BBB penetration and therapeutic efficacy, validating the platform against *in vivo* mouse models [251]. Together, these examples illustrate how microfluidic models provide a relevant platform to study NP diffusion, stability, and therapeutic effects in a controllable yet biologically relevant environment. Furthermore, they highlight that the ECM and the surrounding microenvironment critically influence NDDS performance (Fig. 5).

5.2. Antibody and biologic therapeutics

Beyond NDDSs, matrix-integrated microfluidic tumor models provide unique opportunities to investigate how antibody-based

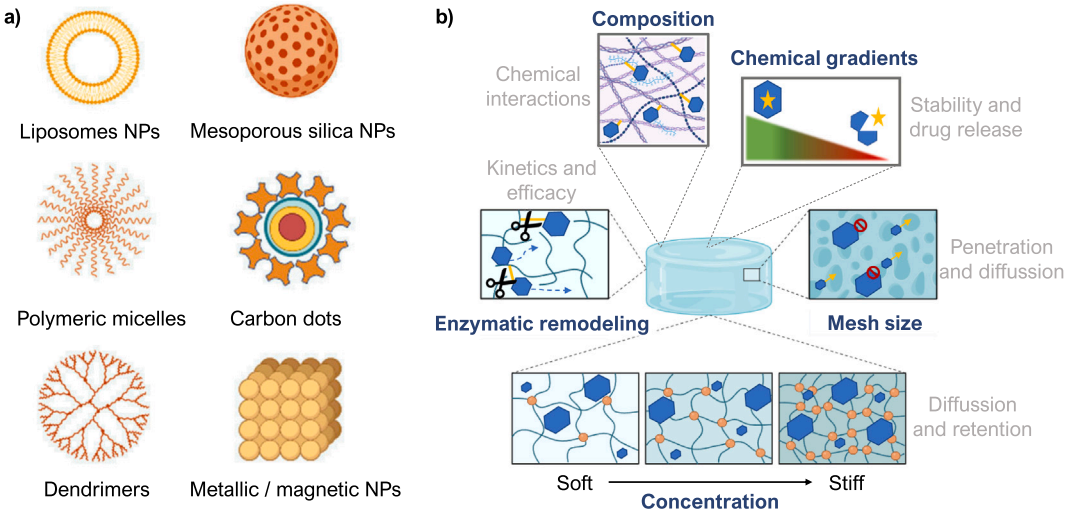

Fig. 5. Schematic illustration of factors influencing NDDSs. (a) NPs commonly used for NDDS fabrication. Adapted from Xu et al., Molecules (2023) [240]. (b) Overview of how ECM properties affect NDDS behavior, including diffusion, penetration, stability, release kinetics, and NPs–matrix interactions.

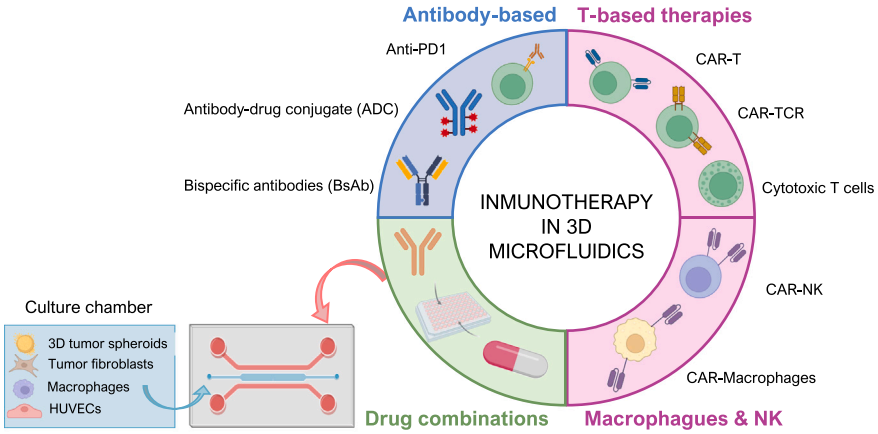

Fig. 6. Schematic representation of the main immunotherapeutic strategies applied in 3D hydrogel-based microfluidic chips. In these systems, immune cells or immunotherapeutic agents are typically introduced through the lateral perfusion channels, while 3D tumor spheroids and other components of the TME, such as HUVECs, fibroblasts, or macrophages, are embedded within the central culture chamber.

therapeutics distribute, penetrate, and exert activity within a biomimetic ECM (Fig. 6).

Immune checkpoint antibodies have revolutionized immunotherapy in solid tumors. Specifically, antibodies that block Programmed Cell Death Protein 1 (PD-1), which normally prevent T-cells from attacking cancer cells, have become some of the most widely prescribed anticancer therapies [254]. Several studies have explored the effects of anti-PD1 antibodies on 3D tumor models using hydrogel-based microfluidic systems. For example, Jiang et al. developed a custom-made immunotherapeutic high-throughput observation chamber designed to test the effect of anti-PD-1 antibodies on breast cancer spheroids and T cell interactions [255]. Beyond these advances, dynamic single-cell RNA sequencing of murine organotypic tumor spheroids performed by Sehgal et al. revealed immunotherapy persister cells that resist PD-1 blockade through baculoviral IAP repeat-containing protein 2 (Birc2) and Birc3-dependent survival. Authors combined PD-1 blockade with Birc2/3 antagonism to enhance tumor cell killing *in vivo* [256]. Additionally, a brain tissue-mimicking hydrogel prepared by interpenetrating growth-factor-reduced Matrigel® matrix and MMP-sensitive HA hydrogels was used to analyze the effects of anti-PD1 therapy in the immunosuppressive microenvironment of glioblastoma [257]. Similarly, Xiang et al. investigated the impact of perfusing an anti-PD-1 antibody in a complex microenvironment, where clusters of differentiation (CD) 8+ T cells flowed through microchannels lined with HUVECs in a chip containing gastric spheroids [258].

Beyond single-agent applications, these platforms have also enabled the evaluation of combinatorial antibody strategies in complex TMEs. For example, Chernyavska et al. reported that co-administration of an anti-epidermal growth factor receptor (EGFR) IgA with an anti-CD47 innate immune checkpoint inhibitor synergistically activated macrophage phagocytic function, thereby promoting selective cancer cell elimination in a collagen-based microfluidic tumor model [259].

Antibody–drug conjugates (ADCs) are particularly relevant in this regard, as their therapeutic success depends not only on antigen binding and intracellular release of the cytotoxic payload, but also on efficient transport across tumor-like barriers. The most representative example is trastuzumab-emtansine (T-DM1), which has been tested in an organoid-slice-on-chip system embedding patient-derived cholangiocarcinoma organoids within 3D decellularized liver matrices, enabling simultaneous evaluation of intratumoral efficacy and off-target hepatorenal toxicity under controlled flow [185].

In addition to ADCs, bispecific antibodies (BsAb) are rapidly emerging as a powerful class of antibody-based therapeutics [260]. Many bsAb are designed to bind tumor-associated cell antigens while simultaneously engaging CD3 on T cells, thereby promoting targeted recruitment and activation of cytotoxic lymphocytes. These CD3-engaging bsAbs have been evaluated in ECM-embedded tumoroid arrays, where hydrogel composition and stiffness modulate T-cell infiltration and tumor killing, underscoring the role of the physical matrix in delivery and therapeutic response [261]. Furthermore, bsAbs have been investigated in bone marrow-on-chip systems to assess safety profiles, enabling evaluation of hematopoietic adverse events under physiologically relevant conditions [262]. Similarly, Kerns et al. evaluated off-target and tumor toxicity of T-cell bispecific antibodies using multi-organ microfluidic systems incorporating immune and stromal compartments [123]. These models enabled simultaneous assessment of immune activation, cytokine release, and tissue-specific damage under dynamic perfusion, illustrating how organ-on-chip technology can enhance the preclinical safety and delivery profiling of complex antibody-based therapeutics.

Although hydrogel-based tumor-on-chip systems are central for studying drug delivery and efficacy in 3D matrices, droplet-based microfluidics offers a complementary upstream platform for high-throughput discovery of therapeutic antibodies and bispecific constructs [263–265]. Integrating both approaches could enable a seamless workflow from antibody screening to evaluation in physiologically relevant 3D models; however, as this review focuses on hydrogel-based microfluidics, droplet-based systems are not discussed in detail.

5.3. Cellular and gene therapy approaches

Adoptive cell transfer (ACT) therapy has revolutionized the treatment of hematological malignancies. This strategy relies on the engineering of cytotoxic immune cells to enhance tumor cell killing. Current ACT modalities include T cell-based approaches such as CAR-T, tumor-infiltrating lymphocytes and T-cell receptor (TCR)-T therapies, as well as the engineering of other cytotoxic populations including NK cells and macrophages [266]. However, the complex microenvironment in solid tumors remains a major obstacle, limiting immune cell entry into the tumor site and reducing cytotoxic efficiency through immunosuppressive mechanisms [267]. Microfluidic tumor models incorporating hydrogels have emerged as powerful platforms to study the delivery, efficacy, and safety of engineered immune and gene-modified cells under physiologically relevant conditions. These systems recapitulate physical barriers and the complexity of the TME that strongly influence CAR-T, T and NK cell infiltration and cytotoxicity at tumor site. Recent microfluidic studies have revealed that biophysical constraints can directly impair immune cell function. In particular, CAR-T cells are more affected than native T lymphocytes by mechanical confinement, displaying reduced deformability, migration speed, and killing efficiency when navigating narrow microchannels that mimic the dense ECM of solid tumors [139]. Similarly, Chen et al. developed a hydrogel-based microfluidic model simulating the physical barriers of the tumor interstitium to evaluate cytotoxic T lymphocyte infiltration and killing efficiency. Their results showed that increasing matrix density and stiffness markedly restricted cytotoxic T lymphocyte migration and reduced target-cell lysis, highlighting the critical role of tumor biomechanics in shaping immune cell-mediated antitumor responses [268]. Complementary modeling approaches have been developed to quantify how such constraints affect immune cell motility in 3D environments. Using an agent-based computational framework integrated with microfluidic imaging data, a recent study estimated 3D cell migration trajectories from 2D measurements and revealed that CAR-T cells exhibit slower and less persistent migration than conventional T cells in dense matrices, highlighting intrinsic mechanical and behavioral limitations that may impact their tumor infiltration [173]. These findings underscore the importance of incorporating realistic physical and mechanical barriers into microfluidic tumor models to accurately assess immune cell delivery, motility, and functional exhaustion.

While physical confinement represents one of the most evident obstacles, metabolic stressors such as hypoxia equally compromise immune cell efficacy within solid tumors. To address this aspect, Ando et al. developed a hypoxic 3D microfluidic tumor model to evaluate CAR-T cell cytotoxicity under oxygen-deprived conditions. Their results revealed that limited oxygen availability significantly reduced CAR-T cell infiltration and killing capacity, underscoring the critical role of oxygen gradients in shaping therapeutic performance [269]. Complementing these approaches, Ronteix et al. used a high-resolution droplet-based microfluidic assay to dissect immune-tumor interactions with single-cell precision. Authors demonstrated with this model that tumor killing by T cells follows cooperative rather than independent dynamics, as early effector cells enhance subsequent T-cell recruitment and cytotoxic activity within confined microenvironment [270].

Different strategies have been implemented to deliver engineered immune cells within microfluidic systems. For example, Suraiya et al. encapsulated CAR-T cells into microgels as delivery vehicles against ovarian adenocarcinoma [271]. However, the most common configuration perfuses immune cells through vascular-like channels adjacent to hydrogel tumor compartments, enabling realistic immune–tumor interactions. These models are powerful for dissecting mechanisms of limited infiltration or immune suppression within solid tumor ECM [272]. For instance, Pavesi et al. introduced modified TCR-T cells through adjacent channels of a collagen-based 3D microfluidic device, achieving specific killing of human cancer hepatocytes [122] and later demonstrated that cytotoxicity could be enhanced using epigenetic regulators [273].

To further reproduce the complexity of the TME, engineered immune cells are often perfused into systems containing multiple cell populations. Lee et al. demonstrated that the presence of monocytes impaired the cytotoxicity of redirected TCR-T cells against 3D tumor spheroids [136]. Wan et al. developed a fibrin-based 3D microfluidic model with sequential addition of fibroblasts to tumor spheroids, creating a vascularized *in vitro* system. CAR-T cells were then perfused under continuous flow, enabling studies of drug delivery and immune cell trafficking [184]. Similarly, Maulana et al. created a vascularized breast-on-chip model with endothelial cells, combining CAR-T cells and controlled dasatinib treatment to reduce cytokine release syndrome, a side effect of CAR-T therapy associated with uncontrolled activation of immune system [188]. Extending this concept, Dey et al. developed a 3D bioprinted vascularized breast tumor model integrating both CAR-T immunotherapy and chemotherapeutic screening, allowing simultaneous evaluation of drug–immune cell synergy under perfused, physiologically relevant conditions [274]. Hydrogel-based microfluidic systems have also been applied to hematological malignancies, such as an organotypic immunocompetent leukemia bone marrow chip used to evaluate CAR-T cytotoxicity [275].

Microfluidic platforms have likewise been applied to NK cell therapy. These cells are excellent candidates for allogeneic immunotherapy because their cytotoxic activity is not restricted by antigen specificity. Instead, NK cells rely on a balance of activating and inhibitory receptor signaling, allowing them to target tumor cells lacking Major Histocompatibility Complex (MHC)-I expression. This property helps overcome one of the major challenges of T cell–based therapies, the requirement to identify a specific tumor-associated antigen as a therapeutic target [276]. Ayuso et al. developed a 3D breast cancer-on-chip model with endothelialized vascular channels and tumor spheroids, demonstrating that NK cells could penetrate and kill tumor cells more effectively than antibodies under perfusion [277]. Building on this concept, a recent study introduced a high-throughput 3D tumor vasculature on-chip platform that enables real-time monitoring of immune cell infiltration and cytotoxicity under dynamic flow [197]. This system provided quantitative insights into NK cell trafficking across vascular barriers and tumor penetration, offering a physiologically relevant tool for evaluating immune cell–based delivery and antitumor efficacy.

Beyond vascularization, microfluidic platforms have also been used to investigate other TME factors influencing NK cell function. Shen et al. used a Matrigel®-based 3D microfluidic chip to show that activated hepatic stellate cells promote NK cell exhaustion and drug resistance in hepatocellular carcinoma [278]. In a glioblastoma–microglia coculture, Hong et al. demonstrated that extracellular vesicle-mediated delivery of miR-124 suppresses tumor growth, reprograms the microenvironment toward an antitumor state, and enhances NK cell recruitment and cytotoxicity [279]. Nguyen et al. developed a triple coculture microfluidic system with colorectal tumor microtissues, NK cells, and cardiac microtissues to assess therapy safety [280].

5.4. Combination therapies and personalized medicine

The convergence of hydrogel-based microfluidic technology and patient-derived models represents a major step toward personalized medicine. By mimicking tumor-specific microenvironments and enabling the simultaneous testing of multiple drugs and immunotherapies within patient-derived 3D constructs, these systems provide a dynamic platform for evaluating treatment responses and optimizing combination strategies tailored to individual tumor biology.

In this context, patient-derived organoids (PDOs), patient-derived cells and induced pluripotent stem cells integrated into hydrogel-based MPS are emerging as predictive tools for individualized therapy testing. Embedding PDOs within defined ECM hydrogels inside perfused chips preserves tissue-specific architecture and allows personalized drug screening by providing valuable insights into an individual such as genetic variants, drug resistance, immune status and disease progression. Different studies have shown a strong correlation between patient-derived organoids-on-chip responses and clinical outcomes, demonstrating their potential for tailoring combination regimens or identifying salvage therapies in resistant cases [281,282]. Building on these advances, Ding et al. introduced patient-derived micro-organospheres, miniaturized systems that preserve key TME interactions while enabling high-throughput functional profiling of individual patient tumors to guide precision oncology decisions [283]. In line with this, Lorenzo-Martín et al. developed patient-derived mini-colons, which retain the structural and cellular complexity of the TME over extended culture periods, allowing long-term evaluation of therapeutic responses and microenvironmental remodeling within hydrogel-embedded microfluidic platforms [284]. In addition, Jenkins et al. developed organotypic tumor spheroids that preserve native immune and stromal components, allowing *ex vivo* profiling of PD-1 blockade within a physiologically relevant 3D context [285]. In a complementary approach, a patient-derived lung tumor-on-chip platform was developed to evaluate personalized responses to anti-PD-1 immunotherapy. This model integrated autologous immune and tumor cells within a collagen-based microenvironment, enabling real-time assessment of T-cell activation, cytokine secretion, and tumor killing under physiologically relevant flow [125]. Similarly, a "chip collection" of hepatocellular carcinoma based on O_2 heterogeneity derived from patient tissue replicated patient-specific oxygen gradients within gelatin matrices, enabling the study of how microenvironmental hypoxia modulates drug penetration and therapeutic efficacy. This work highlights how capturing tumor-specific physiological heterogeneity within microfluidic hydrogels can improve the predictive accuracy of personalized drug delivery assessments and optimize treatment strategies for hypoxic solid tumors [286]. Expanding upon these insights, recent research has highlighted that targeting intracellular resistance pathways can further enhance immunotherapy efficacy. For example, inhibition of TANK-binding kinase 1 has emerged as a promising strategy to overcome adaptive resistance to immune checkpoint blockade [287]. Integrating such molecular targets into hydrogel-based microfluidic systems could allow simultaneous evaluation of checkpoint inhibitors and TANK-binding kinase 1 modulators within patient-specific TME, providing mechanistic understanding and guiding rational combination design for improved drug delivery and immune activation.

In conclusion, matrix-integrated tumor-on-chip models provide a versatile and physiologically relevant platform for evaluating complex drug combinations and personalizing therapeutic regimens. Such approaches not only improve the predictive power of preclinical testing but also support the development of more effective, patient-tailored drug delivery strategies.

5.5. Computational and data-driven approaches in microfluidic tumor models

Computational simulations are useful tools that are commonly employed in biological and biomedical research to complement other modeling strategies, such as *in vivo* and *in vitro* models [288]. Within this context, the concept of digital twins (DTs) has recently gained significant relevance [289]. DTs are particularly attracting considerable attention in the field of disease and biological systems modeling, including patient-specific tumor models [290,291]. However, DTs have not only been utilized for cancer patients, but also for other biomedical applications and to study animal models [292]. In fact, DTs focus not only on body systems and organs, but also on finer components at cellular, subcellular and molecular levels [289]. Here, we focus on those computational simulations or DTs that study *in vitro* tumor models at the cellular level. Normally, these models can be categorized into continuum and discrete models. In continuum models, partial differential equations are typically used to simulate tumor organoids at the cell population level [293,294]. However, in discrete or agent-based models, each individual cell is simulated by means of an agent specifically taking into account the cell–cell and cell–matrix interactions [295]. In both approaches, the transport of nutrients and drugs through the matrix is typically simulated through continuum reaction-diffusion-convection equations [288], considering the matrix that surrounds the tumor organoids as a porous material [293].

These models can be integrated with experimental data to optimize the design of microfluidic platforms, evaluate the impact of ECM composition, properties, and fluid dynamics, and explore scenarios that are difficult or costly to replicate experimentally [296].Indeed, *in silico* simulations can capture diffusion, convection, shear stresses, and interstitial flow within the TME, providing predictions of drug distribution and cellular exposure under different microfluidic configurations [297].

In addition to physics-based modeling, artificial intelligence (AI) and machine learning approaches are increasingly being used to simulate tumor-on-chip systems [173,298,299]. Machine learning algorithms can analyze high-content imaging of tumor models to quantify cell proliferation, apoptosis or drug response at single-cell resolution, enabling rapid, unbiased readouts [300]. Furthermore, predictive machine learning models can integrate experimental data with molecular or patient-derived information to forecast treatment outcomes, drug resistance or optimal dosing strategies [301]. Emerging digital twin frameworks combine experimental tumor-on-chip platforms with real-time computational models to create virtual replicas of the system.

Therefore, combining and integrating experimental and computational results through AI-driven approaches provides researchers with a powerful predictive tool, reduces the need for extensive animal testing and accelerates the development of effective, patient-tailored cancer therapies. This integrative strategy establishes tumor-on-chip platforms as not only tools for mechanobiological investigation, but also as the basis for data-driven, clinically relevant drug evaluation pipelines.

5.6. Regulatory relevance of matrix-integrated microfluidic systems in drug delivery

Recent regulatory initiatives have begun to formally recognize MPS, including organ-on-chip and hydrogel-based microfluidic tumor models, as credible sources of preclinical evidence to support drug development and regulatory submissions. In April 2025, FDA published the *"Roadmap to Reducing Animal Testing in Preclinical Safety Studies"*. It outlined a strategic approach to reduce, refine, and replace traditional animal

testing using scientifically validated New Approach Methods (NAMs); such as organ-on-chip systems, computational modeling, and advanced *in vitro* assays. [117]. This regulatory shift was catalyzed by the FDA Modernization Act 2.0, enacted in December 2022, which amended the U.S. Federal Food, Drug, and Cosmetic Act by removing the longstanding legal requirement for mandatory animal testing in drug development. The legislation replaced the term "preclinical tests (including tests on animals)" with the broader category of "nonclinical tests". This explicitly allows data generated from *in vitro* systems, *in silico* models and MPS (including organ-on-chip platforms), to support Investigational New Drug (IND) applications and biologics licence submissions. Importantly, while animal studies remain permissible, they are no longer legally mandated, enabling a science-based, fit-for-purpose evaluation of human-relevant NAMs as primary sources of safety and efficacy evidence.

Under this legislative and policy framework, the FDA has clarified that non-animal platforms such as MPS can support regulatory decision-making when they demonstrate sufficient biological relevance, robustness, and predictive value for a defined context of use. In oncology drug delivery, hydrogel-based tumor-on-chip systems are particularly well aligned with this fit-for-purpose paradigm, as they recapitulate key physical and biological limitations governing drug penetration, efficacy, and immune cell infiltration in solid tumors.

To facilitate regulatory uptake, the FDA has established dedicated qualification pathways, including the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program and the Centre for Drug Evaluation and Research (CDER) MPS Program, through which developers can demonstrate reproducibility, biological fidelity, and predictive validity of their platforms. Once qualified, such systems can serve as Drug Development Tools (DDTs), enabling their data to be used across multiple regulatory submissions without revalidation, thereby lowering the threshold for systematic incorporation of organ-on-chip data into drug development pipelines.

A critical enabling factor for the regulatory and industrial acceptance of organ-on-chip and related MPS technologies is the emergence of consensus standards that define terminology, performance attributes, and quality benchmarks. Standardization is essential to ensure reproducibility, comparability, and regulatory interpretability of data generated on diverse microfluidic platforms. American Society for Testing and Materials (ASTM) International has taken a foundational step in this direction with ASTM F3570-22 (Standard Terminology Relating to MPS), which establishes a unified vocabulary for MPS and organ-on-chip technologies. This standard defines key concepts such as single-organ and multi-organ systems, body-on-a-chip, and microphysiological models, providing a common language for developers, end-users, and regulatory reviewers [302].

In parallel, the International Organization for Standardization (ISO), primarily through ISO/TC 276 (Biotechnology), is developing a complementary framework of standards for organ-on-chip and MPS technologies. These include ISO/CD 25,448 (Vocabulary for MPS and Organ-on-Chip), which aims to harmonize definitions across international stakeholders [303]; ISO/AWI 26,086 (Flow Control), which addresses fluid handling, perfusion, and transport phenomena critical for biological relevance and reproducibility; [304] ISO/AWI 25,591 (Digital Twins and Computational Modeling), which supports integration of organ-on-chip data with mechanistic in silico models; [305] and ISO/WD 25,693 (Development Process for Organ-on-Chip Used for the Evaluation of Substances), which proposes process requirements for systems intended for regulatory-relevant compound evaluation. [306] Although some of these standards are still in draft or committee stages, they indicate a clear international trajectory toward formalizing the technical and data-quality foundations of organ-on-chip technologies.

Together, these ASTM and ISO efforts complement FDA qualification pathways by providing the technical infrastructure needed to ensure that organ-on-chip platforms generate data that are reproducible, interpretable, and suitable for regulatory review. For microfluidic drug-delivery studies, this is especially important because quantitative predictions of drug transport, tissue penetration, and immune-cell trafficking depend sensitively on fluidic control, material properties, and biological microenvironment, all aspects addressed by emerging standards.

6. Emerging trends and future perspectives

Matrix-integrated tumor-on-chip models are experiencing a rapid surge as an emerging field with extraordinary potential to transform cancer research and to test DDSs. By recapitulating the biochemical and mechanical cues of the tumor ECM, these microsystems enable the simulation and modeling of the TME, providing cancer cells with their native morphology, functionality, cell–cell and cell-ECM interactions. Although we have to keep in mind that a model is always a simplification of the real tumor, this approach opens opportunities to advance the understanding of different matrix-driven phenomena such as drug resistance, invasion, and nanocarrier transport in a controlled yet physiologically relevant context [307]. Table 3 provides an overview of the main emerging trends in this field.

Multi-organ and body-on-a-chip systems have been extensively explored as advanced *in vitro* platforms to study systemic interactions, organ–organ crosstalk, and pharmacokinetics [190,191]. In the context of tumor-on-chip technologies, these approaches represent an important extension toward more physiologically relevant models. However, despite growing interest, their application specifically to cancer microfluidic platforms for drug delivery and nanomedicine remains relatively limited. As such, multi-organ tumor-on-chip systems continue to offer significant opportunities for future development, particularly for studying off-target effects, drug metabolism, and systemic therapeutic responses.

Recent progress in high-resolution, label-free imaging and spectral confocal microscopy now allows real-time visualization of drug diffusion, micelle stability, and cell–matrix remodeling within microfluidic ECM scaffolds. Parallel advances in single-cell microfluidics, cell sorting, and minimally destructive lysis bring the possibility of monitoring cell heterogeneity and rare subpopulations *in situ* [239]. Omics technologies, such as transcriptomics, proteomics, metabolomics, and lipidomics, are increasingly being applied to high-throughput mapping of how cells respond to specific ECM compositions, stiffness, and drug properties [308]. Automation and parallelization are becoming essential to translate matrix-integrated tumor-on-chip systems into drug screening pipelines. Continuous-flow microfabrication, microfluidic assembly lines and integrated gradient generators allow multiplexed testing of drug dose–response relationships under physiologically relevant gradients [309]. Coupled with AI for image analysis and data integration, these high-throughput platforms promise to accelerate the discovery of matrix-dependent mechanisms of efficacy and toxicity [239,310]. Together, these approaches will provide systems-level insights into cell–biomaterial interactions and enable predictive modeling of therapeutic outcomes.

Another promising direction in matrix-based models for DDSs research is 3D bioprinting. This technique is rapidly transforming the way organ-on-a-chip platforms are conceived, enabling the fabrication of MPS that more closely replicate the spatial and functional complexity of native and pathological tissues. Such bioprinted chips can reproduce healthy or disease-specific conditions *in vitro*, improving the predictability of drug toxicity, efficacy, and pharmacokinetics before animal testing, and in some cases offering a superior alternative to animal studies [239,307,310]. Moreover, bioprinting holds great potential for advancing personalized medicine by enabling the fabrication of patient-specific tumor-on-chip models that capture individual tumor heterogeneity and treatment response. It also allows the controlled creation of biochemical and oxygen gradients within compartmentalized constructs [311]. Looking ahead, "4D" bioprinting aims to introduce dynamic behavior into these constructs by exploiting stimuli-responsive bioinks capable of altering their shape or function in response to cues

Table 3
Emerging trends in matrix-integrated tumor-on-chip platforms for drug delivery studies.

Technology	Key features	Relevance for DDS	Outlook
Multi-organ and body-on-chip [190,191]	• Systems interactions • Organ-organ crosstalk • Perfused microfluidic circuits	• Systemic distribution • Off-target effects • Drug metabolism • Pharmacokinetics	• Underexplored in cancer • High potential for predicting systemic responses
Advanced imaging and single-cell/ omics integration [239,308]	• Label free and spectral confocal imaging • Single-cell microfluidics • Transcriptomics, proteomics, metabolomics	• Real-time visualization • Drug diffusion • Carrier stability • ECM remodeling • Cellular heterogeneity	• Understanding of matrix-cell-drug interactions • Identification of rare or resistant subpopulations
Automation and high-throughput [239,309,310]	• Continuous-flow microfabrication • Parallelized chips • Gradient generators • AI-assisted image and data anlysis	• Multiplexed dose-response • Response to ECM composition and stiffness • Physiologically relevant gradients	• Tumor-on-chip models into drug screening pipelines • Accelerated discovery of efficacy and toxicity mechanisms
3D and 4D bioprinting [307,311–313]	• Bioprinted ECM compartments • Patient-specific constructs • Stimuli-responsive (4D) bioinks	• Prediction of drug efficacy, toxicity and pharmacokinetics • Modeling dynamic drug-matrix interactions	• Personalized medicine • Adaptative tumor models
Biosensors [314–318]	• Electrochemical, immuno- and SERS-based sensors • *In situ* monitoring of glucose, lactate, pH, oxygen	• Real-time monitoring • Metabolic response • Hypoxia • Therapeutic effects	• Enhanced predictive power • Need for ECM-aware sensor • Diffusion and interference limitations

such as pH, temperature, or magnetic fields. This evolution from static 3D to adaptive 4D structures opens the possibility of creating responsive tumor-on-chip systems with ECM-mimicking compartments that evolve over time, thereby providing a more faithful representation of drug–matrix and cell–matrix interactions during therapy [312,313].

Finally, biosensor technology is evolving as a powerful complement to matrix-integrated tumor-on-chip systems, enabling real-time and highly sensitive detection of cancer-related biomarkers and metabolic cues within microfluidic platforms. Electrochemical, immuno-, and Surface-Enhanced Raman Spectroscopy (SERS)-based biosensors can convert specific molecular recognition events into quantifiable signals, supporting early diagnosis, therapeutic target discovery, and dynamic monitoring of drug responses [314]. Stretchable "lab-on-a-patch" platforms that combine microfluidics with sensors already enable picomolar detection of hormones such as cortisol during exercise [315]. Recent advances have integrated these sensors directly into microfluidic chips to monitor key metabolic parameters such as glucose, lactate, pH, and oxygen, thereby capturing hypoxia-related adaptations and tumor metabolism *in situ*. Such integrated systems increase the predictive power of tumor-on-chip models and offer unprecedented opportunities for translational research [316–318]. However, the dense and heterogeneous ECM can hinder analyte diffusion and interfere with sensor performance, making it crucial to design ECM-aware sensing strategies and to calibrate or adapt biosensor technologies to the complex microenvironments of 3D cancer models [319].

7. Conclusions

Microfluidic tumor-on-chip systems are emerging as powerful platforms to bridge the gap between traditional *in vitro* assays and animal models. By integrating physiological architecture, dynamic biophysical cues, and ECM-based materials, they enable more reliable studies of tumor progression, drug transport, and therapeutic response. Recent advances in matrix design, coculture strategies, and flow control have greatly improved their biomimetic performance, positioning matrix-integrated platforms at the forefront of translational cancer research. These systems offer a promising route toward more reproducible and potentially personalized drug testing, supporting the development of patient-adapted treatment strategies. However, their broader adoption still requires standardized fabrication protocols, scalable designs, and validated readouts to ensure reproducibility and regulatory acceptance. Continued efforts in these directions will be essential to establish tumor-on-chip models as routine tools for preclinical testing and precision medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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