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Microphysiological Systems in Cancer Research: Advancing Immunotherapy through Tumor Microenvironment-Integrated Organ-On-Chip Models

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The intricate tumor microenvironment (TME) poses a significant barrier to effective cancer immunotherapy, requiring innovative strategies to model and address its challenges. Traditional models, such as 2D cultures and animal studies, often fail to capture the TME's dynamic, multicellular, and spatially complex nature, limiting their predictive power for therapeutic outcomes. To overcome these limitations, this review examines innovative microphysiological systems (MPS) that enhance the understanding of tumor-immune interactions and pave the way for more effective immunotherapeutic strategies. First the complex features of the TME and its key players are detailed, elaborating on their dynamic interplay with tumors. Importantly, it is highlighted how these components contribute to treatment resistance, offering crucial insights into therapeutic failures. Then, state-of-the-art 3D in vitro organ-on-chip (OoC) models are presented that faithfully recapitulate the TME, incorporating patient-derived tumors to enhance clinical relevance. These advanced systems not only overcome the limitations of traditional animal models and 2D cultures but also provide a robust platform for assessing and improving immunotherapeutic regimens. By bridging the gap between bench and bedside, MPS promises to accelerate the development of novel, more effective immunotherapies for solid tumors, potentially transforming cancer treatment in the near future.

1. Introduction

Recent developments in cancer biology research have reshaped our perspective on tumors, demonstrating that they are not merely aggregates of malignant cells but rather intricate ecosystems whose interactions directly modulate disease progression and treatment outcomes.[1] The tumor microenvironment (TME) of solid tumors constitutes a complex network of cellular and molecular variables, incorporating cancer cells, immune cells, fibroblasts, endothelial cells, and the extracellular matrix, all of which collectively dictate tumor behavior.[2] This milieu may not only promote immunosuppression and metastasis but also enable therapy resistance, highlighting its important role in cancer progression.[3] As the TME plays a crucial role in tumor biology, modern cancer research has shifted to emphasize the need for treatments that target not only cancer cells but also the surrounding environment to achieve better effectiveness.

Conventional in vitro models have consistently struggled to replicate the TME's complexity, especially the spatial and

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adtp.202500098

DOI: 10.1002/adtp.202500098

temporal dynamics of cell-cell interactions that critically guide tumor progression. While standard 2D cell culture setups have provided valuable insights into cancer biology, they do not fully capture the TME's dynamic features, particularly the intricate interplay between neoplastic cells and their immune counterparts. This shortcoming has substantially slowed down our knowledge of immune responses in cancer and has limited the refinement of immunotherapeutic strategies. Although mouse models have provided key avenues to translate 2D in vitro discoveries to the in vivo framework, their ability to fully recapitulate the heterogeneity and complexity of human tumors and their surrounding microenvironment remains limited. As highlighted by Mestas and Hughes, fundamental differences between mouse and human immune systems extend far beyond cell composition. These divergences affect both innate and adaptive immunity, influencing leukocyte subtypes, key immune receptors such as Toll-like receptors (TLRs), Fc receptors (FcRs), and natural killer (NK) cell receptors, as well as immunoglobulin classes and B and T cell signaling pathways. Additional disparities in cytokine responses and co-stimulatory molecules further complicate the extrapolation of preclinical findings from mice to humans.[4] Humanized mouse models, generated by engrafting immunodeficient mice with human hematopoietic stem cells or peripheral blood mononuclear cells, represent an additional step toward improving the modeling of human-specific immune responses in vivo.[5] However, these models continue to fall short of fully reconstructing the human immune landscape, often lacking critical immune subsets such as myeloid and NK cells, and failing to support the full spectrum of human cytokine signaling required for accurate immune-tumor interaction studies.[6]

Recognizing these persistent limitations in both traditional and humanized animal models, attention has increasingly shifted toward advanced in vitro platforms capable of better mimicking the human tumor microenvironment.

Microphysiological systems have emerged as impactful platforms to address these drawbacks, providing precise control over the cellular environment while enabling real-time monitoring of biological phenomena. MPS can reproduce crucial aspects of the TME, including chemical gradients, fluid flow, and, importantly, the spatial arrangement of heterogeneous cell populations in vitro. By incorporating multiple human cell types in controlled spatial configurations, these tools facilitate the examination of tumor-immune crosstalk within a more physiologically relevant framework, delivering insights that were unachievable via earlier approaches.[7,8]

These engineered MPS permit the reconstruction of complex tumor microenvironments for high-throughput drug screening, substantially reducing the use of animal models in preliminary testing while ensuring consistent and reproducible results that align closely with human phisyological responses. Recent studies have shown that tumor OoC platforms can predict patient-specific responses to chemotherapy in colorectal cancer by maintaining tumor heterogeneity and dynamic 3D culture conditions.[9] Remarkably, drug efficacy obtained in these in vitro systems showed strong correlation with patient-derived xenograft (PDX) models, while offering faster, scalable, and cost-effective testing without the need for lengthy animal studies.

This capability not only improves translational relevance but also supports regulatory acceptance. Indeed, since 2022, the FDA Modernization Act 2.0 allows the U.S. Food and Drug Administration to consider data generated from new alternative methods, including MPS, in the preclinical evaluation of new drug candidates, marking a significant step toward reducing animal testing while maintaining scientific and regulatory rigor. In parallel with regulatory advances, scientific progress in MPS technology is opening new opportunities for patient-specific disease modeling.

Through employing human cells, these platforms also provide patient-specific models, bringing customized and human-relevant perspectives on tumor progression and therapeutic outcomes in real-time.[10] Nevertheless, although considerable strides have been achieved, multiple challenges persist, including difficulties related to Human Leukocyte Antigen (HLA) compatibility and a lack of standardization, all of which continue to limit the advancement and broader adoption of fully functional immune-competent MPS. These challenges are discussed in greater detail in Section 6 .

In this review, we illustrate how microfluidic platforms have truly reshaped in vitro cancer research by facilitating more precise modeling of the TME. We outline multiple MPS applied in cancer studies and examine different methods to address existing constraints. We highlight how immunoengineering has become essential in overcoming these barriers by integrating concepts from engineering, immunology, and cancer biology to formulate more advanced therapeutic solutions.

2. TME Landscape and Its Implications for Cancer Therapy

Stephen Paget in 1889, through his "seed and soil" hypothesis, postulated that metastatic colonization depends not only on the properties of cancer cells (seeds) but also on a compatible environment (soil) at distant sites.[11] This dynamic "soil" consists of different cellular components, including fibroblasts, immune cells, blood vessels, and extracellular matrix, all interacting with cancer cells to influence tumor growth and survival.[12] When metastasis occurs, circulating tumor cells specifically colonize organs with compatible microenvironments that support their proliferation.[13] The TME thus has emerged as a fundamental player in cancer pathogenesis, orchestrating various processes that mask tumors from immune detection and influence treatment resistance[14] as summarized in **Table 1**. Recent technological advances, particularly single-cell transcriptomics, have revolutionized our understanding of TME complexity, revealing diverse cell subsets and states.[15] For instance, studies of squamous esophageal cancer have demonstrated predominantly immunosuppressive TME profiles, characterizing these as "cold tumors" with implications for immunotherapy response.[16]

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Table 1. Key mechanism impacting tumor behavior and progression.

Mechanism	Cells Involved	ECM Component Involved	Effect on Tumor	References
Metabolic reprogramming	CAFs	Lactate accumulation	Drives hypoxia and acidity; supports tumor survival and immune evasion	[30]
ECM remodeling	CAFs	MMPs, pro-inflammatory mediators	Facilitates invasion and metastasis; promotes pre-metastatic niche formation	[31]
ECM remodeling	TAMs	TGFB1, TNC, FN1	Enhances tumor migration via integrin signaling	[41]
Mechanotransduction via ECM stiffness	Tumor cells	ECM stiffness	Promotes growth and invasion by altering cellular mechanosensing	[33]
Angiogenesis	TAMs, endothelial cells, pericytes	VEGF, ECM-modifying factors	Supports tumor growth and metastatic spread	[34]
Macrophage-induced immunosuppression	TAMs	Indirect	Secrete immunosuppressive molecules (such as IL-10 and TGFβ).	[36,38,39]
PD-1/PD-L1–mediated immunosuppression	TAMs, MDSCs	Indirect	Disrupts T cell function and promotes immune escape	[48]
IL-10 suppressing IL-12 in DCs	TAMs, dendritic cells	Indirect	Inhibits CD8+ T cell priming; reduces chemotherapy efficacy	[40]
CTLA-4 axis–mediated immunosuppression	TAMs	Indirect	Blocks co-stimulation of T cells; enables immune evasion	[42]
LAG-3–mediated immunosuppression	T cells, tumor cells	Indirect	Suppresses T cell activity through MHC-II interaction	[44]
ROS and NO–mediated immunosuppression	MDSCs	Indirect	Causes oxidative stress; impairs T cell receptor signaling	[45]
Methylglyoxal transfer to T cells	MDSCs	Indirect	Blocks T cell metabolism; impairs effector function	[46]
CD39/CD73 upregulation	MDSCs	Indirect	Generates adenosine; fosters immunosuppressive microenvironment	[47]
Physical barrier (dense ECM)	CAFs	Hyaluronan, collagen, fibronectin	Reduces drug penetration; contributes to therapy resistance	[51–53]
Physical barrier (abnormal vasculature)	Endothelial cells	Indirect	Leads to poor drug distribution and therapeutic resistance	[49,50]
Paracrine signaling bypass	Stromal fibroblasts	Indirect	Activates alternative pathways; enables drug resistance	[54]
Cancer stem cell niche (perivascular)	Pericytes, endothelial cells, CAFs, TAMs, MDSCs, Tregs	Collagen, fibronectin, integrins, IL-6, TGF-β	Preserves CSC survival and resistance to therapies	[55,56]
Cancer stem cell niche (bone marrow)	Mesenchymal cells, endothelial cells, macrophages, osteoblasts, fibroblasts	Collagen, fibronectin, integrins, TGF-β	Protects CSCs; facilitates therapy resistance	[58]

Previous works by other groups, such as those by Prof. Johanna Joyce, have extensively explored and described the complexity and dynamics of the TME, providing valuable insights into its role in cancer progression.[17–19] This deeper understanding marks a shift from traditional cancer treatment approaches, which often relied on "one size fits all" chemotherapy and radiation treatment without considering their impact on the TME.[20] Although not all the effects of conventional treatments on TME are known, studies have shown that chemotherapy and radiation therapies induce significant changes in the TME.[21] These changes may enhance tumor survival and metastatic potential by activating pro-tumorigenic pathways and modifying immune responses.[22] Modern therapeutic strategies increasingly focus on taking advantage of the TME modulation to enhance treatment efficacy, representing a more targeted and case-specific approach to cancer therapy that addresses the entire tumor ecosystem rather than just the malignant cells.[23] For instance, therapies targeting immune checkpoints, such as PD-1/PD-L1 inhibitors, have demonstrated clinical success by reactivating anti-tumor immunity within the TME.[24] Similarly, anti-angiogenic agents such as bevacizumab target the tumor's abnormal vasculature to modulate tumor progression. At low doses, bevacizumab has been shown to promote vascular normalization, improving tissue perfusion and oxygenation, which in turn enhances immune cell infiltration and the overall therapeutic response. This strategy not only reduces vascular support through vessel pruning but also contributes to tumor microenvironment reprogramming by restoring vascular function, ultimately offering a multifaceted approach to limiting tumor progression.[25]

2.1. TME Complexity and Its Role in Cancer Progression and Therapeutic Resistance

Understanding the TME in cancer is challenging due to its dynamic and multifaceted nature. This complexity stems from a cellular heterogeneity (different cell types), a genetic heterogeneity (diverse genetic material within a population), a spatial heterogeneity (variations between different regions within the tumor) and a temporal heterogeneity (changes in the TME as the tumor grows and responds to treatment).[2] As mentioned before, the dynamic network of stromal cells, immune cells, extracellular matrix (ECM) components, and signaling molecules of the TME actively interacts with cancer cells to shape their behavior. Tumor-stromal interactions are mediated through a range of mechanisms, including the secretion of growth factors, biomechanical cues, cytokines, and extracellular vesicles, which can act as either tumor supporters or suppressors.[26] For instance, Lv et al. show how the interplay between cancer cell expressing the cyclic GMP–AMP synthase, cGAS, and endothelial cells expressing the stimulator of interferon genes, STING, facilitates lymphocyte recruitment and migration in liver cancer through endothelial activation. This process enhances tumor vascularization and immune infiltration, underscoring the potential of targeting the TME with strategies such as vitamin C and combinational immunotherapies to improve treatment efficacy.[27] On the opposite side, the TME drives metabolic changes that generate hypoxic and acidic settings, thereby fostering tumor aggressiveness and therapy resistance.[28] Cancer-associated fibroblasts (CAFs) play a crucial role in this metabolic reprogramming.[29] By transitioning from oxidative phosphorylation to glycolysis, CAFs generate lactate that supports tumor cells and exacerbates hypoxia within the TME.[30] Furthermore, CAFs secrete pro-inflammatory mediators and matrix metalloproteinases, facilitating tumor invasion and metastasis while also contributing to the formation of the pre-metastatic niche.[31] Understanding the heterogeneity and spatial distribution of CAFs provides opportunities for targeted cancer therapies. Factors such as hypoxia and metabolic reprogramming also affect the TME, fostering immune evasion and allowing cancer cells to evade immune surveillance by inhibiting cytotoxic T-cells and recruiting regulatory T-cells.[32] The ECM stiffness, typically higher in tumor tissues, influences tumor cell growth, motility, and invasion. Tumor cells respond to changes in the ECM's mechanical properties and actively modify them, influencing downstream signaling through mechanotransduction pathways.[33] These biomechanical interactions trigger complex cellular responses involving mechanoreceptors, cytoskeletal rearrangements, and chromatin remodeling, representing a critical aspect of cancer progression. Additionally, the TME promotes angiogenesis, particularly through tumor-associated macrophages (TAMs), leading to the formation of new blood vessels that enhance nutrient and oxygen delivery, while also providing potential routes for metastatic spread.[34] Understanding these mechanisms and the subsets of cells involved is crucial for developing effective anti-cancer therapies. Although the heterogeneity and dynamics of the TME complicate research and result interpretation, a multidisciplinary approach that combines molecular biology, computational modeling, and bioengineering is gradually untangling these complexities and driving advancements in cancer treatments.

2.2. Immune Suppression in the Tumor Microenvironment

The TME exerts its immunosuppressive effects through various mechanisms, including the recruitment of regulatory immune cells and the release of inhibitory factors that undermine anti-tumor defences. DeNardo et al. demonstrated how macrophages, ordinarily involved in tumor suppression, are reprogrammed by tumor-derived signals to become TAMs.[35] These cells acquire immunosuppressive phenotypes that aid tumor immune evasion.[36] TAMs suppress T-cell–mediated immunity through direct and indirect means, such as expressing checkpoint proteins (e.g., PD-L1),[37] secreting immunosuppressive molecules (such as IL-10 and TGFβ),[38] and recruiting regulatory T-cells via CCL22, as noted in gastric cancer[39] (**Figure 1A**). As shown by Ruffell et al., inhibiting TAMs-associated IL-10 signaling boosts chemotherapy effectiveness by reinstating IL-12 production in dendritic cells (Figure 1A), thereby promoting CD8+ T-cell responses.[40] Further, TAMs actively remodel the tumor environment,[35] as observed in ovarian cancer, where they release Transforming Growth Factor Beta-Induced protein (TGFBI), Tenascin-C (TNC), and Fibronectin 1 (FN1) (Figure 1B). These proteins facilitate tumor cell migration via integrin interactions and intracellular signaling pathways.[41] In addition to PD-L1 expression, TAMs are also involved into the CTLA-4 axis that hinders T-cell responses by binding to CD80 and CD86 with higher affinity compared to CD28, essentially denying T-

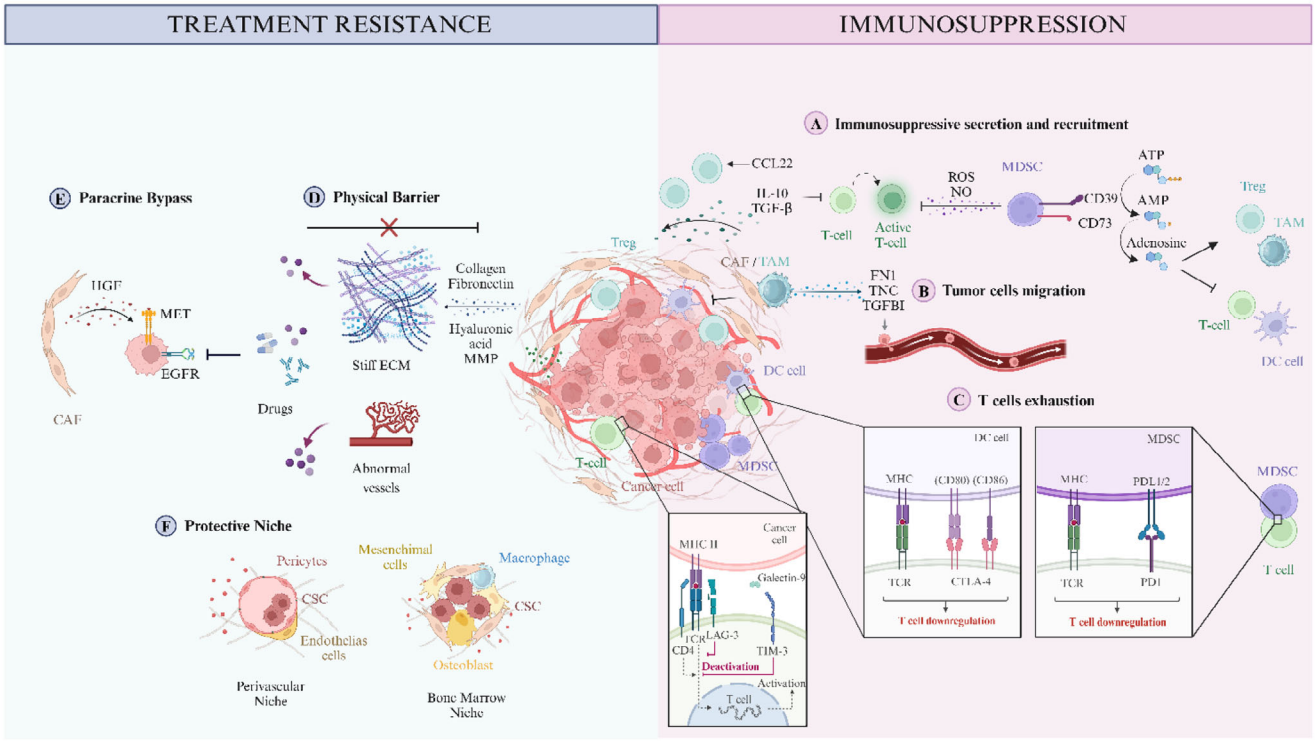

Figure 1. The tumor microenvironment (TME) plays a central role in promoting immunosuppression and cancer treatment resistance. Key cellular components of the TME include cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and dendritic cells (DCs). These cells are reprogrammed by tumor-derived signals to promote immune evasion, therapy resistance, and metastatic progression. A) TAMs and MDSCs contribute to immunosuppression by releasing cytokines such as IL-10 and TGF-β,[38] chemokines like CCL22 that recruit Tregs,[39] and adenosine produced via CD39/CD73,[47] which impairs T cell and DC function. B) TAMs remodel the ECM and facilitate tumor cell migration by secreting TGFBI, Tenascin-C, and Fibronectin-1.[41] C) Immune checkpoint pathways, including PD-L1/PD-1, CTLA-4/CD80/CD86, LAG-3/MHC-II, and TIM-3/galectin-9, suppress T cell activation and induce exhaustion.[42–44] D) CAFs remodel the ECM through the secretion of collagen, fibronectin, hyaluronan, and MMPs, leading to increased stiffness and desmoplasia.[51–53] In parallel, abnormal and leaky tumor vasculature limits the effective delivery of drugs and immune cells.[49,50] E) Paracrine signaling in the TME, such as HGF-MET activation by stromal fibroblasts, enables cancer cells to bypass inhibited pathways and resist targeted therapies.[54] F) Cancer stem cell (CSC) niches—including the perivascular and bone marrow niches—offer survival advantages and therapy resistance via ECM-integrin signaling.[55,56,58] Created with BioRender.com.

cells the essential co-stimulatory signals (Figure 1C). This phenomenon has been documented in melanoma[42] and ovarian carcinoma.[43]

Other negative regulators, including the Lymphocyte Activation Gene-3, LAG-3 and the T-cell Immunoglobulin and Mucin-domain containing-3, TIM-3, are primarily expressed on T cells and intensify T-cell exhaustion. LAG-3 binds to MHC class II, expressed on the surface of antigen-presenting cells (APCs), with a binding affinity up to 100 times stronger than CD4, curtailing T-cell proliferation and cytokine generation. LAG-3 is co-expressed with other checkpoints in early-stage breast cancer (Figure 1C). TIM-3, conversely, engages ligands such as galectin-9, driving T-cell exhaustion and apoptosis: a mechanism observed in ovarian and breast cancers, often correlating with unfavorable prognoses.[44]

Myeloid-derived suppressor cells (MDSCs) constitute another critical component of immune suppression. They promote the expansion of other regulatory immune cell types, obstruct T-cell migration to lymph nodes and tumors, and produce reactive oxygen and nitrogen species that harm immune function.[45] MDSCs impair T-cell function by transferring the glycolytic by-product methylglyoxal into T cells, disrupting their metabolic activity and suppressing cytokine production,[46] while expressing enzymes like CD39 and CD73, thereby converting ATP into adenosine and facilitating a tumor-centric immunosuppressive niche[47] (Figure 1A). Their expression of checkpoint molecules, such as PD-L1, further dampens T-cell activity and supports immune evasion.[48] Recognizing the TME's contribution to therapeutic resistance has inspired combination treatments that target both cancer cells and the supporting microenvironment, offering the potential to enhance clinical outcomes.

2.3. TME-Mediated Drug Resistance: Physical Barriers and Chemical Interplay

The TME affects immune responses and also plays a crucial role in drug resistance and treatment outcomes through various mechanisms, both physical and chemical. Physically, abnormal tumor blood vessels are a major barrier to effective drug delivery[49] (Figure 1D). Tumor vessels differ significantly from normal ones, with irregular shapes, excessive branching, and walls that are weak and leaky. This irregular structure causes uneven blood flow and creates areas where drugs cannot effectively

reach with poorly oxygenated regions often becoming resistant to treatment.[50] Beyond the issues with abnormal blood vessels, the tissue surrounding tumors becomes unusually dense, which creates another barrier to treatment. In squamous esophageal cancer, CAFs actively remodel the extracellular matrix by producing collagen, fibronectin, and Matrix Metalloproteinases (MMPs) (Figure 1D). This dense ECM, referred to as desmoplasia, plays a significant role in cancer treatment resistance. A modified ECM creates a protective barrier, reducing radiation effectiveness and promoting cancer cell survival.[51] Furthermore, excessive hyaluronan accumulation within the tumor microenvironment can impair drug delivery and efficacy, as observed in pancreatic adenocarcinoma.[52] Targeting CAFs and their ECM-remodeling activities presents a promising strategy for overcoming radiotherapy resistance in these cancer types. In this context, suppressing hyaluronan synthesis with 4-methylumbelliferone has been shown to enhance the efficacy of gemcitabine treatment in human pancreatic cancer cells.[53] On a chemical level, the TME promotes drug resistance through mechanisms like the paracrine bypass. In this process, cells within the TME release factors that activate alternative signaling pathways in tumor cells (Figure 1E). This mechanism allows cancer cells to circumvent the blocked pathway, enabling continued growth and survival. For example, in triple-negative breast cancer (TNBC), despite the epidermal growth factor receptor (EGFR) overexpression, EGFR inhibitors are ineffective due to MET (also known as hepatocyte growth factor receptor, HGFR) activation. This resistance mechanism involves stromal fibroblasts secreting hepatocyte growth factor (HGF), which activates MET, bypassing EGFR inhibition.[54] The TME also contributes to therapy resistance by supporting cancer stem cells (CSCs). Certain types of CSCs, which are central to tumor regeneration, are protected within specific niches such as the perivascular niche (PVN) and the bone marrow niche[55] (Figure 1F). The PVN, composed of endothelial cells and pericytes, promotes treatment resistance through several mechanisms. These include ECM adhesion, that supports integrin signaling, and the release of cytokines that help cancer cells survive.[56] Bone marrow niches, on the other hand, consist of various stromal cell types, including mesenchymal cells, endothelial cells, macrophages, osteoblasts, and fibroblasts. These niches rely on cell-to-cell interactions that significantly impact treatment response.[57] One notable mechanism involves IL-6 signaling, where IL-6 secreted by stromal cells activates the STAT3 pathway in tumor cells. This activation inhibits apoptosis and promotes survival, even in the presence of chemotherapy.[58]

3. MPS as a Tool for Recapitulating Tumor-Immune Interactions in the Microenvironment

The mutual influence between cancer cells, and TME demonstrates the remarkable complexity of tumor biology. This complexity poses significant challenges for cancer research, as traditional 2D culture methods cannot replicate the 3D architecture or the complex cell-matrix relationships present in vivo. Although animal models remain useful, their ability to predict human-specific responses is limited by interspecies variations. This limitation has driven the development of more sophisticated experimental platforms that can better model TME complexity. MPS technology has emerged, employing microfluidics, microfabrication, and tissue engineering to recreate highly controlled, tissue-like microenvironments. By emulating the fine-scale dynamics of human tissues and organs, MPS provides a robust and adaptable platform that surpasses the capabilities of conventional 2D cell cultures and animal studies. For example, MPS technology has successfully enabled the in vitro reproduction of complex structures like the blood-brain barrier, allowing for more accurate modeling of its selective permeability and interactions with therapeutic agents.[59] The overarching goal is to establish reproducible, cost-effective, and precise ex vivo models to investigate core biological processes and provide feedback to optimize therapeutic solutions.[60] MPS technology leverages microfluidics to achieve efficient analysis with reduced reagent consumption, high resolution, and rapid results. It allows the precise control of fluid flow at the microscale, enabling the creation of gradients and the delivery of nutrients and drugs in a manner that closely mimics the in vivo microenvironment. Studies have used microfluidic platforms to investigate the migration of splenocytes toward cancer cells through a network of microchannels. These studies first showed that immune-deficient interferon regulatory factor 8 (IRF-8) knockout mice develop more tumors with less immune cell infiltration than wild-type animals[61] and subsequently used a microfluidic assay to investigate the underlying mechanism in greater detail. Specifically, they showed that immune spleen cells lacking IRF-8 do not migrate toward cancer cells nor interact with them as efficiently as wild-type immune cells,[62–64] suggesting a mechanism to explain why IRF-8 knockout cells fail to exert proper immune surveillance, leading to a heavier metastatic burden. In recent years, numerous MPS or OoC platforms have been developed, reproducing fundamental functions of organs on a micro-scale.[65] As an example, a microfluidic gut-liver on a chip was designed to co-culture gut and hepatic cells to reproduce the first-pass metabolism.[66] Specifically, to study systemic repeated doses of apigenin as a model compound[67] and the functionality of the gut epithelial barrier monitored over time.[68] Another example is the development of lymphoid organs on a chip to give major insights into the function and structures that can help in understanding better their role in inflammation, cancer and infectious diseases. To give an example, in a study by Brian J Kwee et al., they develop an on-chip human lymph node stromal network to evaluate the dendritic cell (DC) and T-cell trafficking into the lymph node paracortex, also known as the T-cell zone.[69]

As we previously discussed, the TME has a fundamental role in the understanding of cancer biology and in the development of new therapeutic strategies. Moreover, the TME has a key role in onco-immunology research as it displays heterogeneity and complex microenvironmental signaling that occurs through cancer and immune cells. Immunotherapy has emerged as a promising approach to cancer treatment, but its success depends on an understanding of immune cell interactions within the tumor microenvironment. Immunocompetent Cancer-on-Chip (iCoC) models facilitate this by providing a platform to study tumor-immune cell interactions in a controlled and reproducible manner.[70] MPS and iCoC models are particularly valuable in evaluating various immunotherapy modalities.[71] By incorporating patient-derived cells and co-culturing them with immune cells, these models can assess the efficacy of immunotherapies

on a specific cancer patient prior to treatment. This personalized approach is crucial for developing tailored therapies that are more likely to succeed in clinical settings.[71,72] Additionally, these models can simulate the tumor microenvironment to study immune checkpoint blockade therapy, providing a platform to test new treatments and understand their mechanisms of action.[73]

Another important aspect of tumors is their ability to metastasize. By the end of metastatic dissemination, circulating tumor cells (CTCs) need to extravasate from blood vessels at metastatic sites to form new colonies. Although cancer cell extravasation is crucial in cancer metastasis, it has not been successfully targeted by current anti-metastasis strategies due to a lack of thorough understanding of the molecular mechanisms that regulate this process. Initially, cancer cells can be effectively monitored and recognized by the immune system. However, due to the immune-editing effects of cancer, they will eventually enter an immune escape state where the tumor uses the immune system to achieve faster metastasis. Tumor immune escape is also one of the bottlenecks in improving the efficacy of current tumor therapies. Traditional in vitro techniques fail to reproduce and study these events, while OoC and MPS, with their high-precision control of the microenvironment, offer better insights into possible strategies to understand these mechanisms.[74–76] For example, Bersini et al.[77] investigated the extravasation potential of cancer cells with a multi-approach of transcriptomics and microfluidics. They used two approaches for the study: first, combining a Transwell assay and Affymetrix microarray analysis to study the impact of CTC gene expression on metastatic progression and vascular barrier reorganization. Second, to further investigate cancer cell extravasation beyond the interplay between cancer cells and endothelium, they studied cancer cell transmigration across the endothelium in the presence of a secondary tissue. For this purpose, they chose a microfluidic assay to mimic a bone-like environment and observe the organ-specific metastatic potential of three different cancer cell lines in a more physiological setting. Moreover, they highlighted the importance of both CTC-endothelium and CTC-secondary tissue interactions in cancer cell extravasation with a microfluidic system, elucidating some aspects of the complex cellular interactions involved in cancer cell extravasation by examining two different aspects of heterotypic intercellular communication.

In summary, MPS and iCoC models offer significant advantages over traditional 2D cultures and animal models. Their ability to provide human-relevant data, controlled microenvironments, and high-content analysis makes them indispensable tools for studying complex cell interactions, evaluating immunotherapies, and reducing drug development time. These models hold great promise for advancing personalized medicine and improving outcomes for cancer patients.

4. Advantages of Organ-On-A-Chip Technology Over Traditional In Vitro and Animal Models

4.1. Regulation of the Cellular Environment and Real-Time Monitoring of Interactions Between Cells

MPS and iCoC models offer notable advantages over traditional 2D cultures and animal studies, with the principal benefit being the generation of more human-relevant data. Conventional 2D cell culture cannot adequately replicate the TME and its inherent processes—such as inflammation, immune responses, cytokine activity, and tumor-stromal cell interactions.[78] By contrast, the 3D configuration of OoC models allows for a more lifelike simulation of tissue organization and complexity, while the embedded engineering design of MPS and OoC systems enables precise control over the cellular milieu.[79] This level of regulation is essential for manipulating parameters like fluid flow, nutrient gradients, and mechanical forces, which collectively contribute to physiological relevance. For example, continuous perfusion during spheroid development can speed up spheroid formation and promote greater uniformity in size.[80] In one study, Shim et al. devised a multi-compartment microfluidic chip that co-cultured two tissue sections under constant circulating flow to recreate tumor-induced immunosuppression, effectively mirroring complex tumor–lymph node communication in vitro.[81] Mouse lymph node tissue was co-cultured alongside tumor or healthy tissue, exchanging soluble mediators through a circulated medium. Such precise microenvironmental control is simply not achievable in standard 2D cultures, which typically lack dynamic flow and exhibit inconsistent cell growth and behavior.[71] OoC systems also integrate diverse human cell types with artificially engineered yet biologically relevant microenvironments, enabling investigations into intricate cellular behaviors, such as chemotaxis.[78] Adjusting variables, such as concentration gradients, ECM composition, and cell-cell contact, using microfluidics provides a robust approach to explore tumor heterogeneity and corresponding therapeutic responses.[80]

For example, a study by Hong et al. addressed glioblastoma (GBM) –microglia interplay, specifically examining how microRNA influences this interplay and its implications for novel cancer treatments.[82] The authors utilized an MPS platform to co-culture patient-derived GBM cells and microglia in a 3D, three-compartment chip. They assessed how extracellular vesicles (EVs) carrying miR-124 modulated tumor progression via STAT3 regulation. The findings indicated that miR-124 disrupts M2 microglial polarization, suppresses GBM proliferation, and reduces metastasis. Quantifying cytokine levels in the TME model further confirmed that miR-124-loaded EVs can constrain tumor growth and migration, thereby promoting a tumor-suppressive, immune-supportive environment. In line with these findings, our group used an MPS to demonstrate that both the blood-brain barrier (BBB) and cancer cell spatial organization contribute to Temozolomide (TMZ) resistance in glioblastoma. We showed that TMZ sensitivity decreases with increasing cancer cell spatial organization, and that the presence of a dysfunctional BBB further reduces treatment efficacy.[83] Overall, the refined microenvironments made possible by MPS and iCoC systems represent an important advancement in designing more realistic and human-appropriate cancer models. By faithfully recapitulating the biophysical and cellular complexity of the human tumor micro-environment, microphysiological platforms permit mechanistic investigations of tumor biology, pharmacodynamics, and cell–cell crosstalk that lie beyond the scope of conventional models. This enhanced physiological relevance improves the predictive accuracy of pre-clinical testing, accelerates the optimization of therapeutic regimens, and ultimately facilitates the translation of laboratory findings into personalized, patient-specific treatment. **Table 2** presents an overview of these advantage over tra-

Table 2. Key Advantages and Applications of MPS/iCoC Models in Cancer Research.

Feature/Aspect	Traditional 2D Culture	Animal Models	MPS/iCoC Models (3D, OoC)	Examples and References
Physiological Relevance	Low	Moderate	High	Tumor microenvironment (TME) simulation, inflammation, immune response[81]
Control of Microenvironment	Limited	Limited	Precise (fluid flow, gradients)	Perfusion for spheroid uniformity, microfluidic chips for immunosuppression studies[80,81]
Integration of Multiple Cell Types	Poor	Good	Excellent	Co-culture of tumor, stromal, immune, vascular cells[82,85,87]
Real-Time Monitoring	Limited	Limited	Advanced (imaging, sensors)	Confocal microscopy, flow cytometry, spatial transcriptomics[91]
Vascularization/Angiogenesis	Absent	Present	Engineered, controllable	Perfusable vascular networks,[85] angiogenesis studies
Immune System Modeling	Poor	Good	Tunable, human-specific	TCR-engineered T cell studies, immune migration assays[95,97]

ditional in vitro and animal models, for a comprehensive guide for the ongoing development and eventual clinical application of MPS platforms in cancer immunotherapy.

4.2. Mimicking Human Physiological Conditions

One of the key advantages of the MPS and CoC is the possibility of integrating multiple cell types, including immune, cancer, stromal cells, and vascular cells, in a controlled microenvironment. This integration is essential for studying the dynamic phenomena that occur in the TME. The stromal cells provide structural support and release extracellular matrix components that are critical to the architecture of the tissue and its functions.[80,84] The ECM provides support and biochemical cues that are crucial for cell behavior, differentiation and tissue organization. Usually, the ECM is reproduced via hydrogel in the 3D models that encapsulate the cells and give scaffolds that mimic the natural tissue.[80] Vascular cells, particularly endothelial cells, are essential for forming blood vessels within these systems. By facilitating angiogenesis, the process of forming new blood vessels from existing ones, these cells supply oxygen and nutrients to tumor cells, thereby mimicking tumor physiology. Nashimoto et al. constructed a vascular network on a tumor-on-a-chip platform by culturing tumor spheroid where human umbilical vein endothelial cells (HUVEC), human lung fibroblast (hLF), and human breast cancer cell (MCF-7) were grown.[85] Here, the fibroblasts triggered angiogenesis and formed a perfusable vascular network in the spheroid. Another example of the importance of MPS models completed with vasculature is the study of Campisi et al., who developed a novel microfluidic model to study KRAS-LKB1 (KL) tumor-vascular interactions.[86] They previously demonstrated that KL mutant lung cancer, which is PD1 blockade resistant, exhibits a silencing of the STING pathway, impairing tumor cell production of immune chemoattractants and T-cell exclusion. Notably, dsDNA priming of LKB1-reconstituted tumor cells activates the microvasculature, even when STING is deleted in tumor cells. cGAS-driven extracellular export of 2′3′ cGAMP by cancer cells activates STING signaling in endothelial cells and cooperates with type 1 interferon to increase vascular permeability and expression of E selectin, VCAM-1, ICAM-1, and T-cell adhesion to the endothelium. Thus, the cGAS-STING signaling in tumor cell not only produces T-cell chemoattractants, but also prepare tumor vasculature for immune cell escape. In another study, Scott AL et al., studied how the relationship between cancer and macrophages interaction mechanisms establish this pro-tumorigenic microenvironment and how it is critical for developing of new strategies. A 3D microfluidic assays and patient-derived xenografts were utilized to define the role of cancer-derived colony stimulating factor 1 (CSF1) on macrophage infiltration dynamics toward ovarian cancer cells. Their results demonstrated the abilities of microfluidics to study the dynamic interactions of cancer cells with macrophages in a 3D microenvironment and provide critical insights on targeting CSF1 to control macrophage trafficking.[87] Overall, a fully vascularized model with the multiple integration of different cell types and ECM can fill the gap that 2D in vitro and animal models have on predictive clinical outcomes and provide an important improvement.[88]

4.3. Combination of Multiple Analysis and Readout Flexibility

MPS allows researchers to integrate a wide range of analytical techniques to gain a more complete understanding of tissue and organ function. This includes real-time imaging to monitor cellular processes, high-resolution imaging to quantify cellular morphology and protein expression, multiplex assays to measure secreted factors, and the increasingly powerful approach of spatial transcriptomics to map gene expression within the microenvironment itself. Indeed, as also shown by other groups, the combination of many analytical techniques compatible with engineered iCoC enables the analysis of processes such as immune cell recruitment, migration, and infiltration, which are essential for understanding the TME related to immunosuppression, accurately mimicking the physiological and biological patterns that occur in vivo.[89,90] Our recent study, described in Vasudevan et al.,[91] effectively demonstrates this multi-faceted analytical capability by integrating confocal microscopy, flow cytometry, Luminex-based multiplex assays, and spatial transcriptomics to provide a detailed characterization of how the tumor vasculature modulates both drug efficacy and immune cell responses in a 3D vascularized liver cancer model. This integrated approach highlights the power of MPS/OoC platforms to bridge the gap between in vitro

experiments and in vivo observations, offering significantly improved preclinical models with enhanced translational potential.

4.4. Impact on Preclinical Studies and Therapeutic Development

The precise control of the microenvironment, the real-time cell-cell interactions, the mimicry of human physiology, and the variety of available analyses are all advantages of MPS and OoC that influence the effectiveness of the preclinical model and the discovery of new therapeutic strategies. These advantages have a key role in enhancing drug development processes.[92] Traditional animal models often fail to accurately replicate human physiological responses, leading to lengthy and costly validation processes. In contrast, MPS and iCoC models can simulate human tissue responses more precisely, thereby reducing the need for extensive animal testing and expediting the transition to clinical trials.[89,90] In a study by Trevor Sasserath et al., they demonstrated a multiorgan human model on a chip, integrated with an immune component represented by monocytes capable of responding to either indirect activation from cytokines produced by damaged tissue or broad activation that directly responds to signaling molecules.[93] In another study conducted by Al-Samadiet al. they developed a humanized in vitro microfluidic chip assay to test immunotherapeutic drugs against HNSCC patient samples. This assay could be used to predict the efficacy of immunotherapeutic drugs for individual patients. They manufactured an in vitro 3D microfluidic chip to test the efficacy of, PD-L1 antibody and IDO 1 inhibitor. The assay was first tested using a tongue cancer cell line (HSC-3) embedded in a human tumor-derived matrix "Myogel/fibrin" and immune cells from three healthy donors. Next, the chips were used with freshly isolated cancer cells, patients' serum and immune cells analyzing the migration of immune cells toward cancer cells and the cancer cell proliferation rate. As a result, Immune migration toward HSC-3 cells was dependent on the cancer cells density. IDO 1 inhibitor induced immune cells to migrate toward cancer cells, both in HSC-3 and in two HSNSCC patient samples. The efficacy of PDL-1 antibody and IDO-1 inhibitor was patient dependent, showing how this assay and the MPS can be used into predicting the efficacy of immunotherapeutic drugs for individual patients.[94] The ability of this human-organ-on-a-chip model to replicate a subset of the diverse and dynamic interactions between immune cells and their organ system counterparts, as well as their responses to novel drug compounds, enhances the sophistication of preclinical models and ultimately advances the development of personalized medicine strategies. In several studies conducted by our group, we have utilized a MPS for the preclinical evaluation of TCR-engineered T cells against solid tumors.[95,96] In one study, we incorporated human Hepatitis B Virus (HBV)-associated hepatocellular carcinoma (HBV-HCC) cells as either single cells or tumor cell aggregates within a 3D collagen gel region of a microfluidic device. Human T cells engineered to express HBV-specific tumor-specific T cell receptors (TCR–T cells) were then added in adjacent areas channels. This study showed the impact of oxygen levels and the inflammation environment on the engineered T-cell function and highlighted the ability of these models to ameliorate the lack of flexibility in controlling individual parameters in anatomical locations compared to animal models, potentially reducing the time for the development of new therapies.[73] Building up on the cellular complexity, in Lee SWL et al.[97] we presented an MPS to study how monocytes affect the killing efficacy of HBV-specific TCR-T cells produced by various methods, identifying the key role of monocytes in inhibiting T cell effector functions via PD-L1/PD-1 expression. This 3D microfluidic in vitro immunogenic TME closely resembled the physiological in vivo environment with HBV-HCC cells organized into aggregates within a 3D matrix, allowing to compare different TME-based features or cellular players in cancer therapies.

5. Applications of Microphysiological Systems in Tumor Immunotherapy

5.1. Role and Relevance of Immune Competent CoC

Immune cells play a dual role in cancer, acting as both tumor promoters and tumor suppressors. The dynamic process of recruiting and programming immune cells in cancer depends on intercellular communication.[98] To fully understand the complex and conflicting roles of immune cell populations within the tumor microenvironment, it is essential to identify the communication network between cancer cells and immune cells.[99]

The tumor-immune microenvironment (TIME) refers to the different subpopulations of the immune system and their interactions within a tumor microenvironmental niche. These interactions uniquely influence tumor initiation, development, and response to therapies.[100] The predominant immune populations include TAMs, natural killer (NK) cells, DCs, and T and B lymphocytes. These cells have a close relationship with endothelial cells and the network of collagen and fibers that make up the ECM. The unique signatures of its cellular components, the associated signaling, and the diversity of the TIME have been targeted in cancer therapy to develop new strategies, especially for immunotherapy.[101] In this field, using MPS and OoC to mimic and study the correlation between cancer cells and immune cells within the TIME has enhanced our understanding of potential new therapeutic strategies. For example, investigating the role of NK cells in a CoC model can provide major insights into their effective anti-cancer activity and their role in immunotherapy. In a study by Nguyen et al.,[90] a CoC was used to analyze NK cell phenotype and function in the context of colorectal adenocarcinoma. They included functional cardiac tissue to investigate tolerability and demonstrated selective NK cell-mediated tumor cell killing with varying responses. This was achieved with no structural defects but a decreased beating frequency of the cardiac tissue. Such a model exemplifies how tolerability can be evaluated in microfluidic tumor models alongside efficacy. Another study on NK cells was conducted by Ayuso et al. where they develop a microfluidic tumor on chip model to evaluate the role of tumor environmental stress on the NK cell exhaustion. Their tumor-on-a-chip model includes a microchamber where breast cancer cells were embedded in a 3D matrix and a lumen located at one end of the microchamber lined with endothelial cells and perfused with medium to nourish the cells, mimicking the vasculature present in the tumor and allowed to mimic nutrient, pH, proliferation, and necrosis gradients. Their work demonstrated that NK cells initially exhibited promising cytotoxic capacity, destroying a significant percentage of tumor cells. The device culture decreased

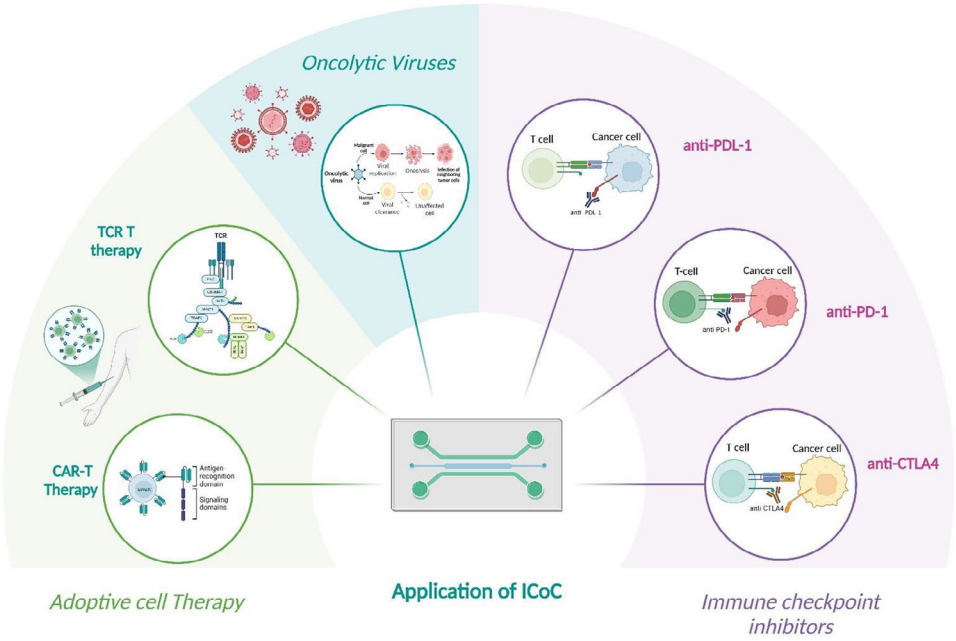

Figure 2. Graphical representation of the recent and most relevant application of Cancer on chip models in immunotherapy. The using of MPS and OoC have bring advantage in the study of new possibly therapeutic strategies focusing on the intimate cancer -immune cell interaction. For this reason, the application of these technologies focuses in improving immunotherapies such as adoptive cell therapy (such as CAR-T and TCR-T therapies), application of oncolytic viruses and immune-checkpoint inhibitors (anti-PD-1, anti-PDL-1and anti-CTLA4). Created with BioRender.com.

their cytotoxic capacity, leading to NK cell exhaustion. Further, they explored the plasticity of NK cells and their capacity of reversing immune exhaustion showing that NK cells did not revert from the exhausted phenotype, expressing multiple molecular and functional alterations despite removing the environmental stress generated by the tumor.[102] In another study, Es et al. used a commercially available three-lane microfluidic chip to investigate the effects of pirfenidone (PFD), an antifibrotic agent with anti-inflammatory and antioxidant properties, on CAFs in a breast cancer model.[103] They found that PFD treatment reduced CAF and cancer cell migration and invasiveness by decreasing the production of immunosuppressive cytokines. Lastly in a study conducted by Park D et al. it was shown the aspiration of a microdluidic device to study the effect of cytotoxic chemotherapy on the dendritic cells and how it induce their recruitment and trogocytosis of cancer cells. In their devices, the cancer cells were encapsulated in a collagen gel blocks and treated with Oxiplatin (OXP) at various concentrations. DCs were attached on the side of the collagen gel blocks, and migration of DCs within the 3D gels was quantitatively analyzed Active infiltration of DCs was predominantly observed when OXP was administrated, indicating OXP-treated cancer cells release factors promoting DC motility. High-resolution video microscopy revealed that DCs employ trogocytosis to disassemble dying/dead cancer cells and acquire antigens, as opposed to phagocytosing the entire cancer cells.[104] These studies highlight how MPS and iCoC technologies provide sophisticated and precise tools to study immune-tumor cell interactions and better understand the mechanisms of tumor immunity, opening possibilities for new therapeutic strategies.

Within the mechanisms of tumor biology, the cancer-immunity cycle plays a relevant role, involving many different tissues and organs.[71] The immune response is often initiated at a tumor site by antigen-presenting cells that capture antigens released by cancer cells. However, the activation of other immune cells (e.g., T and B cells) in the lymph nodes and trafficking through blood vessels is needed to sustain the anti-tumor response. In fact, the activated T cells must travel to the tumor site, where they kill tumor cells, releasing additional tumor antigens and reinitiating the cycle. The diversity of tissues and 3D structures (e.g., vessels, lymph nodes) presents considerable obstacles to investigating these steps in the tumor immunity cycle using traditional in vitro systems. For this reason, an important application of iCoC is integrating lymph nodes into the model. This allows researchers to analyze the intimate relationship between tumor immunity and metastasis formation to develop alternative therapeutic strategies.[81,105] In a recent study, Sergei et al. designed a lymph node-on-a-chip (LNOC) as a tissue engineering model of secondary lymph node tumors formed due to metastasis. The chip employs a collagen sponge to mimic the morphology and porosity of the ECM of human lymph nodes, with a 3D spheroid of cancer cells inside, simulating secondary tumors in lymphatic tissue. To demonstrate the applicability of the LNOC for pharmacological and diagnostic applications, they used it to evaluate the effect of polymer capsules at submicron and micron sizes on their internalization into metastasizing cells.[106]

Overall, MPS and OoC technologies provide a sophisticated and precise means to study immune cell-tumor cell interactions. Here we provide a description of the latest applications of these technologies for the study of new immunotherapies (**Figure 2**) including a summary table with the most relevant application of MPS and iCoC in immunotherapy (**Table 3**).

Table 3. List of MPS/iCoC Models in immunotherapy applications.

Study/MPS Platform	Cancer Type(s)	Immune Components	Special Features	Key Findings	Limitation/Remarks
Shim et al[81]*	Various solid tumor tissues + LN co-culture	Mouse lymph node tissue, tumor tissue	Multi-compartment microfluidic chip- Circulating medium for soluble mediator exchange	Demonstrated tumor-induced immunosuppression mirrored in LN tissue- Showed complex cross-talk in vitro	Mouse LN tissue vs human tumor tissue may have species mismatch- short culture timeframe
Nguyen et al.[90]*	Colorectal adeno-carcinoma + cardiac tissue	NK cells	3D microfluidic co-culture (tumor + functional cardiac tissue)- Real-time readouts on both tumor & heart	Observed selective NK cell-mediated tumor killing- Evaluated cardiotoxicity (beating frequency changes)	Specific to NK cell cytotoxicity- May require further vascularization modules
Preece et al.[115]*	Hepatocellular carcinoma (HBV-related)	TCR-engineered T cells (CRISPR-based)	Collagen-based 3D regions for tumor cells- Adjacent channels for T-cell seeding and migration	TCR-engineered T cells showed improved recognition & killing of HBV+ tumor cells- Potential personalized therapy testing	Focused on TCR engineering; not generalizable to all solid tumors- Requires precise HLA or antigen matching
Chen et al.[111]*	Leukemia (B-ALL)	CAR-T cells	Radial perfusable chip mimicking bone marrow niche- Glass bottom for microscopy	Demonstrated eradication of B-ALL by anti-CD19 CAR-T- Platform to compare different CAR-T products	Requires careful control of flow rates- Bone marrow niche complexity is simplified
Jiang et al.[116]*	Breast cancer (MDA-MB-231 spheroids)	T cells + anti-PD-1 mAb	High-throughput observation chamber (iHOC) with 288 wells- Tracks spheroid viability, infiltration	Anti-PD-1 reversed T cell inhibition in size-dependent manner- Enabled parallel measurement of IL-2 (activation marker)	2D well format with 3D spheroids (not fully microfluidic)- Short-term readouts mainly
Meccantini et al.[124]*	Lung carcinoma	T cells + oncolytic vaccinia virus	PDMS-based microfluidic – Live imaging of viral infection & immune cell recruitment	Enhanced T-cell recruitment, prolonged contact with cancer cells in presence of oncolytic virus- Increased cancer cell death	Focus on single virus strain- Specialized imaging set-up required
Hong et al.[82]*	Glioblastoma	Microglia	extracellular vesicles (EVs) carrying miR-124	miR-124 disrupts M2 microglial polarization, suppresses GBM proliferation, and reduces metastasis.	MPS platform to co-culture patient-derived GBM cells and microglia in 3D three-compartment chip
Jenkins et al.[117]*	Various solid tumors	autologous lymphoid and myeloid cell populations	murine and patient-derived organotypic tumor spheroids MDOTS/PDOTS	MDOTS profiling demonstrated that TBK1/IKKϵ inhibition enhanced response to PD-1 blockade secreted cytokines in PDOTS captured key features associated with response and resistance to PD-1 blockade.	*ex vivo* response to ICB with specific autologus immune population from tumor spheroids

5.2. Adoptive Cel Therapy (ACT): CAR-T Cells and Engineered T-Cell Receptors (TCRs)

Adoptive cell transfer (ACT) involves transferring lymphocytes with anti-tumor activity to cancer patients to stimulate an anti-cancer response.[106,107] This method involves extracting, manipulating, and reinfusing immune cells, such as T-cells, to enhance their tumor-killing capability. In one approach to ACT, pioneered in patients with metastatic melanoma, tumor-infiltrating lymphocytes (TILs) are cultured from resected tumors, selected for antitumor activity, expanded to large numbers ex vivo, and finally reinfused back into the patient.[107,108]

Two prominent adoptive cell therapies include engineered T cells and NK cells with chimeric antigen receptors (CAR-T), as well as engineered T cell receptors (TCR-T). CAR-T therapy represents a significant advancement in ACT and has shown promising results in treating various cancers. These engineered T-cells express chimeric antigen receptors (CARs) that specifically target tumor-associated antigens, enhancing the immune system's ability to recognize and destroy cancer cells. This comprehensive analysis is crucial for understanding CAR-T cell therapy dynamics and developing strategies to overcome resistance and improve therapeutic outcomes.

One study explored CAR signaling related to cytotoxicity, proliferation, and memory formation in human primary T cells targeting a commonly expressed solid tumor antigen, IL-13Rα2. This research identified CAR signaling that produces enhanced cytotoxicity in vitro and substantially improved persistence both in vitro and in vivo against human glioblastoma, a feature likely necessary to combat antigen-positive disease recurrence. While its off-target activity precludes it from clinical consideration without further optimization, this study demonstrated the utility of CARPOOL, a pooled screening platform, in discovering CARs that elicit improved function.[109] In an another study, Paterson et al. developed a novel microfluidic immunoassay to evaluate CAR-T cell cytotoxicity and specificity in 3D spheroids co-cultured with cancer and stromal cells, modeling triple-negative breast cancer (TNBC). The assay used microfluidic devices to

generate and monitor multiple 3D cancer-stromal spheroids, enabling real-time imaging of CAR-T cell homing and cytotoxic effects. Moreover they combined CAR-T therapy with anti-PD-L1 immunotherapy and carboplatin chemotherapy to assess synergistic effects on tumor cell killing. Results showed that CAR-T cell cytotoxicity was significantly enhanced when combined with anti-PD-L1 therapy and chemotherapy compared to monotherapies. The study demonstrates that this microfluidic platform is a cost-effective, miniaturized preclinical tool for quantifying and optimizing CAR-T cell-based combination therapies in solid tumor models.[110] Chen et al. developed an ex vivo organotypic and immunocompetent human leukemia MPS exemplifying a bona fide leukemia bone marrow niche integrating both stroma and immune cells. The PDMS-based radial chip recapitulated the key components of the vascularized bone marrow stromal and immune niches, while a glass bottom allowed for microscopic analysis. The authors perfused anti-CD19 CAR-T cells and reported the eradication of B-cell acute lymphoid leukemia with potent induction of inflammation in the niche, which was not observed for mock T cells. Finally, they used their model to test available CAR-T cell products and demonstrated different responses in efficacy, suggesting the potential use of microfluidic models as a "pre-clinical-trial-on-a-chip" tool for CAR-T cell therapy development.[111] Cho Y et al. provided a study where a microwell array platform was used to investigate CAR T cell interactions with 3D HER2+ breast cancer spheroids, enabling high-resolution live imaging. They used a universal, switchable adaptor CAR system (SNAP-CAR) to precisely control antigen recognition by CAR T cells. Addition of a HER2-specific adaptor antibody significantly increased CAR T cell infiltration, clustering, and cytotoxicity within the spheroids. Larger spheroids showed greater hypoxia and suppressed CAR T cell activity, with reduced effector cytokine and protease expression, especially in the spheroid core. The platform allowed quantitative, spatiotemporal analysis of CAR T cell function, identifying initial T cell-to-spheroid ratios and spheroid size as key predictors of immunotherapy efficacy in solid tumors.[112] Lastly, Ando et al. engineered a 3D microfluidic device that recreates the hypoxic gradients found in solid ovarian tumors by embedding cancer cells in a hydrogel matrix and delivering CAR-T cells through microchannels. Using live imaging and immunostaining, they observed that CAR-T cell infiltration and cytotoxicity were significantly impaired in hypoxic tumor regions compared to normoxic areas. The study's findings highlight how oxygen deprivation within tumors can hinder CAR-T cell effectiveness. This advanced model offers a physiologically relevant platform for investigating and improving CAR-T cell immunotherapy in solid tumors.[113] TCR-T therapy represents an alternative adaptive cell therapy with several advantages and has proven effective against solid tumors. Engineering TCRs involves introducing TCR genes into T cells, enabling them to express receptors that specifically bind to tumor-associated antigens (TAAs). TAAs are overexpressed in cancers but have limited expression in normal tissues. Their expression can be restricted to tissues of tumor origin or to germline tissues.[107]

The use of MPS and OoC models has emerged in studying and applying TCR-T therapy, providing more accurate human-relevant models. Our team has pioneered and extensively tested cell immunotherapy targeting solid tumors using MPS systems, especially in adoptive cell therapy with engineered immune cells and TCR-T applications. Our earlier work used a microfluidic chip to investigate the preclinical evaluation of TCR-engineered T cells against solid tumors.[93] In another study, we analyzed how immunosuppressive drugs interfere with the in vivo function of TCR-T cells in liver-transplanted patients with HBV-HCC recurrence receiving HBV-TCR T cells. This was also studied in vitro in the presence of clinically relevant concentrations of immunosuppressive tacrolimus (TAC) and mycophenolate mofetil (MMF). Immunosuppressive drug-resistant armored TCR-T cells of desired specificity (HBV or Epstein-Barr virus) were engineered by concomitantly electroporating mRNA encoding specific TCRs and mutated variants of calcineurin B (CnB) and inosine-5′-monophosphate dehydrogenase (IMPDH). Their function was assessed through intracellular cytokine staining and cytotoxicity assays in the presence of TAC and MMF. We engineered TCR-T cells of desired specificities that transiently escape the immunosuppressive effects of TAC and MMF.[114]

Preece et al. investigated the efficacy of T cell receptor (TCR)-engineered T cells to kill hepatoma tumor cells in a commercialized standard chip. They adopted an engineered endogenous TCR (eTCR) using CRISPR-Cas9 and introduced a HBV-specific recombinant TCR (rTCR). The study revealed increased expression of the rTCR compared to eTCR, along with enhanced cytokine production and killing of HBV antigen-expressing hepatoma cells on-chip.[115] The integration of MPS and OoC technologies in CAR-T cell and TCR-T engineering research has significantly contributed to advancing adoptive cell therapy. These systems provide a more accurate and dynamic platform for studying CAR-T cell behavior, TCR-T cell interactions, functionality, and antitumor activity, ultimately improving our understanding of tumor-immune interactions and enabling the development of more effective immunotherapy strategies.

5.3. Immune Checkpoint Inhibitors (ICIs): Targeting PD-1, PD-L1, and CTLA-4

Immune checkpoint inhibitors (ICIs) represent a promising class of immunotherapies. The identification of immune checkpoint proteins, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and PD-1 ligand 1 (PD-L1), has facilitated the development of monoclonal antibody (mAb)-based drugs that inhibit their activation. These checkpoints regulate the innate and adaptive immune systems, influencing their functionality. Tumors exploit these inhibitory checkpoints to evade immune eradication. While blockade of the T-cell checkpoints CTLA-4 and PD-1 has demonstrated remarkably durable clinical responses, it remains effective in only a subset of patients. Despite these limitations, ICIs play a crucial role in uncovering new strategies for immunotherapy.

MPS and OoC technologies provide sophisticated platforms for studying the mechanisms of action of ICIs. These advanced models offer a more accurate representation of the human tumor microenvironment compared to traditional 2D cell cultures and animal models. For example, microfluidic systems enable the creation of controlled microenvironments that mimic the complex interactions between immune and tumor cells.

In a study by Jiang et al.,[116] the effects of an anti-PD-1 antibody on T-cells within a breast cancer model were investigated. The authors developed a custom immunotherapeutic high-throughput observation chamber (iHOC) featuring 288 wells to measure various parameters, including the viability of MDA-MB-231 breast cancer spheroids and T-cells, tumor infiltration, and IL-2 secretion. IL-2 concentration served as a marker of T-cell activation, as it is an established biomarker for activated T-cells in vivo, promoting the recruitment, growth, and stimulation of immune cells. The results indicated that administration of the anti-PD-1 monoclonal antibody reversed PD-L1-mediated T-cell inhibition in a tumor spheroid size-dependent manner. Furthermore, the anti-PD-1 antibody increased IL-2 production, preventing T-cell deactivation, and improved T-cell infiltration and survival within the TME.

In another study, Jenkins et al. evaluated ex vivo responses to immune checkpoint blockade (ICB) using murine and patient-derived organotypic tumor spheroids (MDOTS/PDOTS).[117] These MDOTS/PDOTS, isolated from mouse and human tumors, retain autologous lymphoid and myeloid cell populations and respond to ICB in short-term 3D microfluidic culture. MDOTS profiling demonstrated that TBK1/IKKϵ inhibition enhanced the response to PD-1 blockade, effectively predicting tumor response in vivo. Systematic profiling of secreted cytokines in PDOTS captured key features associated with response and resistance to PD-1 blockade.

Sehgal et al. investigated the efficacy of an anti-PD-1 antibody combined with targeted inflammatory cytokines in collagen-embedded murine colon cancer spheroids derived from ex vivo tissue.[118] Using a commercially available microfluidic device compatible with immunofluorescence staining, they performed manual media exchanges and extracted cellular material for RNA analysis. Parallel assessments of viability, gene expression, and protein expression identified a subpopulation of immunotherapy-resistant cells that evaded T-cell-mediated killing. They also demonstrated the enhanced effectiveness of combining PD-1 blockade with Birc 2/3 (inhibitors of apoptosis) antagonism to promote cancer cell elimination.

Cui et al. explored PD-1 blockade in the context of GBM by designing a PDMS-based perfused chip integrating patient-derived GBM cells, functional vasculature, TAMs, and allogeneic T-cells.[119] Using various analytical methods, including assessments of cell viability, migration, and gene expression, they investigated how different GBM subtypes influenced T-cell kinetics, infiltration, and interactions with tumor cells. They demonstrated that dual blockade of PD-1 and CSF-1R, a survival-promoting receptor, could reverse the immunosuppressive effects of aggressive GBM. Furthermore, their GBM-on-chip model's immunohistochemistry and cytokine profile aligned with those of patient-derived GBM samples.

Gopal et al. used a high-throughput microfluidic device to assess the effects of combining trastuzumab (anti-HER2) and atezolizumab (anti-PD-L1) with doxorubicin and/or paclitaxel on NK cell-mediated killing of pancreatic or breast cancer cells.[120] Their microfluidic system comprised 330 micropillar-microwell sandwich units for spheroid culture. They observed hypoxia induction within the TME of the spheroids and noted a reduction in the EC50 dose of small-molecule chemotherapeutics when combined with NK cells and antibodies.

Overall, the application of MPS and OoC models in investigating the PD-1/PD-L1 pathway offers considerable advantages for cancer research. These models generate human-relevant data, provide controlled microenvironments, and enable high-content analysis, all essential for understanding complex cell interactions and accelerating drug development timelines. By leveraging these advanced technologies, researchers can enhance their understanding of the underlying mechanisms of immune checkpoint inhibition and develop more effective therapeutic strategies. Despite the innovative advancements of OoC and MPS technologies for studying ICIs, several key questions remain that could significantly impact future research and therapeutic outcomes. One major concern is patient heterogeneity. Accurately predicting individual responses to ICIs using OoC models that account for genetic and phenotypic diversity is critical. Additionally, the variability of the TME poses challenges; optimally designing these models to mimic the complex interactions of different cancer types is necessary for generating relevant results. There is also a need to explore the long-term effects of chronic exposure to therapies within OoC systems, particularly how this influences T-cell behavior and tumor evolution. Moreover, the optimal combination of ICIs with other immunotherapies or chemotherapeutic agents within OoC settings remains to be clearly defined, as does the identification of specific mechanisms contributing to ICI resistance. Addressing these knowledge gaps is crucial, as is the challenge of ensuring scalability and reproducibility of OoC models to provide consistent and reliable results across research settings. Finally, developing methodologies for real-time immunological profiling within these dynamic models could offer valuable insights into treatment responses, ultimately enhancing the effectiveness of checkpoint therapies.

5.4. Oncolytic Viruses (OVs): Using Viruses to Target Cancer Cells

Oncolytic viruses (OVs) represent a promising avenue in cancer immunotherapy, leveraging their ability to selectively infect and lyse cancer cells while simultaneously stimulating an anti-tumor immune response. OVs were initially developed to preferentially kill tumor cells without harming healthy cells, beyond their direct cytotoxic activity.[121,122] These viruses, whether naturally occurring or genetically modified, selectively infect and destroy cancer cells. Furthermore, evidence suggests that OVs can stimulate the host's immune system to combat tumors.[123] Multiple viruses are currently under investigation as potential oncolytic treatments, including herpesvirus, adenovirus, poxvirus, picornavirus, and reovirus. In 2015, Talimogene laherparepvec became the first OV approved by the U.S. Food and Drug Administration (FDA) for human use.

Given the complexity of OV therapeutic strategies, using a human-relevant model such as a tumor-on-chip or organ-on-chip system offers advantages in reproducing an immunocompetent microenvironment. For example, Meccantini et al. used a microfluidic platform to study oncolytic vaccinia virus (OVV) in a lung carcinoma model. Through novel video-microscopy analysis, they observed enhanced immune cell recruitment and prolonged immune-cancer cell interactions in the presence of OVV, resulting in increased cancer cell killing.[124]

6. Challenges and Opportunities in Cancer Immunotherapy

6.1. Biological and Technical Challenges in Modeling the TME

The development of MPS and iCoC systems has opened new avenues in cancer immunotherapy research. However, recreating physiological cancer models that closely mimic patient conditions presents several significant challenges requiring careful consideration. While earlier work explored incorporating basic elements such as cancer cells, fibroblasts, and endothelial cells into MPS models,[125] research now faces the challenge of capturing the full range of cellular interactions and signaling networks present in vivo. A key limitation is representing the complete diversity of immune cell populations found in tumors. While current models often include T-cells,[126] macrophages,[127] dendritic cells,[128] and NK cells,[129] other crucial immune components, such as helper T-cell subsets, regulatory T-cells, myeloid-derived suppressor cells, and various types of innate lymphoid cells, are often missing. This omission is critical because the success of anti-tumor immune responses depends on the coordinated action of numerous immune cell types, each with specific roles in either promoting or suppressing tumor growth. Furthermore, while research on MPS modelling specific immune cell interactions, such as in CAR-T,[130] is progressing, these models still struggle to replicate how immune cells naturally migrate, activate, and differentiate in response to evolving tumors. The absence of these dynamic cellular interactions can lead to an incomplete understanding and limit the predictive value of these models for clinical applications.

A significant challenge is creating immune-competent in vitro systems for immune cell therapies, particularly concerning human leukocyte antigen (HLA). HLA, the "ID card" of every cell, plays a crucial role in determining self from non-self.[131] Mismatched HLA types can lead to severe complications, including host rejection or donor cell attacks, such as graft-versus-host disease (GvHD),[132] making it difficult to accurately assess treatment impact. This issue is particularly significant in MPS, where identifying suitable donors with matching HLA profiles can be time-consuming and sometimes impossible for certain patient biopsies. The challenge is compounded by the fact that patient-derived cells are not always accessible for all tumor types, and these systems are often created with commercially available cell lines where immune cell matching is not feasible. Recent research has increasingly focused on using autologous cells—derived from the patient—whenever possible, as this approach bypasses the complexities of HLA matching. Nevertheless, this strategy is not universally applicable, making HLA matching a persistent challenge in advancing immunotherapy.

Long-term culture maintenance presents another significant hurdle for developing MPS and iCoC systems. Primary cells are particularly difficult to maintain long-term due to their limited lifespan. While immortalized cell lines can help address this issue.[133] Complications arise when integrating multiple cell types, as each cell population has unique survival and functional requirements. For example, one cell type might require a specific growth medium, while another cell type in the same chip might find those conditions unsuitable. These challenges can limit the efficacy of these model in predicting the clinical responses. Addressing these biological and technical challenges, with a focus on cell sourcing, culture conditions, and immune system integration, will be a key feature of next-generation research to improve the physiological relevance and predictive power of these models.

6.2. Standardization and Reproducibility Challenges in MPS Technology

A major limitation of the widespread use of iCoC technology is the lack of standardization across laboratories. Varied protocols for cell culture, chip fabrication, data collection, and matrix composition make it difficult to compare results between research groups or validate findings on a larger scale. This challenge is exacerbated by the fact that many device designs are not commercially available or patented, hindering other laboratories from validating them with different cancer types and treatments, a crucial step for future clinical translation. This could lead to variability and difficulties in reproducibility across laboratories and platforms resulting in one of the major limitations into predicting the clinical responses, Despite these challenges, the future of MPS in cancer immunotherapy holds considerable promise, offering predictive and physiologically relevant alternatives to traditional research methods.[134] These systems can facilitate the evaluation of innovative therapeutic approaches, such as compounds or interactions that enhance immune cell recruitment or HLA-independent immunotherapies.

6.3. Commercial Scale-Up and Clinical Integration of MPS Platforms

Ongoing advancements in these technologies will improve MPS capabilities by allowing the integration of more TME components. This will result in even more accurate representations of cancer tissues. Recently, MPS platforms have been more widely adapted for high-throughput applications. This advancement enables statistically robust studies with multiple replicates, as exemplified by the Organix system,[135] which is essential for both basic research and drug development. This scaling capability, combined with the use of patient-derived cells, positions MPS as a powerful tool for conducting preliminary "human trials on chips." Such capabilities could revolutionize the cancer immunotherapy development pipeline by enabling more precise candidate selection before proceeding to full clinical trials. These challenges, along with the current technological advances and perspectives solutions, are summarized in **Table 4** to provide a clear roadmap for the future development and clinical translation of MPS platforms in cancer immunotherapy.

7. Conclusion and Outlook

MPS, particularly OoC technology, have revolutionized cancer research by offering more physiologically relevant models for studying tumor biology and treatment efficacy. These platforms enable highly controlled cellular microenvironments and real-time visualization of cell-cell interactions, crucial for understanding cancer cell behavior and evaluating therapeutic interventions.

Table 4. Challenges and future perspectives for MPS in cancer immunotherapy.

Challenge	Current Advances	Future Perspectives	References
Incomplete representation of immune cell diversity	Models already include T-cells, macrophages, dendritic cells, and NK cells.	Expand models to include additional immune populations such as T-helper cells, T-regulatory cells, MDSCs, and ILCs	[123–127]
Limited modeling of immune cell dynamics (migration, activation, differentiation)	CAR-T cell migration and activation studies	Incorporation of microfluidic flow, live-cell imaging, and multi-compartment designs, platforms to better simulate the temporal dynamics of immune responses and tumor progression	[130]
HLA mismatching complications for immune-competent systems	Autologous patient-derived cell use is gaining traction, reducing HLA mismatch issues	Advance the use of universal donor cells or HLA-independent immunotherapies to overcome matching limitations.	[129,130]
Long-term co-culture maintenance of multiple cell types	Protocols for using immortalized cell lines and primary cell cultures exist, but not universally applicable	Implementation of optimized cell culture procedures, particularly through the use of tailored, cell-specific media formulations	[133]
Limited access to patient-derived cells for certain tumor types		Establish tumor biobanks and optimize expansion protocols to ensure broader cell and biopsy availability	[133]
Lack of protocol standardization across laboratories	Individual labs have developed robust methods, but they remain non-standardized	Community-driven standardization of protocols for reproducibility and regulatory alignment	[134]
Limited access to validated and commercial MPS designs	Proprietary and academic models are in use, but open access is limited	Promote commercially available validated platforms	[134]
Limited scalability for high-throughput testing	Platforms like Organix enable some level of high-throughput experimentation	Develop industry-grade, high-throughput MPS systems for drug screening and patient-specific testing.	[135]

Furthermore, MPS allows for precise control of fluid dynamics, effectively mimicking in vivo physiological conditions. By recreating gradients of nutrients, oxygen, and mechanical stimuli, these platforms maintain cell viability and functionality during preclinical assessments. Their flexibility and customizability allow researchers to replicate diverse physiological and pathological conditions by manipulating flow configurations, ECM composition, cellular components or molecular factors, leading to more robust predictions of human treatment outcomes.

A key advantage of OoCs lies in their ability to integrate multiple cell types, mirroring the intricate cellular interplay within the TME. For example, including immune cells allows investigation of tumor-immune interactions, shedding light on immune evasion and immunotherapy effectiveness. MPS holds particular promise for immunotherapy investigations, enabling in-depth study of immune cell activity within the TME. This reveals mechanisms of therapy resistance and informs the optimization of treatment strategies, a necessary capability for personalized cancer treatments where understanding patient-specific responses is crucial for targeted therapeutics. Incorporating more complex TME features into microfluidic platforms will further improve physiological accuracy. Enabling vascularization and perfusion within these models is another key advancement. Replicating the body's vascular complexity is essential for maintaining tissue viability, nutrient delivery, and waste removal. Vascularized MPS are indispensable tools also to accurately capturing dynamic immune cell infiltration and functions. Microfluidic channel design ensures precise control over flow conditions, crucial for reproducing physiological shear forces and blood vessel pressures.

The use of patient-derived cells and tissues in MPS is another critical step toward more accurate disease modelling and drug evaluation. Leveraging the genetic diversity of primary samples obtained from patient biopsies or surgical resections enables the creation of models with greater clinical relevance. Integrating patient-derived tumor organoids or tumor fragments, for instance, serve to obtain powerful human-relevant platforms for drug testing and therapeutic profiling.

Establishing standardized protocols and guidelines is essential for improving reproducibility across platforms. Challenges often arise from the complexities of integrating multiple cell types and establishing biochemical gradients within these devices. Transparent protocols detailing sample preparation, maintenance, and monitoring will facilitate the adoption of these technologies for clinical applications, ultimately advancing the translation of effective therapeutic strategies.

Despite their revolutionary potential, translating MPS platforms from preclinical research laboratories to clinical applications faces several challenges. Addressing these through collaborative efforts between researchers, industry partners, clinicians, and regulatory bodies is critical for realizing the full potential of these promising technologies. As these barriers are systematically overcome, MPS will transform personalized medicine, particularly in complex diseases like cancer where traditional methods have significant limitations. They provide robust platforms patient-specific disease modelling for evaluating novel immunotherapies and perform functional drug screening for precision medicine.

Acknowledgements

F.M. and F.N. contributed equally to this work. This work was supported by: i) the Institute of Molecular and Cell Biology, Agency for Science Tech-

nology and Research (A*STAR), ii) the Singapore Immunology Network, iii) the Mechanobiology Institute, National University of Singapore, iv) the Lee Kong Chian School of Medicine – Ministry of Education Start-Up Grant and by v) the Ministry of Education, Singapore, under its Academic Research Fund Tier 1 (RS28/24).

Conflict of Interest

G.A. and A.P. are co-inventor of the OrganiX^{TM} plate (WO/2022/197254), licensed to AIM Biotech. A.P. is a member of the scientific advisory board and equity holder of AIM Biotech Pte. The other authors declare no competing interests.

Keywords

immunocompetent tumor micoenvironment, lab on a chip, microfluidic, microphysiological systems

Received: February 28, 2025
Revised: June 1, 2025
Published online:

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Francesco has co-authored several peer-reviewed publications including journals such Journal of Experimental and Clinical Cancer Research, Methods in Cell Biology and iScience, and has presented their research at numerous international conferences, receiving multiple awards for both oral and poster presentations. His work is dedicated to advancing preclinical cancer models to elucidate mechanisms of therapy resistance, with the goal of developing new innovative cancer immunotherapies.

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