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Ex Vivo Tumor-on-a-Chip Platforms to Study Intercellular Interactions within the Tumor Microenvironment

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Abstract

The emergence of immunotherapies and recent FDA approval of several of them makes them a promising therapeutic strategy for cancer. While these advancements underscore the potential of engaging the immune system to target tumors, this approach has so far been efficient only for certain cancers. Extending immunotherapy as a widely acceptable treatment for various cancers requires a deeper understanding of the interactions of tumor cells within the tumor microenvironment (TME). The immune cells are a key component of the TME, which also includes other stromal cells, soluble factors, and extracellular matrix-based cues. While in vivo studies function as a gold standard, tissue-engineered microphysiological tumor models can offer patient-specific insights into cancer-immune interactions. These platforms, which recapitulate cellular and non-cellular components of the TME, enable a systematic understanding of the contribution of each component toward disease progression in isolation and in concert. Microfluidic-based microphysiological platforms recreating these environments, also known as “tumor-on-a-chip,” are increasingly being utilized to study the effect of various elements of TME on tumor development. Herein are reviewed advancements in tumor-on-a-chip technology that are developed and used to understand the interaction of tumor cells with other surrounding cells, including immune cells, in the TME.

Keywords

microphysiological systems; tumor-on-a-chip; tumor-immune interactions; co-culture systems

Conflict of Interest

The authors declare no conflict of interest.

1. Introduction

Advancements in 3D cell culture systems have played a key role toward the development of microphysiological systems. Such systems have been widely used to study the dynamic interplay between cells and their microenvironment given that cells in their native state reside within a 3D environment. This 3D environment is composed of different cell populations, extracellular matrix (ECM), and soluble molecules like growth factors and cytokines. In recent years, integration of 3D cell/tissue culture systems with microfluidics has led to the development of new platforms known as microphysiological systems or organ-on-a-chip.^[1,2] Microfluidics ensures that the system is continuously perfused with fresh media, which gives it an advantage over corresponding static 3D cell cultures. The ability of these systems to capture several organ level architectures and physiologies such as tissue barriers, mass transport, and vascular perfusion gives them a significant lead over conventional 2D and 3D cultures to not only study various processes associated with organ development and function but also to be used as a platform to advance drug discovery.^[3–5]

In their simplest form, organ(s)-on-a-chip consist of a single cell type lined along a microchannel or seeded in a single chamber while being perfused with media. More advanced models with multiple microchannels separated by membranes to mimic tissue interfaces and barriers such as air–liquid interface (ALI), blood–brain barrier (BBB), and blood–retinal barrier (BRB) have led to the development of lung-on-a-chip,^[6,7] BBB-on-a-chip,^[8–15] and BRB-on-a-chip,^[16] respectively. Optical transparency of these devices allows real time visualization and imaging enabling easier in situ characterization. By employing biomaterials with the necessary chemical and mechanical properties, the cells can be provided with tissue-specific ECM-based nonsoluble cues.^[17] Furthermore, tunable channel dimensions and perfusion rates aid in the recreation of physiologically relevant shear stresses. While the biomaterials can provide static mechanical properties (e.g., matrix rigidity), sophisticated fabrication designs can be incorporated to introduce dynamic mechanical strains emulating organ functions such as breathing,^[7] peristalsis,^[18] heartbeat,^[19] and blinking,^[20] among others. Incorporating such complexities has allowed for the development of intricate technological platforms recapitulating tissue-specific functions ex vivo, which has so far not been possible using conventional in vitro models. Harnessing such models can provide insight into organ-specific drug efficacy or response to perturbations. For example, the lung-on-a-chip model has demonstrated the effect of breathing on the inflammatory response against nanoparticles in the airway.^[7]

Continued advancements in this field has led to the development of several organ-on-a-chip platforms. There now exist microfluidic chips for lung,^[6,7] liver,^[21,22] heart,^[23,24] intestine,^[18] kidney,^[25–29] brain,^[8–15] artery,^[30] skin,^[31–35] eye,^[36–38] pancreas,^[39,40] and skeletal muscle,^[41–43] among several others. Studies have started to interconnect different engineered organs to generate microphysiological systems with a larger aim of creating integrated multiorgan-on-a-chip platforms (often termed as “body-on-a-chip”).^[44–51] In addition to being a tool to understand tissue development and maturation, such integrated systems could also function as a more physiologically relevant technological platform to assess organ-dependent drug toxicity, which is very important for many cancer drugs as well as for pharmacokinetics and pharmacodynamics studies.^[50]

2. Tumor-on-a-Chip Models

Conventional in vitro methods to study tumors include 2D cultures, transwell assays, 3D spheroid cultures, and scaffold-assisted 3D cultures. Each of these methods offer certain advantages and disadvantages that have been reviewed in other articles.^[52,53] These in vitro cultures integrated with microfluidics, widely termed as tumor-on-a-chip models, have been used extensively to study various characteristics associated with tumor progression such as growth, angiogenesis, metastasis, and drug response. Studies over the years have shown the role of the tumor microenvironment (TME), including both cellular and noncellular components, on tumor growth and metastasis.^[54,55] The primary cellular components of the TME are cancer associated fibroblasts (CAFs) of the stroma, immune, and inflammatory cells, cancer stem cells, and endothelial cells of vascular networks.^[55] The noncellular part is mainly composed of the surrounding ECM, soluble factors, and interstitial pressure.^[56] The ECM of the TME provides various biophysical cues (e.g., mechanical and topographical cues) due to the changes that the ECM undergoes with tumor growth. For instance, most solid tumors manifest a change in tissue mechanical properties, which is a result of excessive ECM accumulation, as well as the changes in the ECM architecture. Various studies have demonstrated the key role played by the ECM on growth and metastasis of cancer.^[57–62] These understandings suggest that a deeper insight into cancer development is not feasible by studying cancer cells in isolation but should rather be studied in terms of their interaction with associated cells and the ECM changes in the TME. Furthermore, pertaining to the critical role it plays in cancer progression, the TME is increasingly being investigated as a potential therapeutic target to treat various cancers.^[54]

Tumor-on-a-chip allows for the recreation of many of the cardinal TME features such as multicellular interactions, ECM-based biochemical properties (by using biomaterials to encapsulate the cells), biophysical cues^[63] and their gradients, hypoxia,^[64] and others. Below we discuss some of the recent studies that have examined interactions of cancer cells with the cellular components of the TME individually or in concert within a microfluidic chip. Owing to several other interesting reviews looking into the interactions of tumor with noncellular components,^[53,56] we do not discuss the tumor–ECM interactions in this review.

2.1. Tumor–Stroma Interactions on a Chip

Devices that allow spatiotemporal control of multicellular cultures serve as an invaluable tool to study the crosstalk between cancer cells and other cells found in the stroma. Among the nonmalignant cancer cells in the TME, cancer-associated fibroblasts (CAFs) are the most common. Unlike normal fibroblasts (NFs) which are activated as a response to injury or stress, CAFs remain in a prolonged activated state. Several studies have shown the critical role of CAFs in cancer progression, although the mechanisms by which they promote disease progression have yet to be unraveled.^[65] Given that fibroblasts are one of the key cell populations in the stroma, a number of studies have examined tumor–fibroblast interactions on chip.^[66–71] Integration of microfabrication along with biomaterial-assisted cell encapsulation can be used to compartmentalize different cell populations.^[72] Coculture systems facilitating reciprocal interactions of different cell populations are better than the

corresponding conditioned medium cultures to study the effect of cell–cell communication.^[73,74] Compared to 3D culture systems, microfluidic coculture systems provide a better spatiotemporal control and can be used to study cell–cell communications via direct contact or through exchange of soluble factors in a systematic and defined manner. Below we discuss in detail some of the studies that employed microfluidics along with 3D cultures to generate cocultures of cancer and stromal cells.

A multichannel microfluidic coculture system was used to study the effect of pancreatic stellate cells (PSCs) on human pancreatic cancer cells.^[69] Specifically, human pancreatic cancer cells (PANC-1 cell line) were cocultured with pancreatic stellate cells (HPaSteC). PSCs mostly consist of CAFs and are found in the stroma of pancreatic ductal adenocarcinoma.^[75] The cancer cells were embedded in collagen and housed in a central channel while two channels on either side contained collagen-embedded PSCs. In the presence of PSCs, cancer cells displayed increased migration, growth, endothelial to mesenchymal transition (EMT), and drug resistance. In another study,^[67] bone marrow stromal cells (HS5) (which are of fibroblastic origin) and a liver tumor cell line (HuH7) were cultured in distinct compartments separated by a hydrophobic barrier within a microfluidic device. During the initial culture period, the hydrophobic barrier was used to separate the two cell populations from coming in contact with each other directly. After the initial segregated culture, the hydrophobic barrier was removed upon the cells reaching confluence, which enabled direct contact of the two different cell populations. Contact of tumor cells with HS5 cells resulted in a more aggressive and proliferative behavior of tumor cells and a faster death rate of the stromal cells as compared to those lacking a direct contact. The physical contact between the two cell types was mediated by HuH7 cells developing membrane protrusions called tunneling nanotubes (TNTs). Further, it was observed that the concentration of reactive oxidative species (ROS) in the coculture was fivefold higher than that in the corresponding monocultures. This increase in ROS concentration in the coculture could have contributed to the death of stromal cells. Increase in ROS concentration upon physical contact of tumor cells with endothelial cells is known to cause endothelial cell apoptosis via cell membrane oxidation.^[76]

Another microfluidic-based coculture system has used openings along the channel walls to separate the compartments containing cancer cells and fibroblasts (Figure 1A).^[68] Spheroids of human colorectal cancer cell line HT-29 (Figure 1B) were cocultured with CCD-18Co fibroblasts (Figure 1C) in parallel channels separated by a channel filled with medium. The openings in the channel walls permitted exchange of soluble factors for cell–cell communication in the absence of their direct contact. Moreover, because of intermittent perfusion (by turning the flow on and off), concentration gradients were likely to be generated between the cell channels. This concentration gradient could promote migration of fibroblasts toward the tumor spheroids. Compared to monocultures, tumor spheroids in the cocultures were found to be of larger size and had more fibronectin expression (Figure 1D) with reduced drug uptake. These observations are consistent with previously reported studies, which showed low drug penetration with increased ECM deposition.^[77] A recent study^[78] that looked into cancer cell invasion into an enclosed tumor–stroma microenvironment used a microfluidic device with compartmentalized chambers for tumor and stroma. SUM-159 breast cancer cells encapsulated in a 1:1 mixture of Matrigel and

collagen were seeded in the inner chamber (tumor region) and CAFs encapsulated in collagen hydrogel were confined in the surrounding outer chamber (stroma region). The presence of CAFs resulted in increased cell invasion as evident by a greater invasion distance compared to control with no CAFs.

Microfluidic-enabled tumor-on-a-chip systems have also been used to evaluate the interactions between cancerous and corresponding noncancerous cells. For instance, normal breast cells (HMEpiC) and breast cancer cells (MDA-MB-231) were cocultured in three different ratios to create tumor models with varying severity (mild, moderate, and severe).^[66] Migration of MDA-MB-231 cells was found to be significantly increased in the presence of HMEpiC. This was confirmed by measuring the migration distance as well as expression level of IL-6 protein, which is associated with cell migration.

2.2. Tumor–Endothelial Cell Interactions on a Chip

Angiogenesis, the formation of new blood vessels from existing vasculature, is a key facilitator of cancer growth and metastasis. Besides facilitating supply of nutrients to cancer cells, the newly formed vessels within the tumor serve as a route for cancer cells to break from the tumor and enter the circulation system.^[79] The metastatic process, also called the metastatic cascade, consists of three sequential steps: invasion, intravasation, and extravasation. Several tumor-on-a-chip models have been employed to gain insights into these interactions and mimic several of these processes in vitro.^[80–87] In this review, we focus on multicellular cultures to study angiogenesis and metastasis via cancer–endothelial cell interactions.

In an effort to study the role of glioblastoma in neovascularization, Kim et al.^[80] used a microfluidic device where the fibrin gel embedded glioblastoma cells, U87MG, were placed within a microchannel with endothelial cells (HUVECs) within a side channel. The endothelial cells were allowed to form a vertical monolayer wall at the fibrin gel boundary. The HUVECs were found to invade the adjacent fibrin matrix and formed vascular sprouts. Over time, the sprouts started fusing with neighboring vessels instead of extending directionally towards the cancer cells which is akin to in vivo observations.^[79] Another recent study by Aung et al.^[83] utilized the ability of HUVEC cells to migrate against the concentration gradient generated by the perfusing medium in a microfluidic device to create an endothelial wall around the cancer cell spheroid-laden matrices.

In a recent work by Nagaraju et al.^[86] to study cancer cell invasion and intravasation into 3D stroma, a three layered microfluidic device was used. The three interconnected layers were meant to compartmentalize tumor, stroma, and vasculature. Cells used for the tumor region were embedded in collagen gel while endothelial cells used for the vasculature network were embedded in fibrin gel. The stromal region, middle compartment, consisted of acellular collagen. MDA-MB-231 breast cancer cells displayed elongated morphology and enhanced invasion into the stromal region in the presence of vascular networks. Over time, cancer cells were able to cross the stroma and successfully intravasate into the vascular networks, whereas in the absence of vasculature, no such invasion was observed into acellular fibrin gel. Distinct but interacting compartments in this device enabled the study of reciprocal

relationships between tumor–endothelial interactions on key processes involved with cancer progression.

Tumor-on-a-chip platforms incorporating endothelial cells have been used to study extravasation. One such study has compared the metastatic potential of different cancers.^[84] A multichannel device containing endothelial cells (HUVECs), normal lung fibroblasts (NHLFs), and medium placed intermittently was used to study extravasation of different cancer cell lines, where the cancer cells were introduced into the medium. Prior to the introduction of cancer cells, the endothelial cells were allowed to organize into vessels and form lumens. The device was fabricated to allow the lumen to establish a continuum with the channel containing medium allowing the perfusion of the formed vascular network. The tumor cells in the medium which entered the vascular network were found to breach the endothelium and invade into the outer 3D matrix region. A positive correlation was found between the extravasation efficiency and metastatic potential of three different cell lines: HT1080>MDA-MB-231>MCF-10A. Another study^[87] by the same group looked into the extravasation of breast cancer cells into organ-specific microenvironments within a microfluidic device. Human bone marrow-derived mesenchymal progenitor cells (hBM-MSCs), osteo-differentiated (OD) primary hBM-MSCs, and endothelial cells (HUVECs) were embedded in a fibrin gel within a microchannel (Figure 2A). Upon perfusable vascular network formation by endothelial cells as depicted in Figure 2B, breast cancer cells (katushka-expressing bone seeking clone (BOKL) of the MDA-MB-231) were introduced into the medium which flowed into the vasculature, adhered to the endothelium, and subsequently transmigrated into various microenvironments (Figure 2C). The extravasation rate (into the bone tissue) was found to be significantly higher as compared to that in a muscle mimicking microenvironment which was created by co-culturing endothelial cells with C2C12 myoblasts. It was hypothesized that C2C12-secreted adenosine was responsible for the antimetastatic effect in the muscle microenvironment. This was confirmed by addition of external adenosine to the bone microenvironment, which resulted in decreased extravasation.

Microfluidic devices were also used to study angiogenesis in liquid tumors such as leukemia.^[82] Leukemic cells and endothelial cells (HUVECs) were seeded into two parallel microchannels separated by an acellular channel filled with collagen gel. When the channel designated for leukemic cells was maintained acellular, very few endothelial cells were found to invade the collagen matrix that separates the two channels. On the contrary, in the presence of leukemic cells, the endothelial cells invaded the collagen matrix and formed sprouts and neovessels. Furthermore, the angiogenic patterns were found to be leukemic cell specific, where U937, HL60, and K562 leukemic cells were tested. In another study involving renal cell carcinoma-on-a-chip, clear cell renal cell carcinoma (ccRCC) cells and normal-adjacent renal cortex cells were seeded in a collagen matrix surrounded by endothelial cells.^[81] Minimal sprouting was observed in the case of acellular collagen and normal-adjacent renal cortex cells, whereas in the presence of ccRCC cells, the endothelial cells showed more sprouting. The afore-described studies demonstrate the potential of tumor-on-a-chip systems on studying cancer-induced angiogenesis as well as metastasis.

2.3. Tumor–Immune Interactions on a Chip

As mentioned in the introduction, immune cells are a key component of the TME and play a crucial role in tumor growth and maintenance. Interactions between the immune cells and the tumor cells have been of great interest due to the potential of the immune cells to both attenuate and promote tumor progression. One of the premises of cancer immune therapy is in the molecular identification of tumor antigens, and studies over the years focusing on development of tumor vaccines have resulted in various levels of success. Sipuleucel-T is an FDA-approved vaccine for prostate cancer.^[88] Another approach is adoptive cell therapy (ACT), which involves patients' own or engineered cytotoxic T cells to eliminate cancer cells.^[89] CART-T cell therapy, a type of ACT, has recently been approved for the treatment of leukemia and lymphoma.^[90]

Another approach is the use of immune checkpoint inhibitors to activate a patient's own immune cells for targeting tumor. Herein, the drugs block the checkpoints placed by the immune system that are meant to prevent immune response by T cells. Programmed cell death protein 1 (PD-1) is a major protein expressed on the T cell surface which by binding to the programmed death ligand-1 (PDL-1) on dendritic cells helps the cancer cells to evade immune response.^[91] Several anti- PD-1 compounds have been developed as a class of immune checkpoint inhibitors, which prevent the protein–ligand binding, thus making the cancer cells vulnerable to immune response by T cells. Recently, FDA has approved some of these checkpoint inhibitors for non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and metastatic melanoma.^[92]

Although the aforementioned success points toward the promise of immunotherapies, there are several challenges that need to be resolved for the widespread use of these therapies. For instance, while many of these approaches have been found to work in some cancers, they fail to work in others and also tend to exhibit a patient-specific response.^[93–95] These observations, along with the prevalent role of cellular and molecular characteristics of the tumor microenvironment on immune cell function, call for a detailed and systematic understanding of the dynamic interactions between various immune cells and cancer cells. The key to further advance such immunotherapies will be to gain a deeper understanding of the tumor immune microenvironment (TIME)^[96]—a sub-microenvironment within the TME. Composition, localization, and functions of the immune cells form the basis of the broad classification of TIME. Infiltrated–excluded (I–E) TIMEs consist of tumors where cytotoxic lymphocytes (CTLs) are found around the boundary of the tumor mass but not at its core, largely because of their inability to infiltrate. On the contrary, infiltrated–inflamed (I–I) TIMEs have a high infiltration of PD-1 positive CTLs. A subclass of (I–I) TIMEs is TLS–TIMEs, where there is infiltration by CTLs along with the presence of tertiary lymphoid structures (TLSs) which consist of a mixed population of T cells, B cells, and dendritic cells.^[97]

Continued detailed analysis of the immunologic features of the TME, as well as the characteristics of the tumor microenvironment that promote infiltration of cytotoxic T cells, will advance immunotherapeutic strategies for treating various tumors. Tumor-on-a-chip offers a technological platform to study tumor–immune interactions within the TME in a

systematic and defined manner akin to tumor–stroma interactions, which cannot be easily achieved by simple tissue cultures or animal models.^[98]

Recently, a number of studies have used microfluidic-assisted tumor-on-a-chip systems to examine tumor–immune cell interactions. For instance, a study by Zervantonakis et al.^[99] has shown enhanced breaching of the endothelial cell barrier in the presence of macrophages by creating an intravasation model on a chip. Human breast carcinoma cells were seeded in a 3D matrix in a microchannel adjacent to another microchannel containing endothelial cells such that the endothelial cells formed a layer adjoining the 3D matrix. Upon intravasation, cancer cells migrated within the 3D matrix, reached the basal surface of the endothelial layer and subsequently breached the endothelial barrier to appear on the apical side. To study the role of macrophages in intravasation, macrophages were seeded in contact with the endothelial layer. In the presence of macrophages, ninefold more cancer cells breached the barrier to reach the apical surface of the endothelium as opposed to in the absence of macrophages where cells primarily remained on the basal side. The barrier permeability was confirmed by using 70 kDa dextran molecule. The macrophage-induced permeability of the endothelial wall was further confirmed by antibody blocking experiments. Antibodies against TNF- α mitigated the endothelial leakiness and thereby breaching by the cancer cells. Taken together, the results suggest that TNF- α signaling by the macrophages was involved with altering the barrier function to make it more permeable to cancer cells. The role of immune cells in promoting extravasation has also been demonstrated on a chip^[100] using a similar vasculature model as described in extravasation-on-a-chip study.^[84] The device consisted of multiple hydrogel regions—each with its own endothelial vascular network—within a single chip as shown in Figure 3A. Perfusion was achieved by creating a hydrostatic pressure drop in the reservoir (Figure 3B). Lipopolysaccharide-treated neutrophils (PMNs) were used to simulate an inflamed state within the chip. Once tumor cells (MA2) and neutrophils were inside the vascular networks, sequestration of neutrophils around the tumor cells was found to facilitate tumor cell extravasation via IL-8 and CXCL-1 (Figure 3C,D).

In a series of studies^[101–103] carried out to evaluate the role of interferon regulatory factor-8 (IRF-8) on the migratory response of immune cells toward tumor cells, a microfluidic device consisting of parallel channels seeded with mouse melanoma cells (B16) and mouse spleen cells was used. The channels occupied by the cells were separated by an acellular channel. The channels were connected by an array of perpendicular microchannels to facilitate migration. The spleen cells consisted of several immune cells including T lymphocytes, B lymphocytes, and phagocytes, among others. The role of transcription factor IRF-8 was evaluated on the leukocyte migration towards cancer cells. Wild-type splenocytes from immunocompetent mice exhibited drifted random walks that gave rise to a net movement towards melanoma cells as opposed to IRF-8 KO cells which were found to move in uncorrelated random walks that were not pointed at melanoma cells. These observations were consistent with in vivo studies that have demonstrated the migrational inability of IRF-8 KO cells toward tumor sites.^[104] In recent studies,^[105,106] the role of the formyl peptide receptor 1 (FPR1) gene in the migration of human peripheral blood mononuclear cells (PBMC) towards cancer cells was studied on a chip by using PBMCs with different levels of FPR1 expression—PBMCs with normal FPR1 expression, with one of the two copies of FPR1 functional (heterozygous mutants), or PBMCs lacking the FPR1 gene

entirely (homozygous mutants). The results showed a graded migrational response where the cells with normal FPR1 expression displayed a biased random walk towards MDA-MB-231 breast cancer cells. The biasness toward the cancer cells decreased in the case of heterozygous mutant cells, while the migration was reduced to an uncorrelated random walk in the case of homozygous mutants.

A modified microfluidic platform consisting of a recreated tumor and immune microenvironment was used to study the dynamic interactions of cancer cells and dendritic cells and factors involved in facilitating dendritic cell migration towards cancer cells.^[107] Dendritic cells are known to recognize dying tumor cells, take up tumor antigens, and deliver them to T cells to generate an immune response against tumors. Colorectal cancer (CRC) cells—untreated and treated with antitumor compounds—were seeded in the tumor compartments and interferon- α -conditioned dendritic cells (IFN-DCs) were cultured in the immune system compartment within the microfluidic device. Directed migration of IFN-DCs was observed toward treated cancer cells rather than toward the untreated cells, and the CXCR4/CXCL12 axis was found to guide the directed migration. Another study utilized a three-chambered microfluidic device to evaluate the effect of immune cells and TNF- α on cancer cells by coculturing lung cancer cells, macrophages, and myofibroblasts. TGF β -1 secreted by cancer cells was found to increase cancer cell motility, while TNF- α limited migration by restraining myofibroblast functions.^[108]

In a recent ex vivo study, tumor tissue fragments from mice and patients were housed in a multiplexed microfluidic device made of cyclic olefin copolymer (COC).^[109] The tissue-laden chip was perfused with media dispersed with tumor infiltrating lymphocytes (TILs) to study TIL infiltration and their potential to induce tumor cell apoptosis. A comparison study examining the role of TILs in the presence and absence of a checkpoint inhibitor, which used cultures exposed to media (without TILs), media containing TILs treated with isotype control antibodies, or media containing TILs treated with anti-PD-1 immune checkpoint inhibitor, showed significantly higher tumor cell death in the presence of the inhibitor. These findings are consistent with the previous observation where anti-PD-1 immunotherapy was found to be effective against the MC38 tumor model.^[110] Other recent ex vivo studies^[111,112] have also demonstrated microfluidics as a valuable tool to screen immune checkpoint inhibitors based on their response to murine-and patient-derived organotypic tumor spheroids (MDOTS/PDOTS). MDOTS/PDOTS are distinct from conventional spheroid cultures and PDX (patient-derived xenograft) models as unlike the latter, they consist of stromal and immune cells along with the tumor cells thus allowing for the study of multiple cell interactions within a single culture and in a closer-to-native environment.^[111] Table 1 summarizes various studies that incorporate multicellular cultures within microfluidic devices to study different processes associated with tumor progression.

3. Summary and Future Perspective

Despite tumor-on-a-chip being a promising tool to study the tumor microenvironment, there only exist a few examples that have utilized such microfluidic-assisted microphysiological systems to study the role of immune cells in tumor growth and destruction. Most of these studies have focused on cancer–ECM interactions or cancer–stromal interactions. Another

area where tumor-on-a-chip has been utilized is in recreating and evaluating tumor-associated angiogenesis and metastasis in vitro. Although the studied components are essential to the TME, they are mostly investigated in isolation. A natural next step would be to incorporate multiple aspects of the TME including stromal layer, multiple cell types, vasculature, and macrophages to replicate the complex and heterogenic nature of the TME. When incorporating immune components into these models, there is also a need to consider different types of immune cells and their contribution to the TME.

Integration of microfluidics, microfabrication, and biomaterial design could be used toward the development of a more realistic microphysiological system to replicate the TME and TIME. While the advancements in microfabrication can facilitate the confinement and spatial organization of cells and thereby the incorporation of various cell types relevant to TME, biomaterials can be designed to mimic the tumor-specific ECM properties. Furthermore, the flow profiles along with the microfabrication design could be used to achieve physiologically and pathologically relevant flow behaviors such as shear flow. In addition to understanding the reciprocal interactions of cancer cells and their surrounding environment, such systems could be redesigned in a modular fashion such that the contribution of each component towards cancer progression or dormancy could be systematically studied.

The cellular and molecular characteristics of the TME play a key role with regard to tumor escape. Understanding of the immunological features of the TME suggest strong correlations of immunophenotypes present in TME and disease prognosis. While in vivo studies involving animals provide crucial insights, tumor-on-a-chip platforms that are modular could be designed and utilized to study the contribution of various immunophenotypes on cancer eradication (or progression). Such an understanding along with animal studies could aid the development of new immunotherapeutic strategies.

Adoptive cell therapy such as chimeric antigen receptors (CAR) T-cell therapy involving cytotoxic T-cells armed with CAR were shown to be efficacious in treating leukemia.^[89,90] However, improving the effectiveness of T-cell-based therapies for solid tumors is still a challenge. Tumor-on-a-chip systems offer a unique platform to study the effects of various components of the TME on T cell infiltration. Dense ECM is a characteristic of solid tumors, and recapitulating such structural changes of the ECM and determining how physical properties of the ECM can be tuned to promote infiltration of T cells is needed. Identification of the key factors that promote infiltration of cells into the tumor site will increase the success of ACT approaches such as CAR-T. In addition, tumor-on-a-chip platforms could be used as a screening platform to study the efficiency and function of CAR-T cells and their batch-to-batch variability associated with ex vivo biomanufacturing and processing.

Like any other biological tissue, tumor is also evolving.^[113] While recapitulating all aspects of the evolution of cancer ex vivo is challenging, tumor-on-a-chip system can be used to study the real time changes that the tumor-associated ECM undergoes and/or compromised ECM remodeling with tumor progression and its influence on cellular composition and function. Biomaterial-based 3D encapsulation enables manipulation of matrix properties to

mimic various physicochemical changes of the ECM. Advancements in biomaterials that enable reversible interactions could provide a tool to recapitulate some of the dynamic features of the ECM. Similarly, stimuli-responsive multifunctional hydrogel could be used to introduce multiple dynamic cues to the cells simultaneously.

Another important concern is the patient-specific response to immunotherapies. Genetic predisposition as well as acquired mutations can lead to patients developing a resistance to immunotherapies.^[94,95] This calls for a need to conduct studies using a patient's biopsy samples and patient-derived induced pluripotent cells rather than immortalized cell lines. Combined with studying gene expression patterns in patients, such platforms can be utilized to understand the cellular and molecular mechanisms underlying efficient immunotherapies.

Another major hurdle in developing more robust tumor-on-a-chip platforms is the incorporation of crosstalk between the tumor and other organs. Molecular signaling from distant sites is known to affect cancer progression, specifically metastasis. While several studies^[44–49] have already begun to connect different tissue engineered organs (multiorgan-on-a-chip systems) to model crosstalk between organ systems, there are several challenges that need to be addressed. Creation of a universal circulating media that can maintain cell viability and functionality across different chips has not yet been achieved.^[114] Maintaining nutrient and oxygen levels for individual organs in these systems is also a challenge.^[114] Such integrated chips coupled with patient-specific cells could advance pharmacogenomics and precision medicine.

Tumor-on-a-chip platforms could also play an important role in improving the efficiency of combinatorial treatment, which is being considered as a promising approach to treat various cancers. However, examining the efficiency of the large number of candidates available in a systematic manner requires combinatorial screening. In the absence of mass production during the drug screening stage, low availability of materials can be a major hurdle. Limited primary cells and patient biopsy samples compound these challenges. Microfluidic technology helps to overcome these deficiencies because of the low volume requirement and ability to replicate complex cell–microenvironment interactions which are not possible in conventional high throughput screening (HTS) platforms.^[115] High throughput screening tumor-on chip platforms could serve as an ex vivo model to study combination treatment effects on various tumors.^[116–119]

Performing more quantitative analyses and in situ read-outs will enhance the applicability of tumor-on-a-chip platforms. These analyses include real time monitoring of relevant biophysical and biochemical factors, which can be achieved by imaging and the use of biosensors.^[120,121] Continuous measurements of such factors are critical because different cells respond to drugs in different time scales. So far, the approach has been to monitor these factors through collecting the efflux from such chips. However, these measurements are averaged and not instantaneous, they require a considerable volume of efflux, and the collection itself creates disturbances in the system. Functionally integrating biosensors into these systems can bring continuity and automation.^[122–125]

Despite all the advantages offered by microfluidic-assisted tumor-on-a-chip to study cancer–TME interactions, the challenges posed by this system cannot be ruled out.

Polydimethylsiloxane (PDMS) is the most widely used precursor for creating these devices and is known to adsorb certain drugs and proteins,^[126–128] thus limiting its application. A number of alternative candidates have been developed and tested;^[129,130] however, the device needs to be optically transparent, easily moldable, mechanically tunable, nonreactive, and economic, limiting the available candidates. Some of these limitations, such as drug adsorption, have been addressed with the use of polysulphonebased microphysiological systems^[47,51] but such systems need to be micromachined and lack transparency, which limits their imaging capability. While advancements in the areas of microfabrication and biomaterials allow for the creation of sophisticated and complex devices, one should be mindful to avoid over complicated systems. Complexities introduced should be driven by answering biological questions, while simultaneously ensuring sufficient simplicity of the devices to enhance their standardized and widespread applications.

In conclusion, while the development of newer cancer treatment approaches such as immunotherapies is revolutionizing the field of cancer therapy, their precise underlying mechanisms and reasons for lack of robustness evade understanding. Gaining a deeper insight into cancer–immune interactions within the TME will help to develop more targeted therapies. In vitro microfluidic models can help recreate the TME and can thus facilitate the delineation of the roles of individual immune components on tumor growth. The use of such models as an early screening platform could improve the efficiency of drug/ candidate molecule testing. These advantages will ultimately help to bring the costs of immunotherapies down, thereby making cancer care more accessible and affordable.

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Biography

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Figure 1.

Tumor–fibroblast interaction on a chip. A) Microfluidic chip design where each chip consists of four units containing seven channels each for loading either media or cells. Fluorescence images of B) HT-29 tumor spheroids and C) CCD-18Co. Left panel for both (B) and (C) shows nuclei (DAPI) and F-actin (left panel) and right panel shows calcein staining. D) Effect of coculture on fibronectin expression. Adapted with permission under the terms of the CC-BY license.^[68] Copyright 2016, the Authors. Published by PLoS One.

Figure 2.

Extravasation model on chip. A) Endothelial cells (ECs), MSCs, and osteoblast-differentiated cells (OBs) are encapsulated within gel in the central channel. ECs form vascular networks, whereas MSCs and OBs create a bone mimicking microenvironment (BMi) outside the vasculature. Cancer cells are introduced in the vascular network through perfusion. B) Branched vascular network formed by endothelial cells. C) Schematic depicting observed cancer cell extravasation into various microenvironments. Bone microenvironment consisted of osteodifferentiated human bone marrow MSCs (OD hBM MSC) while muscle microenvironment comprised on C2C12 cells. Adapted with permission.^[87] Copyright 2015, National Academy of Sciences.

Figure 3.

A) Each chip consists of eight independent units with vascular network. Inset depicts one of the vascular networks (Scale bar: 200 μm). B) Schematic and exploded view of the chip. Continuous perfusion is maintained by creating a pressure drop in reservoir. C) Microvasculature network within a chip showing tumor cell(TC)–neutrophils (PMN) clusters in microvessels. Magnified images show extravasating and nonextravasated melanoma cells (MA2) in TC–PMN clusters. D) Dispersion of PMNs from TCs over 2 h, in (i) the absence and (ii) presence of anti-CXCL-1 + anti-IL-8. Blocking CXCL-1 and IL-8 resulted in decreased cell dispersion; thus acting as a concentrated source of chemokines. Adapted with permission.^[100] Copyright 2018, National Academy of Sciences.

Table 1.

Summary of cell-cell interactions evaluated on a chip and corresponding phenomenon studied.

Cell/cell interactions	Process studied
PANC-1(pancreatic cancer)/HPaSteC(pancreatic stellate cells) ^[69]	Tumor-stroma interactions
HS5(bone marrow stromal cells)/HuH7(liver cancer) ^[67]	
HT29(colorectal cancer)/CCD18-Co(cancer associated fibroblasts) ^[68]	
SUM159(breast cancer)/CAFs (cancer associated fibroblasts) ^[78]	
MDA-MB-231(breast cancer)/HMEpiC(normal breast cells) ^[66]	Angiogenesis
U87MG(glioblastoma cells)/HUVECs(endothelial cells) ^[80]	
U937, HL60, K562(leukemic cells)/HUVECs ^[82]	
ccRCC (clear cell renal cell carcinoma)/HUVECs ^[81]	Intravasation
MDA-MB-231/HUVECs ^[86]	
HT1080,MDA-MB-231,MCF-10A (breast cancer)/HUVECs ^[84]	Extravasation
MDA-MB-231/MSCs(mesenchymal progenitor cells)/HUVECs ^[87]	
HT-1080, MDA231 (breast cancer)/MVEC, HUVECs (endothelial cells)/RAW264.7(macrophages) ^[99]	Intravasation, immune response
MA2(melanoma)/HUVECs/PMNs(Neutrophils) ^[100]	Extravasation, immune response
B16(mouse melanoma)/mouse spleen cells ^[101–103]	Immune response
SW620 CRC(colorectal cancer)/DCs(dendritic cells) ^[107]	