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Microphysiological Systems for Cancer Immunotherapy Research and Development

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Cancer immunotherapy focuses on the use of patients' adaptive immune systems to combat cancer. In the past decade, FDA has approved many immunotherapy products for cancer patients who suffer from primary tumors, tumor relapse, and metastases. However, these immunotherapies still show resistance in many patients and often lead to inconsistent responses in patients due to variations in tumor genetic mutations and tumor immune microenvironment. Microfluidics-based organ-on-a-chip technologies or microphysiological systems have opened new ways that can provide relatively fast screening for personalized immunotherapy and help researchers and clinicians understand tumor-immune interactions in a patient-specific manner. They also have the potential to overcome the limitations of traditional drug screening and testing, given the models provide a more realistic 3D microenvironment with better controllability, reproducibility, and physiological relevance. This review focuses on the cutting-edge microphysiological organ-on-a-chip devices developed in recent years for studying cancer immunity and testing cancer immunotherapeutic agents, as well as some of the largest challenges of translating this technology to clinical applications in immunotherapy and personalized medicine.

1. Introduction

Cancer, a disease in which abnormal cells grow uncontrollably by disregarding the normal rules of cell division, affects 1 of every 3 individuals in the United States.[1] Cancer has been studied for decades, and the majority of previous studies have classified it as strictly a genetic disease, meaning that it involves changes or mutations in the cell genome.[2] However, more recent studies have shown that immuno-oncology, a focus on the adaptive immune system's behavior in relation to cancer, can further explain the mechanisms and development of cancer in the context of its immune microenvironment.[2] This is evident from the expansion of six hallmarks of cancer, initially proposed by Hanahan and Weinberg in 2000,[3] to ten hallmarks in 2011[4] and fourteen in 2022,[5] two of which involve the immune system, including "avoiding immune destruction" and "tumor-promoting inflammation".

The six hallmarks of cancer proposed by Hanahan and Weinberg in 2000 can be described using an analogy to a traffic light: 1) Immortality: indefinite dividing and limitless replicative potential 2) "Go" signals: produce self-sufficient growth factors from oncogenes to continuously divide; 3) "Stop" signals: insensitive to and override neighboring cells' anti-growth signals to continue dividing; 4) Evading apoptosis: resist programmed cell death; 5) Sustained angiogenesis: induction of new blood vessel growth to keep nutrient supply lines open; 6) Tissue invasion and metastasis: migration and spread of cancer cells from the primary tumor site to different parts of the body.[1,3] Among these, metastasis is the leading cause of death in patients with malignant neoplasms.[6]

More recent studies have shown that immuno-oncology, a focus on the adaptive immune systems' behavior about cancer, can further explain the mechanisms and behaviors of cancer and its microenvironment. The immune system can initially play a role in opposing tumor formation, but it can grow tolerant and even helpful to tumor growth and proliferation.[7] The immune system is responsible for defending the body against invaders through its innate and acquired immunity. The former, which is also called non-specific immunity, is present from birth and as its secondary name suggests, defends against foreign cells without identifying them. The latter, which is often called specific or adaptive immunity, is built on specific exposure and memory of antigens of various invaders. The function of the adaptive immune system is primarily performed by lymphocytes, which are a type of white blood cell. Of the lymphocytes, key cell types include B cells, which can differentiate into plasma cells that secrete antibodies, a type of protein that binds to foreign invader cells using antigen-specific receptors on the surface.[8] T cells, another type of lymphocytes, work to kill infected or mutated cells found in the body. Specifically, cytotoxic T cells, also called tumor-infiltrating lymphocytes (TILs), target host cells that have become infected or cancerous, making them critical in the field of immuno-oncology.

Therefore, instead of considering it solely a genetic disease, cancer is more appropriately described as a "systemic abnormality." A heterotypic model can be used to evaluate the changes in cell physiology, which ultimately lead to the development of

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cancer.[1,2] Current dogma states that cancer is a multi-gene and multi-step systemic disease, which originates from a single abnormal cell (i.e., clonal origin) with an altered DNA sequence (i.e., mutation).[1] Factors that may cause cancer are hereditary (e.g., gene) and/or environmental (e.g., lifestyle and age). The model looks at tumors as complex tissues, in which cancer cells recruit and utilize normal cells for their survival and proliferation.[1]

Knowing this, immunotherapy, which involves preventing the immune system from helping metastasis and promoting it to detect and fight cancer cells, is growing in the field of cancer research and adopted as a systemic approach to treat cancer.[7] Personalized therapy and precision medicine approaches play a crucial role in the development and implementation of immunotherapy because each patient's tumor microenvironment (TME) is different. These differences include a mix of biophysical and immunological changes in the lesion.[9] Recent immuno-oncology studies emphasize the cancer-immune cycle, including the interactions between tumor and immune cells,[10] tumor and stromal cells,[11] tumor and endothelial cells,[12] as well as immune and endothelial cells.[13] Understanding how different cell types work synergistically to either promote tumor growth or suppress antitumor immunity is a necessary step to improve the therapeutic efficacy of immunotherapy.

Cancer research has long been relying on in vivo models. Current technology for in vivo imaging of individual cells includes intravital microscopy (IVM), which provides the ability to visualize lymphocyte trafficking in real-time in the context of tissue complexity. Direct imaging of individual immune cell types in their native context provides the most accurate spatiotemporal assessment of immune effector functions, but the complexity of the milieu also makes it challenging to parse out individual molecular contributions.[14] Furthermore, high-resolution multiphoton microscopy uses single or dual laser systems that limit the number of fluorophores that can be simultaneously excited, therefore restricting the ability to visualize more complex structures and multicellular components in wider fields, or in longer timeframes.[15] In addition to imaging issues, in vivo models often fail to isolate causes and behaviors, resulting in experiments that cannot be fully decoupled and controlled. Other than having various technological limitations, animal models present ethical concerns, higher costs, longer study time, and many physiological and immunological differences than humans.[7,16] It is estimated that less than 8% of successful animal trials for cancer drugs translate to successful human clinical trials, primarily due to inter-specific differences in physiology and cell biology.[17]

Microphysiological system (MPS), also known as Organ-on-a-Chip, is an integrative microfluidics-based modeling system that merges biological, physical, and chemical knowledge and technologies to address specific biological and clinical problems in disease modeling and drug development fields (**Figure 1**).[18] The microphysiological system, by its name, is an engineered in vitro or ex vivo culture system upon which multiple types of cells are controllably cultured with organotypic architectures, biochemical factors, and mechanical cues within a 3D extracellular matrix (ECM) to recapitulate the physiology and/or pathophysiology of the in vivo tissues and organs.[19] The origin of MPS can be traced back to three decades ago, beginning with the fabrication and application of microfluidic devices to culture cells of interest for biological interrogations.[20] As a type of microfluidic device, MPS is fabricated with materials such as polydimethylsiloxane (PDMS) using the soft lithography approach. First, silicon master molds of the designed structure are fabricated by photolithography. Different layers of the microfluidic device are then generated with PDMS polymers using replica molding, which replicates the micro-size features from the silicon molds to PDMS slabs. The PDMS slabs are finally assembled either with each other or with glass slides via oxygen plasma-assisted bonding.[21]

Microphysiological systems make use of their compact size and microchannels to precisely manipulate various fluidic and chemical parameters, such as flow rate, pressure, oxygen, and pH level, providing controllable cell culture conditions. Microphysiological devices, designed and manufactured using a reductionist approach, do not strive for reproducing the whole tissue or organ at the original scale, but instead mimicking the major organotypic characteristics, such as basic and functional units of organs in a 3D setting at a micrometer to millimeter scale. MPS shares some similar approaches but is different from the concept of tissue engineering, which is a broader approach focusing on replacing large deficient tissues or even whole organs.[22]

Microphysiological system particularly fits the application in drug development and disease modeling and is a good medium between in vivo and in vitro studies. MPS also provides a 3D environment critical for immune response studies.[27] Most reviews focus on cancer studies and cancer drug development from a biological perspective using either 2D or animal models. Here we present a comprehensive and critical review on the application of MPS on immuno-oncology and immunotherapy research from both biological and engineering perspectives. We first summarized the MPS used for mechanistic studies of immune-cancer cell interaction and tumor microenvironment, followed by its application in preclinical development and testing of immunotherapies, including adoptive T cell therapy and immune checkpoint inhibition. Finally, future trends and challenges in its use in cancer drugs and immunotherapy development were discussed.

2. Microphysiological Systems as an Emerging Tool to Study Immuno-Oncology

A microphysiological system is an emerging tool that provides a robust and efficient platform for immuno-engineering study (**Figure 2**). Aiming to quantitatively analyze and modulate the immune response and cancer development, MPS is not only precise in the single-cell analysis of immune cells[28] but also powerful for the spatiotemporal identification of interactions between tumor cells and immune cells.[11b–e] The microfluidic single-cell analysis allows the heterogeneous study of immune cells including cellular communication and signaling pathways, which is usually overshadowed by conventional bulk study methods.[29] In addition to the single-cell and cell-cell interaction analyses, MPS is also used in the recapitulation of the tumor microenvironment (TME) to study cancer cell migration and invasion[6,12a,30] as well as immune cell trafficking and infiltration.[31] Providing a 3D environment that is cell-specific, organ-specific, or patient-specific, MPS is particularly useful in isolating and studying the multifactorial processes of tumor intravasation and metastasis; this warrants MPS is a powerful tool for the development of novel

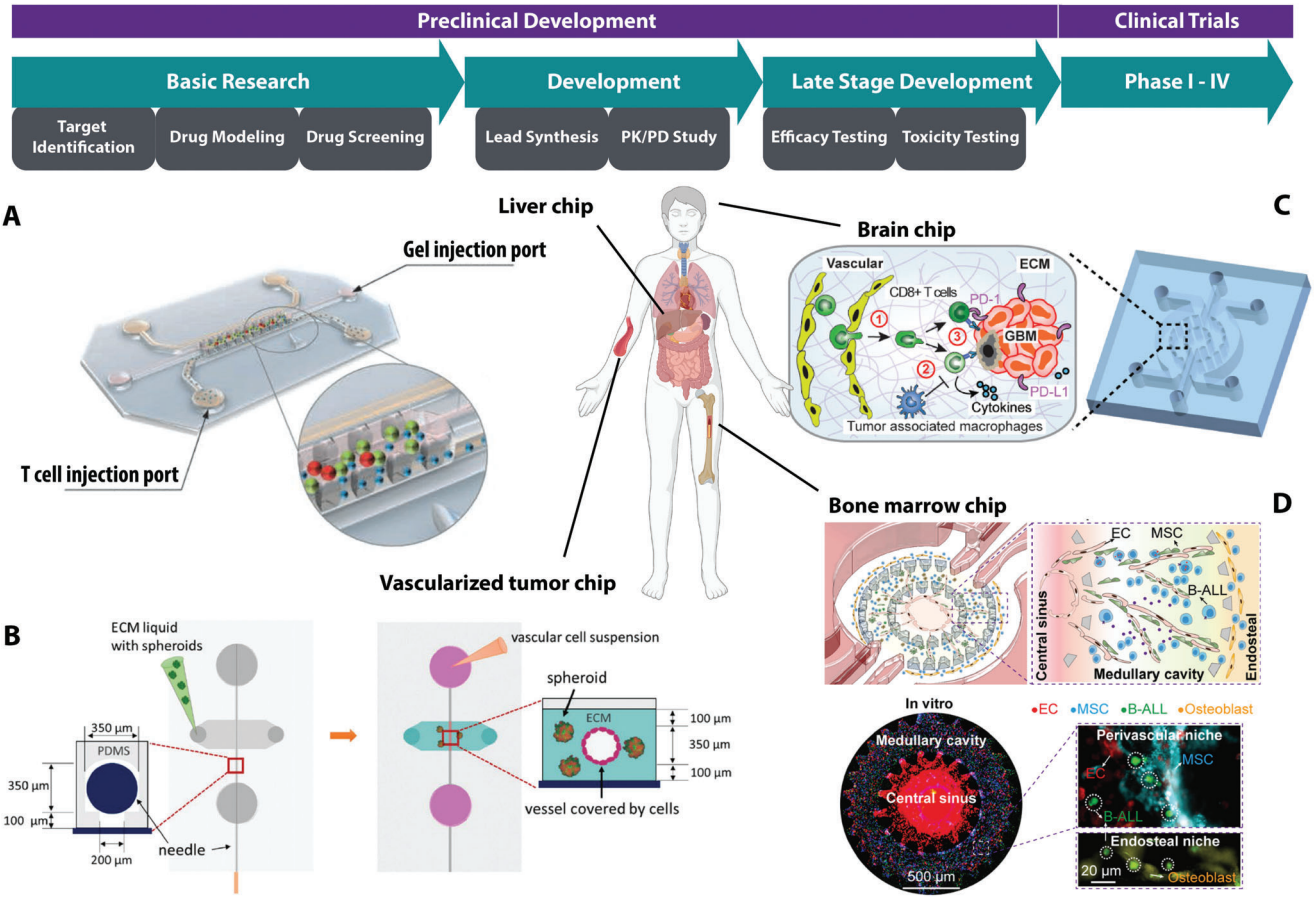

Figure 1. Organ-specific microphysiological systems for immunotherapy research and drug development. A) A liver chip developed by Pavesi et al. to assess the efficacy of different TCR T cells on human cancer hepatocytes. Reproduced with permission.[23] Copyright 2017, American Society for Clinical Investigation. B) A vascularized breast tumor chip developed by Kwak et al. to study tumor interaction with perfused blood vessels and tumor-induced angiogenesis. Reproduced with permission.[24] Copyright 2020, Springer Nature. C) A brain chip developed by Cui et al. to study the tumor-immune environment and ICB therapy outcome in glioblastoma. Reproduced with permission.[25] Copyright 2020, eLife. D) A bone marrow chip developed by Ma et al. to dissect the chemotherapy resistance in leukemia. Reproduced with permission.[26] Copyright 2020, American Association for the Advancement of Science. Part of the figure generated using BioRender.com.

therapies and vaccines to manipulate and modulate the antitumor immune response in vivo.[27]

2.1. MPS for Single Immune Cell Analysis

Single immune cell manipulation and analysis using MPS may delineate the heterogeneous behaviors of immune cells overshadowed by conventional bulk study methods. Microfluidic technique in single-cell immunology studies was well summarized by Jammes and Maerkl.[32]

For instance, Huang et al. developed a PDMS-based microfluidic immunophenotyping assay (MIPA) to profile cytokines secreted by human monocytes.[28b] The device consisted of two layers separated by a surface-micromachined PDMS microfiltration membrane (PMM), which is a rapid and robust platform for immunophenotyping compared to conventional cell-stimulation assay using enzyme-linked immunosorbent assay (ELISA) or intracellular cytokine staining (ICCS) assay in terms of efficient cytokine diffusion time, reduced sample volume, and increased detection sensitivity.

Common techniques used in microfluidics-based single-cell analysis include microfluidic chips with i) cell traps, ii) droplet microfluidics, and iii) valve-based microfluidics.[29] i) Cell traps, as its name suggests, take advantage of carefully considered geometries to trap and confine cells passively. This type of microfluidic device is relatively easy to design but is hard to conduct complex fluid control and manipulation. Xue et al. developed a single-cell, multiplexed microfluidic device to evaluate the cytokine release profile of CD19 CAR-T cells upon antigen-specific stimulation.[28a] CAR-T cells were stimulated and incubated in a single-cell barcode chip (SCBC), which consists of a microchamber array, and a glass slide patterned with antibodies. Single CAR-T cells trapped within the microchamber then underwent imaging and downstream proteomic analysis. Combined with advanced bioinformatic tools and software, researchers were able to visualize and compare the functional landscape of the CAR-T cells between donors. This platform can be used to study

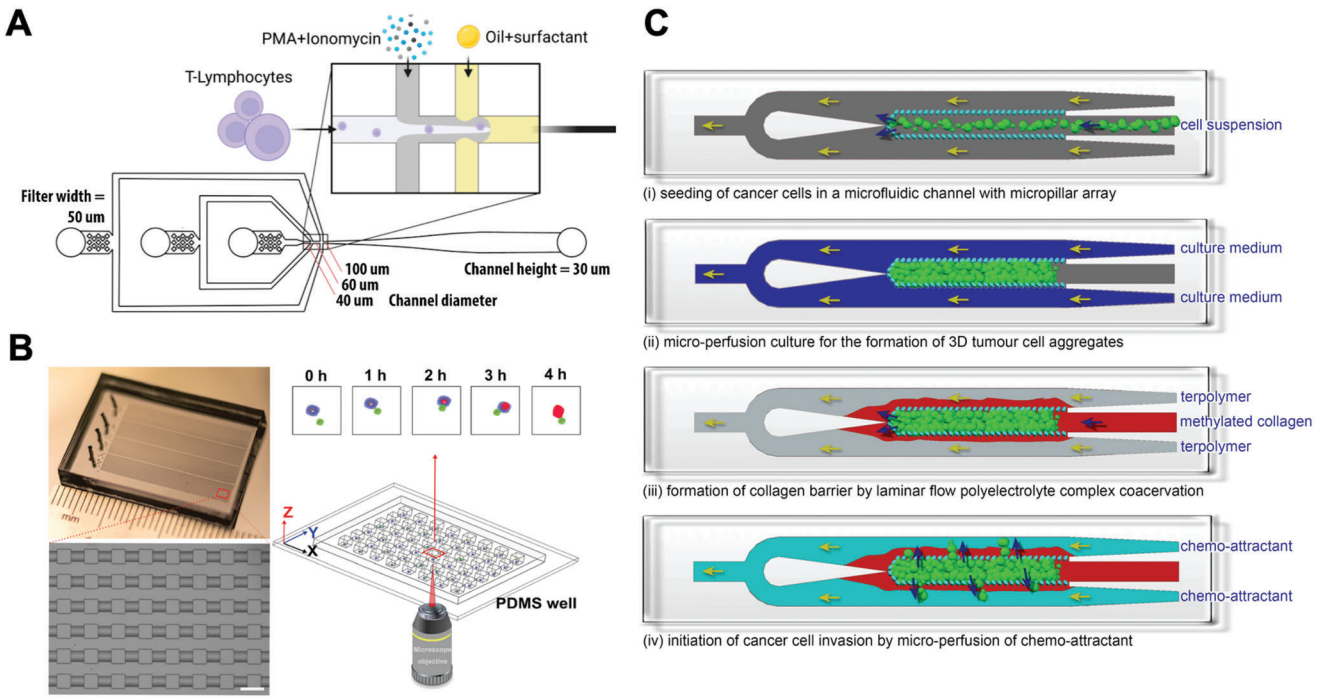

Figure 2. Microfluidics-based microphysiological systems as an emerging tool to study immuno-oncology. A) Layout of the microfluidic device used to generate droplets for single immune cell analysis. Reproduced with permission.[28c] Copyright 2022, Multidisciplinary Digital Publishing Institute. B) Working principle of the microfluidic device for immune-cancer interactions at the single cell level. The tumor cell (green color) killed by the T cell (blue color) expresses the red fluorescence (scale bar: 200 µm). Reproduced with permission.[10d] Copyright 2020, Royal Society of Chemistry. C) Establishment of a 3D cancer cell migration model in a microfluidic channel. i) Cancer cells are seeded into the microfluidic channel and ii) perfusion-cultured for 3 days to allow the formation of 3D cellular aggregate. iii) A collagen barrier is formed around the 3D cellular aggregate by laminar flow complex coacervation of a positively charged collagen and a negatively charged HEMA-MMA-MAA terpolymer. iv) Cancer cell migration/invasion is then initiated by perfusing chemo-attractant through the side perfusion channels. Reproduced with permission.[6] Copyright 2018, Multidisciplinary Digital Publishing Institute.

and prevent cytokine release syndrome (CRS) and improve the safety and efficacy of future CAR-T therapy.

ii) Droplet microfluidics is advantageous in a way that allows the encapsulation of single immune cells to interrogate the heterogeneity of immune cell activation and cytokine secretion in a more controlled manner. Tiemeijer et al. developed a hydrogel-based droplet microfluidic device for phenotypic and functional analysis of cytotoxic T lymphocytes (CTL) (Figure 2A).[28c] Single CTLs were encapsulated in agarose microgel with either Dynabeads or soluble stimuli like phorbol 12-myristate 13-acetate (PMA) and ionomycin. Oil and surfactant were used to generate single droplets. Compared to other conventional droplet microfluidic techniques, this platform allows multiparameter phenotypic and functional measurements after the T cells were recovered from the microgel via emulsification for high-throughput downstream analysis and sorting using flow cytometry.

iii) Valve-based microfluidics is large-scale integration of pneumatic valves arrayed as fluidic multiplexers for complex fluid manipulation.[10d] They are highly controllable and programmable, but the design and fabrication processes are usually laborious. Briones et al. designed a high-throughput microfluidic chip with 5000 hydrodynamic traps and control microvalves.[28d] The microchambers in the chip were compartmentalized by the circular microvalves, which had a sealing pressure as low as 0.04 MPa and were able to confine enzymes and substrates in picoliter volumes. The device was successfully used to profile the enzymatic activity and granzyme B expression of single immune cells individually trapped and isolated in the microchambers from patient peripheral blood mononuclear cells (PBMC).

2.2. MPS for Studying Immune Cell and Tumor Cell Interactions

The anti-tumor immune response often starts with the uptake of tumor antigens by the antigen-presenting cells like the dendritic cells (DC) that carry antigens from the tumor environment to the tumor-draining lymph node (TdLN) for T cell priming and activation.[9] This process involves complex interactions of multiple types of immune cells with tumor cells. The ability to quantitatively analyze individual T cell activation status, formation of immune synapse, as well as the efficacy of tumor cell killing at the single-cell level is critical in understanding, regulating, and enhancing the tumor immunosurveillance and immune response. Techniques used in the single-cell analysis, which involve complicated interaction of fluids in different phases, are not suitable for culturing different cell types at the same time.[10d] Current 3D microfluidic devices for immune-cancer cell interaction studies are normally made up of multiple compartmentalized chambers to house various cell types and incorporated with Matrigel or

collagen scaffold to create a more physiologically relevant environment for cells to attach and grow.

Mattei et al. developed an on-chip model of B16F10 melanoma to study the mechanism of interferon regulatory factor (IRF)−8 in regulating immune and tumor cell migration behavior.[10b] The device has two main culture chambers, a melanoma, and a spleen cell compartment, which were connected to the central end-closed chambers via an array of narrow capillary migration microchannels. They discovered a more durable and functional interaction of wild-type splenocytes with the tumor cells compared to their IRF-8 knockout counterpart. The knockout of IRF-8 in the immune cells resulted in their poor migratory capacity and thus a more invasive behavior of the melanoma cells. The group hypothesized that the up-regulation of IRF-8 expression led to the secretion of soluble factors like interleukin (IL)−27, a cytokine with anti-tumor and anti-angiogenic activity.

Tu et al. created a high-throughput microwell array device to interrogate CD8+ T cells' killing capacity, kinetics, and immune synapse formation affected by their initial distance and cell ratio to the leukemia cancer cells (Figure 2B).[10d] The device consists of a single PDMS layer containing well arrays connected to a flow channel. Using this device, they were able to observe in real-time the time required between TCR engagement and T cell-mediated killing. They found that T cells could move toward the leukemia cell and kill the target cell at an initial distance as far as 15 μm and that most (51%) T cells were able to kill tumor cells within 50 min. They also found that anti-PD-1 treatment could significantly reduce the T cell inertia and killing time needed against leukemia cancer cells.

Parlato et al. created a microfluidic device with three chambers to study the migration of DCs toward colorectal cancer cells (CRC).[10e] The device consists of a central immune culture compartment, where DCs are in floating motion, and two side narrow chambers with tumor cells embedded in a type I collagen matrix. The immune and tumor chamber were connected via a series of capillary migration channels to mimic the extravasation of DCs into the peripheral tissues, and the two side tumor chambers were treated with different conditions. They found that DCs showed increased phagocytosis and antigen cross-presentation activities as well as tended to recognize and migrate toward the cancer cells treated with interferon (INF)-α and romidepsin, which was mostly regulated by the CXCR4/CCL12 axis.

2.3. MPS for Studying Tumor Microenvironment and Metastasis

Many research groups endeavor to develop more robust, complex microphysiological systems to better recapitulate the in vivo physiology and pathophysiology of the organ-specific microenvironment. These systems often compose of delicate geometries and microarchitectures with different cell types that may self-assemble into complex structures and vascularized functional units. Many MPS have been developed to dissect and interrogate the cancer-immune cycle; this includes trafficking and migration of T cells, extravasation and colonization of tumor cells, APC processing and presentation of tumor-associated antigens (TAA), T cell priming and activation by APCs, as well as the interaction between cancer cells and macrophages.

2.3.1. Reconstructing Tumor Lymphatics on MPS

One of the major components of the tumor microenvironment is tumor lymphatics. The majority of cancers metastasize initially through the lymphatic system, but the mechanisms of lymphogenous metastasis remain poorly understood and understudied compared to hematogenous metastasis.[33] Furthermore, the lymphatic system not only acts as a route for cancer metastasis but also mediates fluid flows that affect antigen distribution, lymph node organization, leukocyte trafficking, and immune activation.[34] O'Melia et al. and Greenlee et al. summarized in their reviews various engineering tools that enable quantitative analysis of lymphatic transport, lymphatic metastasis, and anti-tumor immune response.[33,35] Since metastasis is a critical stage in cancer development dissemination, creating the right TME with necessary biochemical and biophysical cues as well as microarchitecture is essential for successfully recapitulating tumor-specific metastatic niche.

Henderson et al. developed an engineered lymphatic vessel model to understand lymphatic endothelial cell-cell junction formation and junction tightening in fibronectin via integrin alpha 5,[36] which can be adopted to model tumor invasion to lymphatics or immune cell trafficking to tumor lymphatics. There was another study showing that head and neck tumor-derived fibroblasts promote tumor lymphangiogenesis in their lymphatics and tumor co-culture model.[37] A lymphoid follicle (LF)-on-a-chip was developed by Goyal et al. to recapitulate human seasonal vaccination and adjuvant responses.[31c] B and T cells autonomously assemble into ectopic LFs on the chip when cultured in a 3D ECM gel, with superfusion via a parallel channel separated by a microporous membrane. The functional LF was able to support plasma cell differentiation and display antigen-specific IgG production as well as secretion of clinically relevant cytokines when added autologous dendritic cells (DCs) and vaccinated with the influenza vaccine. Pisano et al. created an in vitro model of the tumor-lymphatic microenvironment using a standard Boyden chamber coupled with a micro-channel and fluid control component.[30a] The system could simultaneously create transendothelial and luminal flows that rendered the model more physiologically relevant to the lymphatic microenvironment. The device revealed mechanisms of flow-enhanced invasion of MDA-MB-231 tumor cells. They found that the luminal flow upregulated tumor cell transmigration rate via the CCL21/CCR7 signaling pathway and when combined with the transmural flow, these biomechanical cues additively increase tumor cell intravasation.

2.3.2. Reconstructing Tumor Metastatic Niches on MPS

The tumor microenvironment directly impacts tumor metastasis. There have been several studies reconstructing tumor metastatic niches on MPS. A miniaturized version of the metastatic model was developed by Toh et al. using a microfluidic-based culture chip to simulate cancer cell migration and invasion across the basement membrane, though their model lacked the endothelial cell (EC) layer described in Pisano et al (Figure 2C).[6] They cultured metastatic MX-1 breast cancer cells in the middle channel surrounded by a micropillar array, which allowed cancer cells to form 3D cellular aggregates

resembling cancer tumors before initiating migration and invasion. A 3D collagen barrier was then formed around the 3D cancer cell aggregate via a polyelectrolyte complex coacervation process. The group was able to monitor cell migration in real time, resolving different aspects of cancer cell intravasation such as loss of cell adhesion, different modes of cell motility, and ECM degradation. They observed both mesenchymal and amoeboid modes of migration as well as collective migration, which is rarely seen in other 2D migration assays including using the Boyden chamber.

Nguyen et al. took a step further and reconstituted ex vivo a human epidermal growth factor receptor 2 positive (HER2+) breast tumor microenvironment consisting of four cell populations including cancer, immune, endothelial, and fibroblasts.[30b] The chip consisted of five parallel microchambers separated by regularly spaced micropillars that allow the confinement of hydrogels, mimicking the original in vivo architecture in tumors. The group managed to characterize the ecosystem-level responses to the drug trastuzumab (Herceptin) and dissect the roles of stromal components including immune cells and fibroblasts. They found that cancer-associated fibroblasts (CAFs) were antagonists of Trastuzumab, inhibiting the capacity of the chemotherapy to stimulate longer cancer-immune cell interactions. One limitation of this model is that ECs were grown in a 2D monolayer instead of a 3D conduit for immune cell trafficking.

There have been studies preparing rudimentary blood vessels and showing tumor cell interactions with the engineered vessels in 3D as part of the tumor metastatic cascade. Kutys et al. used a biomimetic vascularized mammary duct platform to investigate the mutation-specific morphogenic phenotypes of endothelial vessels. They discovered that PI3Kα^{H1047R} led to IL-6 secretion from mammary duct paracrine signaling and drove vascular dysfunction and remodeling.[38] Nguyen et al. created an organotypic pancreatic ductal adenocarcinoma (PDAC)-on-a-chip culture model that emulates vascular invasion and tumor-blood vessel interactions.[12a] The device is featured by a perfusable endothelial lumen and a cancerous pancreatic duct, and they found the activin-ALK7 pathway a mediator of endothelial ablation. Kwak et al. developed an organ-specific breast tumor extravasation model by creating an engineered vessel surrounded by bone or lung parenchymal cells and introducing bone- or lung-tropic breast tumor cells into the vessel lumen. The model shows organ-specific tumor cell extravasation and colonization showing regression of lung-tropic breast tumor cells in bone microenvironment.[39]

3. On-Chip Testing of Cancer Immunotherapy

Previously, tumor immunotherapy was categorized into active (e.g., immune checkpoint blockade) and passive (e.g., adoptive T cell transfer) types depending on the ability to engage the host immune system.[40] More recently, the boundary between active and passive immunotherapy has become blurry with an expanding appreciation of the complex tumor immune microenvironment and its dynamic effector and suppressor responses that dictate the terms of cancer remission.[41]

Adoptive cell-mediated cancer immunotherapy, or adoptive cell therapy (ACT), utilizes either endogenous TILs or genetically engineered T cells that express either highly specific T cell receptors (TCRs) or chimeric antigen receptors (CARs).[42] ACT has the growth of development from 2017–2019, with nearly 1200 new cell therapy products that are currently in the preclinical and clinical evaluation.[42b] Besides TCR and CAR T cell therapy, immune checkpoint blockade (ICB) currently represents another promising cancer therapeutics. The number of preclinical and clinical trials evaluating T cell-targeted modulators has increased to more than 4000 in 2020.[42b]

All these novel immunotherapies have shown great performance in early clinical trials, though patient response varies. The underlying mechanisms that suppress anti-tumor immune responses are suggested to be from the immunosuppressive milieu within the local TME.[43] Preclinical evaluation of genetically engineered T cell products and ICB within the TME may reveal the underlying mechanisms compromising the efficacy of different immunotherapies and lead the way for clinicians toward developing better-personalized therapeutics.[41] The application of microfluidic tissue-engineered tumor immunity models for the testing of potential therapies may greatly help predict the efficacy and clinical outcome of personalized immunotherapies.

3.1. MPS for Screening T Cell Receptor-Redirected T Cells

T cell receptor-redirected T cells (TCR T cells) are isolated cancer-reactive immune cells from the body, enhanced and expanded ex vivo, and reinfused back into the patient.[42b,44] Common strategies to test engineered T cells for cancer immunotherapy include using 2D standard tissue culture models and murine tumor models.[42a] However, murine models fail to represent central mediators in human immunity, while in vitro 2D tumor models cannot faithfully capture the spatiotemporal dynamics of T-cell infiltration and tumor eradication.[17,41,42] Many studies have reported discrepancies in their findings between 2D and 3D models.[45] MPS is a valuable tool to perform preclinical tests of the therapeutic efficacy of engineered T cells as well as their on-target off-tumor toxicity in a fast and reproducible way compared to the patient-derived xenograft (PDX) mice models (**Figure 3**).[46]

Pavesi et al. developed a 3D human liver cancer microphysiological model to assess the efficacy of different TCR T cells (Figure 1A).[23] The central region of the device was confined by two rows of trapezoidal pillars that confine the type I collagen and tumor cells. The cytotoxicity of engineered TCR-T cells was found to be different in 3D from 2D because the chemotaxis and motility of T cells largely impacted their interactions with the tumor cells in 3D. They found that the retroviral-mediated stably transduced TCR-T cells (retroV-TCR-T cells) had higher efficacy than mRNA-electroporated transiently transfected TCR-T cells (mRNA-TCR-T cells) only in their 3D model. Although both TCR-T cell preparations showed reduced killing capacity in the hypoxic condition, the retroV-TCR-T cells showed an increased ability to kill in the presence of inflammatory cytokines such as IFN-γ and TNF-α.

Lee et al. tested TCR T cell therapy for hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) on a modified microfluidic device previously designed by Pavesi et al. through the incorporation of human primary monocytes (Figure 3A).[47] The device

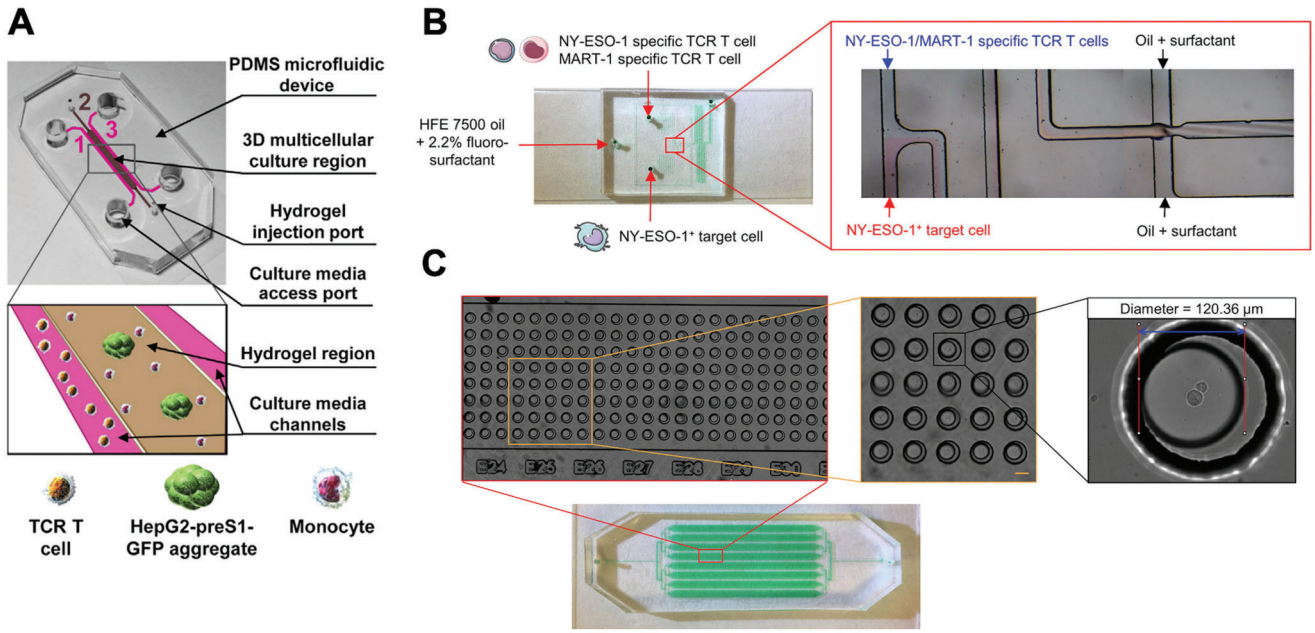

Figure 3. Microphysiological systems for screening T cell receptor-redirected T cells. A) A 3D multicellular tumor microenvironment microfluidic model consisting of a middle hydrogel channel (2) flanked by two media channels (1, 3) for the mechanistic study of the effect of monocytes on TCR T cell killing of tumor cell aggregates. Human monocytes were inserted together with target HepG2-preS1-GFP cell aggregates in collagen gel in the central hydrogel region (2), while HBV-specific TCR T cells were added into one fluidic channel (1) to mimic the intrahepatic carcinoma environment. Reproduced with permission.[47] Copyright 2018, Frontiers Media SA. B) The droplet microfluidic device used to generate 120 µm-diameter droplets containing co-encapsulated TCR T cells and target cells. A flow-focusing droplet generator was used to generate water-in-oil droplets. The two symmetric inlets were loaded with cell solutions containing food dyes for better clarity: blue for TCR cells and red for target cells, before being mixed at the junction where the aqueous phase got pinched by HFE 7500 oil containing 2.2% by weight fluoro-surfactant (PFPE 5000-PEG900) to generate droplets. C) Generated droplets are loaded into a floating droplet array (iFDA) containing 10368 trapping wells for imaging. Droplets occupied all the trapping wells after the loading and flushing of the extra droplets. Right image: higher magnification of a trapping well containing a 120 µm-diameter droplet with a NY-ESO-1 specific TCR T cell interacting with a target cell. Scale bar: 100 µm. B,C) were reproduced with permission.[10c] Copyright 2018, Royal Society of Chemistry.

is made up of a middle hydrogel channel flanked by two media. Tumor spheroids were cocultured with human primary monocytes in the middle channel, while TCR T cells were perfused through the two media channels. They found that monocytes played a suppressive role in retrovirally transduced (Tdx) TCR T cell therapy via a PD-L1/PD-1-dependent mechanism on their 3D microfluidic model, whereas the mRNA electroporated (EP) TCR T cell cytotoxicity was not affected by the presence of monocytes. Such suppressive effect of monocytes on TCR T cell therapy was lost in the 2D cytotoxicity assays, indicating the importance of a 3D environment in maintaining valid outcomes from immunological assays.

Segaliny et al. developed a droplet microfluidics platform for functional screening and real-time monitoring of single TCR T cell activation upon recognition of target tumor cells (Figure 3B,C).[10c] The device has a flow-focusing generator with two types of fluids containing the continuous phase and disperse phase to trap single TCR T cells with single target cells. The droplet then goes to the inverted floating droplet array (iFDA), which contains trapping wells to monitor individual droplet and TCR T cell activities. Activated T cells can be then sorted using the UV-laser microdissection microscope with heat-induced cavitation. This platform is powerful for identifying rare clones of TCR and future TCR discovery.

3.2. MPS for Screening Chimeric Antigen Receptor T Cells

CARs are simulated receptors containing an extracellular single-chain variable fragment (scFv), a transmembrane domain, as well as an intracellular region of immunoreceptor tyrosine-based activation motifs (ITAMs) in association with a co-stimulatory signal.[48] In contrast to TCR T cells, CAR-modified T cells can detect epitopes of tumor-associated antigens (TAAs) independent of the presence of the major histocompatibility complex (MHC) molecules, which cancer cells often mutate and lose to evade the immune system.[42b] CAR delivery has a wider spectrum of functional effects than transduced T cell receptors (TCRs) but is restricted to identifying markers located on the cell surface.[48]

Current CAR-T therapies are mostly clinically approved for hematogenous tumors. CD19 CAR T-cell therapy, for example, has achieved a durable clinical benefit in a subset of patients with chronic lymphocytic leukemia and non-Hodgkin lymphoma.[46] Nevertheless, there are currently no approved CAR-T cell therapies for solid tumors.[49] The previous generation of CARs has varied abilities in activities such as IL-2 secretion and CAR affinity. The third generation of CARs, containing two different costimulatory domains collective with a special activation domain in their cytoplasmic section, demonstrated a greater ability to the treatment of solid tumors in several mouse models.[48]

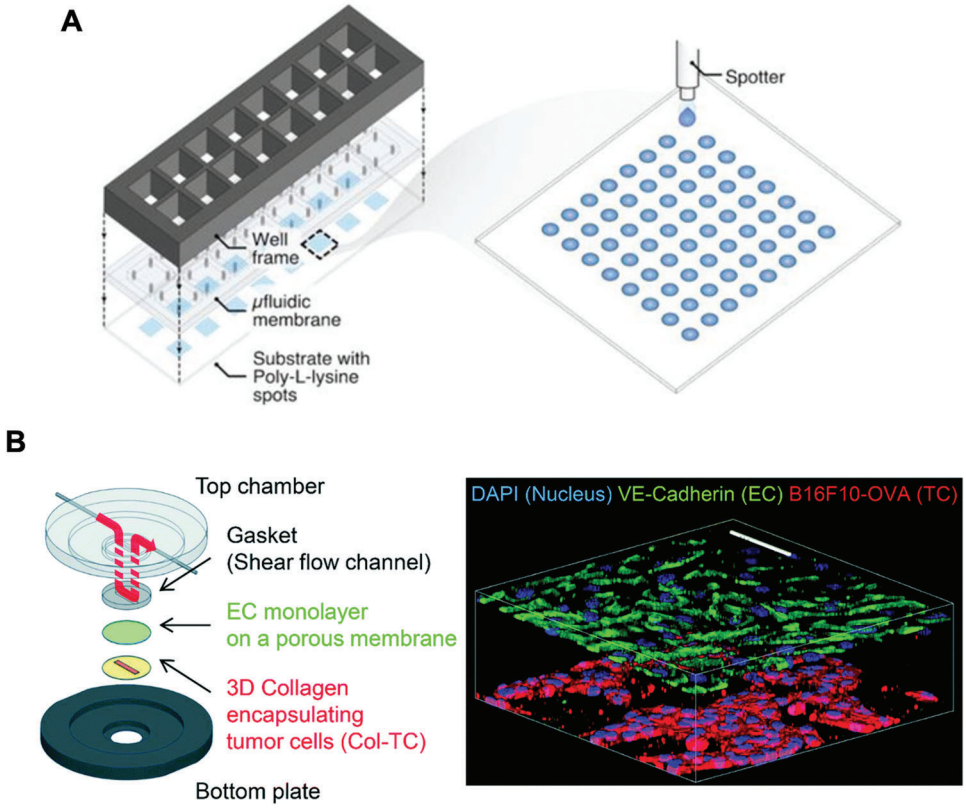

Figure 4. Microphysiological systems for screening chimeric antigen receptor T cells. A) Schematic illustrations showing the assembly of the micropatterned tumor arrays (MiTA) for the quantification of CAR T cell killing. A 16-well device, zoom-in of one well during the printing of the 64 spots, tumor-cell patterning in the wells, and subsequent CAR T cell loading and imaging. Reproduced with permission.[31b] Copyright 2019, Wiley. B) Assembly of the multilayered blood vessel/tumor tissue chip (MBTC) to investigate T cell infiltration into solid tumors and confocal image of ECs and TCs located in the MBTC. An endothelial cell (EC) monolayer cultured on a porous membrane and a 3D collagen gel containing tumor cells (TCs) is located between the top chamber and the bottom plate. EC junctions were stained with FITC-conjugated anti-VE-cadherin antibodies, TCs were labeled with cytoplasmic fluorescence dyes, and nuclei were stained with DAPI. Scale bar: 100 μm. Reproduced with permission.[31a] Copyright 2021, Royal Society of Chemistry.

Despite the promising patient response outcome from ACT, both TCR and CAR-T cell therapies are proven less effective in treating solid tumors than skin cancer (e.g., melanoma) and blood cancer (e.g., leukemia) due to inefficiency in immune cell infiltration. The pivotal challenges in the field of solid tumor T-cell therapies can be summarized into three major parts: recognition, trafficking, and survival in the tumor.[48] Many MPS have been developed in recent years to tackle these challenges (**Figure 4**).

First, recognition of TAAs in solid tumors is challenging due to antigen heterogeneity. While cancer cells in hematological malignancies normally express the special and individual markers, solid tumors often do not express one-specific marker, but instead, tumor-associated antigens (TAAs) that are often also expressed on natural tissues.[48] Wang et al. developed a multifaceted micropatterned tumor array (MiTA) to evaluate the CAR-T trafficking, clustering, and killing dynamics (Figure 4A).[31b] The device was created using a glass substrate patterned with poly-L-lysine and ZETAG to form tumor-cell islands, as well as a top PDMS membrane containing individual compartments that minimize the mechanical perturbation acting on loosely adherent T cells and prevents artificial cell interactions induced by cell drifting. Using this device, the group tested various constructs of CAR-T and found that CAR-T cells merging into large clusters are more effective in tumor cell killing. They also concluded that efficient killing is driven by antigen-specific binding. For example, APRIL-based T cells can eliminate both BCMA-positive and negative multiple myeloma (MM.1) tumor cells, while anti-BCAR T cells can only eliminate BCMA-positive tumor cells.

Second, trafficking and infiltration of T cells into tumor tissues through the vascular endothelium is obstructed due to the overexpression of endothelin B receptors in cancer tissues and downregulation of the ICAM-1,[44a,48] and the lack of chemokine expression to guide T cell trafficking in the tumor.[50] Multiple on-chip studies of T cell infiltration and trans-endothelial migration within the tumor concerning vessel permeability have been developed.[13a,d,31a] For example, Lee et al. added a vascular component to the microfluidic device to study T-cell infiltration into the solid tumor tissue (Figure 4B).[31a] The device, named multilayered blood vessel/tumor tissue chip (MBTC), is composed of a top fluidic chamber, a porous membrane covered with endothelial cell (EC) monolayer, and a collagen gel block filled with tumor cells. Using this device, the group successfully recapitulated T cell extravasation and interstitial movement on a chip. T cell activities such as intraluminal crawling and transendothelial migration (TEM) were observed, and tumor-associated ECs showed

disrupted adherens junctions with increased permeability and NO production. Anergic ECs were unresponsive to inflammatory cytokines such as TNF-α, limiting T cell attachment and TEM. Using anti-vascular endothelial growth factor (anti-VEGF) treatment was able to revert EC anergy and promote T cell infiltration into the tumor.

Third, T cell activation, survival, and proliferation in the immunosuppressive TME are also studied using various novel microphysiological systems. Ma et al. developed a biomimetic leukemia-on-a-chip platform to dissect the immunosuppressive components in the bone marrow microenvironment potentially for testing the CAR-T therapy (Figure 1D).[26] The device was compartmentalized into a central venous sinus, a medullary cavity, and an endosteal region by regularly spaced trapezoid micropillars that confine cell-embedded hydrogels. Using the platform, the group was able to compare how perivascular, endosteal, and hematopoietic niche-derived factors maintained the survival and quiescence of different B cell acute lymphoblastic leukemia (B-ALL) subtypes, including ETV6-RUNX1+ REH B-ALL and Philadelphia chromosome-positive (Ph+) SUP-B15 B-ALL. They found that CXCL12/CXCR4 cytokine signaling axis induced leukemia progression and that VCAM-1/VLA-4 adhesive signaling axis enhanced B-ALL adhesion and clustering as well as their ability to engage with perivascular stromal cells via the nuclear factor (NF)-κB pathway. While ECs may enhance VCAM-1 singling to regulate B-ALL progression, osteoblasts, and mesenchymal stem cells (MSC) promoted B-ALL dormancy via osteopontin (OPN) signaling. Future studies can be expanded on this model by increasing its biological complexity with the addition of patient-derived immune cells and testing different CAR-T constructs.

3.3. MPS for Screening Immune Checkpoint Blockade (ICB)

Immune checkpoints are immune co-stimulatory molecules that regulate the activation and cytotoxicity of T cells.[51] Cancer cells sometimes find ways to use these checkpoints to avoid being attacked by the immune cells. Two negative costimulatory molecules of particular interest found on T cells are programmed cell death 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4).[52] PD-1 acts as an "off switch" that limits the activity of T cells in peripheral tissues.[51b] The programmed cell death ligand 1 (PD-L1) is commonly upregulated on the tumor cell surface from many different human tumors. Forced expression of PD-L1 on tumor cells inhibits local anti-tumor T cell-mediated responses.[51b] On the other hand, T cells' CTLA-4 (inhibitory) competes with CD28 (stimulatory) by binding to B7 on antigen-presenting cells like DCs.[51,53] CTLA-4 checkpoint protein prevents DCs from priming T cells to recognize tumors. The "brakes" on the immune system are released, and T cells can kill the cancer cells when these proteins are blocked. Blockades of PD-1 with nivolumab and pembrolizumab or CTLA-4 with ipilimumab demonstrate highly efficient anti-tumor activity and have been approved by FDA for the clinical treatment of patients with a broad range of tumor types.[51a] The ex vivo 3D tumor immune models that incorporate the local TME-specific features may as well facilitate monitoring of the dynamic responses to various ICB regimens, which will allow the effective profiling and decision-making of more personalized and effective ICB therapies (**Figure 5**).

Peranzoni and coworkers investigated how macrophages impede natural T cell functions via PD- L1/PD-1 signaling in mice models, which took 24–28 days to complete.[58] On the other hand, Cui and colleagues developed a glioblastoma (GBM)-on-a-chip to dissect the immunosuppressive components, including the effect of macrophages on cytotoxic T cells, in different tumor subtypes treated with ICB (Figure 1C).[25] The establishment of an ex vivo tumor chip largely shortened the culture and analysis time to three days. The group found abundant CD163+ tumor-associated macrophages (CD163+ M2 TAMs) were attracted to the GBM niche with elevated expression of PD-1/PD-L1, TGF-β, IL-10, and CSF-1 cytokines. Blocking the CSF-1 receptor with BLZ945 successfully ablated CD163+ M2 TAMs and strengthened CD154+CD8+ T-cell functionality and GBM apoptosis on-chip by enhancing the PD-1 inhibitor nivolumab efficacy.

Ayuso et al. also developed a tumor chip to evaluate the role of TME stress on natural killer (NK) cell exhaustion.[54] The model consists of a central chamber where breast cancer cells (MCF7) with/without NK cells (NK-92) were embedded in a collagen hydrogel (Figure 5A). A lumen located at one end of the chamber was lined with human umbilical vein endothelial cells (HUVECs) and perfused with a medium to nourish the cells, mimicking nutrient, pH, proliferation, and necrosis gradients across solid tumors. They found that NK cells exhibited exhaustion by upregulating the expression of IDO-1, PD-1, and CTLA-4, and the molecular and functional impact on NK cells was permanent and persisted even after removing the environmental stress for an adequate time. Using ICB like Atezolizumab alleviated NK cell exhaustion and improved their killing efficiency, but some tumor cells still managed to survive at regions distal to the blood vessel, suggesting the importance of vessel reconstruction in solid tumors for immune cell trafficking, infiltration, and ultimately complete eradication of tumors.

Jenkins' group used murine- and patient-derived organotypic tumor spheroids (MDOTS/PDOTS) and successfully demonstrated immune checkpoint sensitivity of PDOTS and testing of combination therapy with TANK binding kinase (TBK)−1 or cyclin-dependent kinases 4 and 6 (CDK4/6) (Figure 5B).[55,59] The device was fabricated using cyclic olefin polymer (COP) with three microfluidic chambers, each with a central gel channel flanked by two media channels. The group successfully expanded the evaluation of tumor-immune interactions using RNA-sequencing and recapitulated the results in their animal studies, though they acknowledged the limitation of their platform in capturing T-cell priming. Jiang et al. developed an immunotherapeutic high-throughput observation chamber (iHOC) to study the immune checkpoint molecules on triple-negative breast cancer spheroids (Figure 5C).[56] The device was composed of an array of 3D-printed conical microwells to house the tumor spheroids cocultured with T cells and a micropillar array fabricated by photolithography for sampling secreted biomarkers from the microwells. The device was able to detect the IL-2 level secreted by T cells and monitor T cell infiltration into tumor spheroids. It is worth noting that both Jenkins and Jiang incorporated tumor organoids into microfluidic devices for faster drug screening and testing.

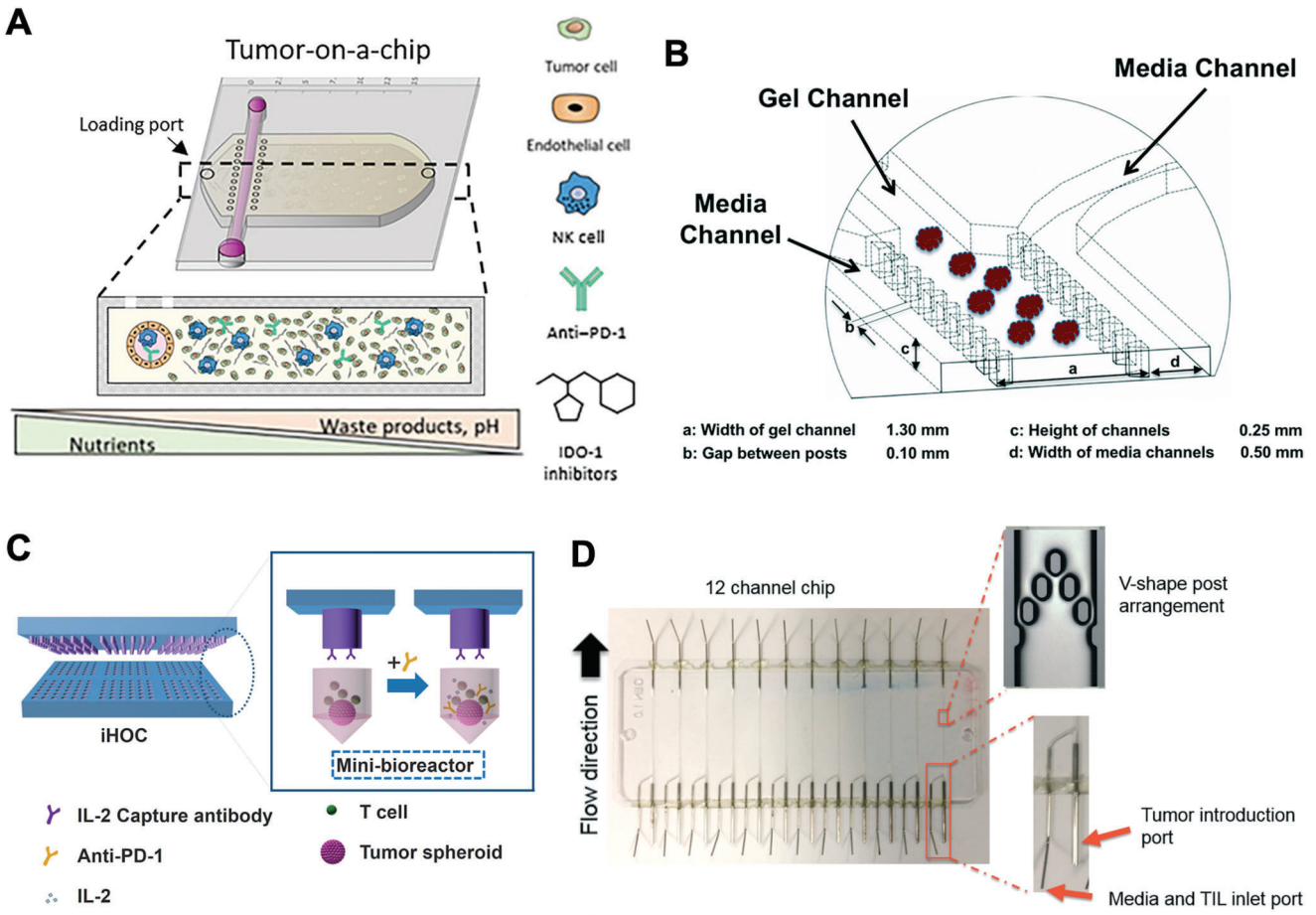

Figure 5. Microphysiological systems for screening immune checkpoint blockade. A) Scheme of the tumor-on-a-chip microdevice. The bottom panel shows the microdevice cross section. The lumen was lined with endothelial cells (e.g., HUVECs) to generate a blood vessel surrogate, allowing the perfusion of the medium, NK-92 cells, anti-PD-L1 antibodies (i.e., atezolizumab), or IDO-1 inhibitors (i.e., epacadostat). Reproduced with permission.[54] Copyright 2021, American Association for the Advancement of Science. B) A microfluidic device to culture organotypic tumor spheroids. Each device contains a center gel region with posts separating the gel region from the anti-parallel side channels. Reproduced with permission.[55] Copyright 2018, Royal Society of Chemistry. C) Schematic illustration of the immunotherapeutic high-throughput observer chamber (iHOC). The iHOC consists of microwell cell cultures and complimentary micropillar arrays for cytokine monitoring. The interactions among tumor spheroids, immune cells, and ICIs can be directly observed in the mini-bioreactor arrays. Reproduced with permission.[56] Copyright 2021, Wiley. D) Cyclic olefin copolymer (COC) bonded assembly with patterned channel layer laminated to thin, flat COC cover film. Upper Right Inset: Embedded 5-post V-trap design for capturing tumor sample in the flow stream, located partway along each channel in the location shown. Lower Right Inset: Branched design with a dual port entry for media and tumor-infiltrating lymphocytes (TILs) at the left and straight portion tumor fragment introduction port. Reproduced with permission.[57] Copyright 2018, Royal Society of Chemistry.

Compared to their models, Moore and coworkers developed a more dynamic microfluidic system, termed "EVIDENT" (ex vivo immuno-oncology dynamic environment for tumor biopsies), to test various ICB treatments and corresponding tumor responses (Figure 5D).[57] The device can directly incorporate patient-derived tumor tissues and create a better physiologically relevant environment under dynamic fluid flow. Such a platform is even more powerful and clinically ready in terms of the usage of low-sorption materials, precise and simple flow control by a single pressure-driven pump, the ease of scaling up the device to accommodate more samples, and machine learning automatic analysis of images and results. The platform can be used to quantify the level of T cell infiltration and tumor death by time and test different ICB regimens for personalized therapy and precision medicine.

All the aforementioned devices can recapitulate T cell extravasation and engraftment events within the TME, perform ex vivo profiling of PD-1 and CTLA-4 blockade for interrogating the tumor immune microenvironment, as well as develop therapeutic combinations and facilitate precision immuno-oncology efforts.[41] Nevertheless, unlike single-cell and pair-cell microfluidic platforms, the purpose and complexity of these microphysiological systems also limit their capability of studying the early events in the cancer-immune cycle such as APC trafficking and T-cell priming. Balancing the need and outcome of the studies is the key to creating MPS for better clinical applications (**Table 1**).

Table 1. A summary of various microphysiological systems used for researching and developing cancer immunotherapies.

Group	Cancer type	Tumor cell	Immune cell	Stromal cell	Application	Technical highlight
Huang et al.[28b]	Leukemia	THP-1	Monocyte		Single cell cytokine profiling	PDMS microfiltration membrane
Xue et al.[28a]			CD19 CAR-T		Single cell cytokine profiling	Antibody barcode array
Tiemeijer et al.[28c]			CD8 T cell		Single cell cytokine profiling	Single-cell droplet
Briones et al.[28d]			Jurkat T cell		Single cell granzyme B activity assay	Valve-based microfluidics
Mattei et al.[10b]	Skin	B16F10	Splenocyte		Immune cell migration	Microchannel array
Tu et al.[10d]	Leukemia	C1498	CD8 T cell		T cell activation and killing	Microwell array
Parlato et al.[10e]	Colorectal	SW620	DC		DC extravasation	Microchannel array
Henderson et al.[36]				LEC[a)]	EC junction formation	Vessel lumen by needle-driven approach
Goyal et al.[31c]			B cell, T cell, DC		Lymphoid follicle formation and vaccine testing	Porous membrane
Pisano et al.[30a]	Breast	MDA-MB-231		LEC	Tumor transendothelial migration	Interstitial flow
Toh et al.[6]	Breast	MX1			Tumor metastasis and invasion	Laminar flow polyelectrolyte coacervation
Nguyen et al.[30b]	Breast	BT474, MCF7	PBMC	HUVEC, CAF	Trastuzumab effects on tumor microenvironment	Microchambers separated by micropillars
Kutys et al.[38]	Breast	MCF10A (noncancerous)		hMVEC[b)]	3D morphogenic behaviors of endothelium	Vascularized mammary duct
Nguyen et al.[12a]	Pancreatic	PD7591		HUVEC	Endothelial ablation by tumor	Vessel lumen by needle-driven approach
Kwak et al.[39]	Breast	MDA-MB-231		hMVEC, hFOB[c)], hMSC, HLF[d)]	Organ-specific tumor cell extravasation and colonization	Multiplayer overhung structure for needle guide
Pavesi et al.[23]	Liver	HepG2	TCR T cell		Engineered T cell testing	Microchannels separated by trapezoidal pillars
Lee et al.[47]	Liver	HepG2	TCR T cell, monocyte		Engineered T cell screening	Microchannels separated by trapezoidal pillars
Segaliny et al.[10c]	Leukemia	K562	TCR T cell		Engineered T cell screening	Inverted floating droplet array
Wang et al.[31b]	Leukemia	RPMI 8226, MM.1s	CAR T cell		Engineered T cell trafficking, clustering, and killing dynamics	Multifaceted micropatterned tumor array
Lee et al.[31a]	Skin	B16F10-ova	OT-1 T cell	bEnd.3	T cell migration and infiltration	Multilayered blood vessel/tumor tissue chip
Ma et al.[26]	Leukemia	Reh, SUP-B15		HUVEC, hMSC, hFOB	Tumor progression and subtype characterization	Circular design mimicking bone marrow niche
Cui et al.[25]	Brain	Patient-derived GBM	CD8 T cell, macrophage	hMVEC	ICB screening on tumor subtypes	Circular design mimicking brain tumor niche
Ayuso et al.[54]	Breast	MCF7	NK-92	HUVEC	ICB screening on immune cell	Asymmetric design generating chemical gradients
Aref et al.[55]	Colorectal, skin, lung, brain	MC38, CT26, B16, GL261, patient	Multiple	Multiple	ICB-based combinatory therapy testing	Fabricated with Cyclic olefin polymer
Jiang et al.[56]	Breast	MDA-MB-231	Jurkat T cell		ICB testing and immune cell monitoring	3D-printed conical microwells
Moore et al.[57]	Colorectal	MC38, patient	CD8 T cell		ICB screening	Cyclic olefin polymer and V-shape post trap

[a)] lymphatic endothelial cell; [b)] human microvascular endothelial cell; [c)] human fetal osteoblasts; [d)] human lung fibroblast

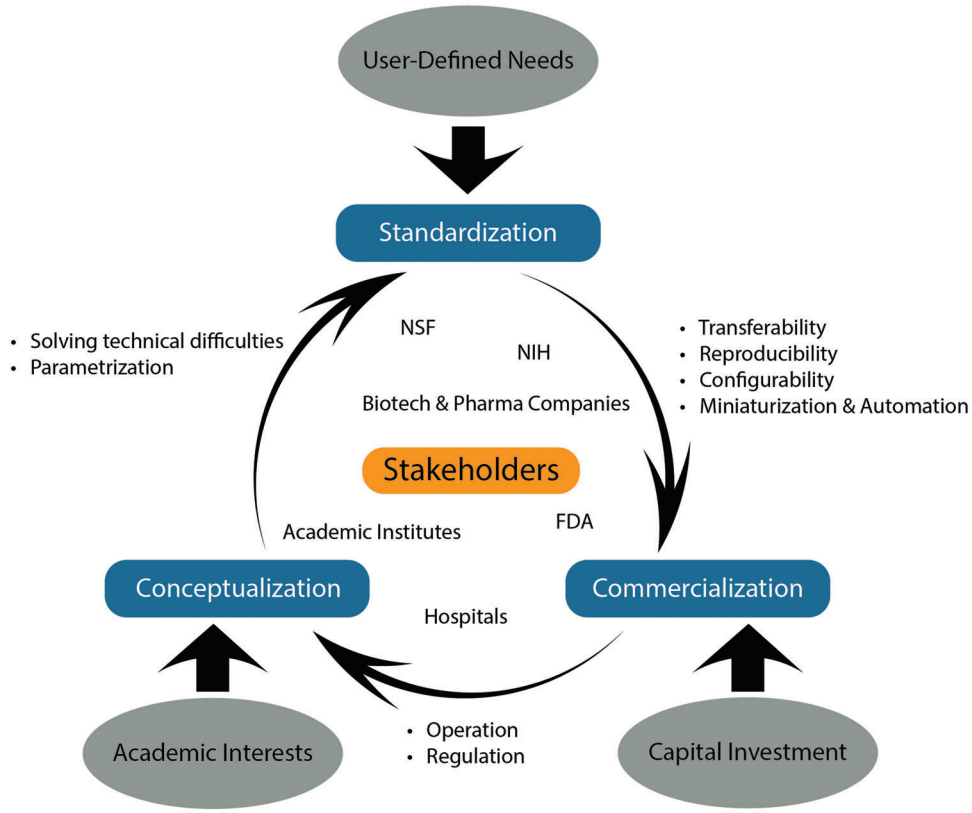

Figure 6. Academic interests promote conceptualization, user-defined needs drive standardization, capital investments initiate commercialization. Early and close collaboration between academic institutes, industrial R&D departments, and healthcare agencies during each stage of MPS development will fulfill different interests and needs and generate a positive feedback loop to corroborate the effectiveness of MPS platforms and maximize their utility in the actual healthcare industry.

4. Current Challenges and Future Perspectives on Clinical Translation of Microphysiological Systems for Cancer Immunotherapy

Despite the revolutionary power that MPS may bring to the cancer field, the overall improvement of immunotherapy development has yet to be determined, with grand challenges existing in the transition from basic research to preclinical applications, and ultimately successful commercialization of MPS products.[21b]

Currently most microphysiological systems are fabricated in research laboratories manually using the soft lithography technique. The low throughput and reproducibility of the manufacturing process is difficult for large-scale production and translation of MPS for the potential market: new technologies to achieve both physiological relevance and high-throughput power would be necessary. Also, the widely used fabrication material, PDMS, requires innovation, and shall aim for more biologically inert, air- and liquid-impermeable materials, like plastics, to reduce absorption of hydrophobic biomolecules and bubble formation.[42a,57] Barriers in reliable cell sourcing, maintaining, and integrating also restrain large-scale production of MPS, and hardships in cell retrieval from the devices further hinder downstream molecular analyses such as flow cytometry, polymerase chain reaction (PCR), and ELISA.[60] Scaling of the systems relevant to normal human organ geometry and dimension is another critical factor to consider during the design of MPS and data analysis. For instance, the cell-to-media ratio and evaporation speed of the media both account for the functional and metabolic dynamics of cells as well as their drug response. Sampling frequency and volume from the devices may also affect study results. Data retrieved from MPS shall be benchmarked against "omics" (e.g., genomics, transcriptomics, proteomics) and animal studies.[21a]

To address these challenges, the current framework for developing MPS may be specified into three phases: conceptualization, standardization, and commercialization (**Figure 6**).[61]

4.1. Academic Interests Promote Conceptualization

Conceptualization first lays a foundation for its physiological relevance to the human body as microphysiological systems aim to emulate the in vivo human physiology and tumor microenvironment. MPS is proven to be a valid platform to screen cancer immunotherapies, and most of the outcomes align with the subsequent animal studies; however, preclinical results obtained from MPS platforms still need to be validated on xenograft murine models, which is the current gold standard for drug development.[62] MPS are mostly published and researched within the field of engineering, and as microfluidic devices are further developed, studies need to approach the broader scientific community across disciplines and industries.[7] Bridging the gap between engineering and medicine is important since we

need more medical applications rather than engineering proof of concept. With a more integrated and collaborative method of research, MPS can be further designed to be more applicable, useful, and cost-effective.

4.2. User-Defined Needs Drive Standardization

Standardization looks from the analytical perspective, which centers on the analysis of large data sets obtained from the MPS devices, validation of their diagnostic and therapeutic usage, and reproducibility of effective and predictable models in any of their practices. A series of requirements for consideration during the development of MPS technology had been described by Sutherland et al.[63] For example, the combination of tunable biomaterials with MPS will allow researchers to modulate the tumor stromal environment and promote tissue regeneration at tumor sites. Advanced biomaterials and fabrication techniques such as 3D bioprinting not only give a more accurate representation of the tissues but also enable more intricate designs, rapid prototyping, and mass production of the devices.[64] With more complex designs of the microfluidic 3D space, modeling individual metastatic steps will become possible, allowing immunotherapies to be tested at each phase of cancer.[65] Implantable MPS have the potential to temporally monitor a patient's cancer metastasis and control drug release to the tumor-specific site.[66] In addition, functional single-cell selection and sequencing may link genomic and transcriptomic profiles to phenotype and cellular characteristics to study metastatic or therapy-resistant cancer cells.[67] The advance of microfluidic devices, in specific single-cell analysis, results in a much higher influx of data than previous 2D or nontemporal methods. To fully use the results of research in this field, new computational advances, such as AI and supercomputers, must go hand in hand with device development.[68]

4.3. Capital Investments Initiate Commercialization

Finally, though still too early to consider, the commercialization of MPS technology emphasizes the industrial perspective, including ease of operation, rapid mass production, and government regulation. Concerns may arise regarding the fulfillment of government regulation in intellectual property protection and patent management, which are key elements in the commercializing process. The standardization of various concepts, such as modular and reconfigurable components of the MPS, may help accelerate the process of acquiring regulatory approval and commercialization.[69] The essence of the MPS guarantees its ability to embrace patient-specific cells and capture genetic as well as physiological differences, therefore making it possible to customize treatments. For those unfit for standard clinical trial designs, MPS and personalized medicine offer new solutions and may optimize the treatment for specific patient biology.[70] In the long run, MPS has the potential to bridge the gap between preclinical cancer drug development and clinical trials.[21b,71]

5. Concluding Remarks

While animal models have been used for a long time in biomedical research, they are genetically different from humans, and there have been ethical issues in using live animals as experimental tools. It is also difficult to use animal models to establish the mechanisms underlying tumor progression and immunity owing to the inability to decouple the relative contributions of biological and biophysical factors. In contrast, 2D cell culture models are highly controllable with no ethical issues but do not recapitulate the 3D organization and function of the tumor microenvironment in vivo. Microphysiological system is attracting attention for their unique advantages in engineering the process of treating cancer and their substantial promise for personalized and precision medicine. As a powerful and robust screening platform for novel and combined therapies, such as the ultrasound-targeted microbubble destruction (UTMD)[72] and tumor-targeted T-cell bispecific antibodies,[73] MPS will greatly improve the therapeutic efficacy, treatment sensitivity, and clinical response rate, and have a broader impact on the drug development process. We believe MPS has the potential to bring revolutionary changes in the cancer research community and become the next-generation standard in scientific research.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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