Tumor-on-a-Chip: Microfluidic Models of Hypoxic Tumor Microenvironment

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Abstract

Cancer is ranked as the deadliest prime health issue, albeit with mammoth efforts to panacea the ailment. To subdue the vast constraints in tumor therapy, one requires a superior cognizance of the tumor microenvironment (TME) including more sophisticated means to screen potential anticancer therapies. Hypoxia or low blood oxygen is a critical component of the TME and is significantly linked to tumor immune response, angiogenesis, metabolism, and cell proliferation. Thereby, detecting tumor hypoxia by employing cutting-edge tools, and emerging tumor-on-a-chip (ToC), is an upthrust area of research. Here, we assessed the state-of-the-art of the ToC tech, which incorporates tissue engineering, biomaterial research, microfluidics, and microfabrication in addition to profound novel contingency for fabrication and application of functional 3D in vitro tumor models for oncology research, immunotherapeutic, and screening. ToC microdevices, in particular, are competent for microscopic analysis of the interplay between tumors, immune, and cells in the TME, which are not

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S. Mukherjee, J. R. Kanwar (eds.), *Hypoxia in Cancer: Significance and Impact on Cancer Therapy*, https://doi.org/10.1007/978-981-99-0313-9_14

amendable with simple tissue cultures or animal models. The difficulties in developing ToC tech for the next generation are also addressed.

Keywords

Tumor-on-chip · Microfluidic devices · Tumor microenvironment · Hypoxia · Microfabrication

14.1 Introduction

Cancer due to its high morbidity and mortality rate remains one of the world's leading health issues after cardiovascular disease, accounting for 18.1 million cases and approximately 10 million deaths in 2020, amid the plethora of research and substantial medication discovery efforts made over the past few decades to combat the malady (Ranjan et al. 2020; Sung et al. 2021; Parihar et al. 2021a, 2022c, e). The global burden of malignancies is presumed to increase more adversely, making it a research platform in the clinical arena (Thun et al. 2010). Patients diagnosed with cancer needs extensive and continuous monitoring. The current medical setup lags in sustaining incessant round-the-clock monitoring competencies and paves an overwhelming challenge for the healthcare department with an utmost burden on the country's capital expenditure. This is due in part to the hefty expense of developing a novel antineoplastic therapeutic, in addition to the necessity for an enhanced understanding of the proliferation of cells and the tumor microenvironment (TME), besides the functions of tumor vasculature, tumor-stromal heterogeneity, various immune effectors, suppressor responses, and inflammation (Labani-Motlagh et al. 2020; Parihar et al. 2021b; Munjal et al. 2022). According to extensive research, TME promotes cancer growth in several ways, particularly treatment resistance. It not only reduces drug penetration but also enables surviving cells the benefit of proliferation and antiapoptosis, aiding resistance and common morphological changes associated with illness (Sun 2016). Unavailability of oxygen or inadequate blood flow is a common TME condition in almost all malignancies due to the tumors' uncontrolled and rapid growth (Muz et al. 2015). Most cancers exhibit reduced oxygen availability (hypoxia), a TME characteristic caused by an imbalance between elevated oxygen consumption and insufficient oxygen delivery. Countless research analyses that a person with tumor hypoxia has a higher chance of mortality and metastasis. Independent of the clinical stage, it has been documented that hypoxia is an unfortunate prognostic indication at the point of diagnosis (Walsh et al. 2014). The transcription factor, HIF-1α triggered by hypoxia, mediates a significant portion of all these effects (You et al. 2021). Therefore, to achieve substantial advancements in cancer therapy and tumor prognosis, more effective techniques for screening anticancerous therapeutics and a deeper knowledge of TME employing sophisticated techniques, such as organs-on-chips technology, are required (Tsai et al. 2017). Cell culture and animal models currently involve in cancer research and antineoplastic drug screening to date and therefore serve as a

crucial link between the cell and clinical trials (Kitaeva et al. 2020). Although cancer animal models (animal experimental objects and related materials) can provide crucial in vivo evidence and testimony on tumor progression and drug molecule responses, the relevant statistics accomplished have immense variations among the animals utilized and are therefore exceedingly expensive (Mak et al. 2014; Parihar et al. 2022d). In laboratory-cultured cells as well as cancers from both humans and animals, hypoxia can be detected utilizing both direct (i.e., needle-type O_2 electrodes) and indirect methods (i.e., HIF-1/2α immunolabeling or downstream HIF-targets) (Godet et al. 2022). Furthermore, in vivo models employed for cancer screening and therapeutic development, such as mouse models, may not accurately depict the interaction since the human tumor cell lines' biological activity and tumor heterogeneity varies significantly from that of the underlying tumor tissue (Onaciu et al. 2020). Contrary to this, 2D and 3D cell culture models have extensively been employed for anticancer drug screening and studies of cell proliferation, signaling, migration, and treatment outcomes, including altered protein/gene expression (Parihar et al. 2022d; Edmondson et al. 2014; Kapałczyńska et al. 2018; Parihar and Dube 2022). These model systems entail the co-cultivation of various cell types in the matrix comprises hydrogel which include cells derived from patient. Although these cellular models are economical and simple to use and commonly exhibit excellent reproducibility, perhaps they couldn't be capable to replicate the TME in an organ or models, making them ineffective for studying the effects of complicated spatial organization and cell interaction (Barbosa et al. 2022).

A practical in vitro site for novel therapeutic designing should foresee drug efficacy in a shorter time frame, accurate, and significant outcomes with practical applicability (Parihar et al. 2010, 2013, 2021c; Parihar and Dube 2022). Microphysiological in vitro techniques are perhaps the utmost sophisticated forums for early clinical drug effect assay till known (Bai and Wang 2020). Nevertheless, existing platforms have limitations which include low throughput, exorbitant costs, and, most importantly, the incapability to procreate physiological drug exposures. As a result, there is an exigency to enhance and strengthen these networks, as well as develop better predictive systems capable of simulating drugs' in-vivo pharmacokinetic (PK) profiles (Norris et al. 2000). Microfluidics-based 3D culture models are coupled in tumoroid/organoids-on-chip technology (Duzagac et al. 2021). The fundamental goal of these techniques is to retain the cellular intricacy of cell models (mainly 3D cell culture with TME) and syndicate with the potential to simulate therapeutic testing in model organisms. The majority of available models investigate tumor invasion, progression, angiogenesis, and metastasis and thereby mimic the pathophysiology of human cancer. The more sophisticated they are, the lower will be their efficiency and the more complicated they become. A microfluidic chip comprising 2D or bare or enclosed 3D cultures continuously perfuses drugs at a consistent flow rate is possible using microfluidic technology (Kumar et al. 2022a; Li et al. 2012; Parihar et al. 2022b; Ranjan et al. 2022). These techniques are capable of simultaneously assessing a multitude of promising therapeutic leads as possible at varied concentration ranges (Cui and Wang 2019). The integration of gradient generators coupled with cell culture chambers is being used by some devices to

solve this challenge (Yahyazadeh Shourabi et al. 2021). Using cultural 2D or 3D models, however, varied chemical concentrations are administered to a limited proportion of replicate wells preventing the cell construct from being exposed to varied drug concentrations emblematic of the plasma PK profile. Moreover, significant advances incorporate PK-PD setups for 2D and 3D cultures with syringe pump-controlled flow rates that use custom-made PDMS chip models (Petreus et al. 2021).

14.2 3D Tumor Models on Chip for Measurement of Hypoxia

Drug discovery is still a difficult and expensive process due to low success rates in clinical trials. Approximately half of all medications couldn't make it to stage II and III clinical trials because of their futile in nature, although about one-third of failures are related to safety concerns such as a NTI (narrow therapeutic index) (Arrowsmith and Miller 2013). As casualty cases continue to escalate, innovative tools that allow for greater precision in drug discovery are considered essential (Dugger et al. 2018). The emergence of novel preclinical testing models that effectively mimic in vivo system and tumor microenvironment, along with targeted therapy, which has the potential to lead to future biomarkers and more precise pharmacological targets, are among the utmost promising fields that are anticipated to enhance drug development efficacies (Langhans 2018). Hypoxic cells have routinely been identified in 2D and 3D cell cultures by HIF-1 immunolabeling utilizing immunohistochemistry (IHC) or immunofluorescent (IF) techniques. Because HIF-1/2α proteins only have a modest half-life when exposed to oxygen, thereby immunolabeling can be difficult. Additionally, nuclear permeabilization is necessary for their detection since HIF-1 and HIF-2 reside in the nucleus in hypoxic settings. Although popular, in vivo three-dimensional models involving animal xenografts have ethical limitations too and cannot accurately replicate the biology and physiology of humans. Thereby, by modifying a multitude of three-dimensional tissue engineering techniques, 3D tumor models are produced that imitate in vivo TME in the body (Lara Rodriguez and Schneider 2013). As pioneered by Mina Bissell and her colleagues demonstrated in the 1980s when they conducted research on the significance of the ECM (extracellular matrix) in cell behavior, cultivating cells in 3D systems that imitate key tissue facets is comparatively more representative of the in vivo environment than simple 2D monolayers (Pampaloni et al. 2007; Ravi et al. 2015). 2D cultures are unable to replicate the intricate 3D tissue organization, biophysical and biochemical characteristics of ECM, and cell-cell interactions encountered in malignancies. High-throughput screening (HTS) cellular assays still frequently use traditional monolayer cultures, although 3D cell culture techniques for drug research are rapidly advancing (Ryan et al. 2016).

Building vascularized tissue in three-dimensional tissue engineering for non-animal substitutes has been a serious challenge for decades (Grover et al. 2018). Vascularization is required to provide sufficient oxygenation to thick tissue ($>$100–200 m) for persistent maintenance and functioning, for instance, assessments of toxins in sub-acute and chronic circumstances (Zhang et al. 2021). Additionally, it

is critical for improving therapies and simulating diseased tissue. Drug leads for neurological disorders, in particular, have a larger rate of failure during the bench-to-bedside transfer compared with former treatment (Gribkoff and Kaczmarek 2017). Only a small percentage of the candidates who made it through stage I of the clinical rigorous assessment got commercial approval, according to research (Sun et al. 2022). The causes for detrimental impacts on cerebral microvascular remain unknown, and it's unclear if they're caused by endogenous pathogenic systems or by the medications themselves. Many neurological illnesses, including brain malignancies, are linked to injury or malfunction of the brain's vascular system. Among the most prevalent and deadly forms of elderly brain tumor is glioblastoma multiform (GBM), which has an average survival span of only 12 months with the right treatment. Additionally, it is among the most vascularized cerebral tumor, and it's linked to a lot of ECM remodeling. Modeling the characteristics of GBM to comprehend their influence on cerebral vascularization, especially relevant to the modulation of angiogenic signaling pathways, has been a focus of research, as microvascular proliferation is a characteristic of GBM (Rodriguez et al. 2012; Hardee and Zagzag 2012). In methods for developing tumor-on-a-chip (ToC), three-dimensional tumor tissues are usually initially prepared according to standard culturing techniques before being introduced to the microfluidic chip for examination. Thus, research into tumorigenesis and the formulation of remedies for targeted therapy can both benefit from the application of a 3D cancer model. The common methods for developing 3D in vitro cancer models are being thoroughly covered.

14.2.1 Conventional Transwell Model

The BBB constituents' endothelial cells (ECs), astrocytes (ACs), and pericytes (PCs) are reconstructed in the co-cultivation transwell model (Fig. 14.1). To evaluate the motility of tumor cells in conjunction with a biological inclination, transwell inserts, often referred to as Boyden chambers, are frequently employed in traditional migration, invasion, and transendothelial migration studies. A polymeric porous membrane makes up a transwell insert, allowing cancer cells to traverse through the pores. The propensity of tumor cells to move through the pores is assessed using a migration assay. In glial cell co-cultures, positive glial input towards initiation of BBB characteristics is often more efficient (Abbott 2002; Garcia et al. 2004). Endothelial cells that were co-cultured with astrocytes in cell-cell interaction elevated the expression of close junction proteins like occludin (EC 1.6) and P-glycoprotein (P-gp). In co-culture settings, pericytes could be used instead of astrocytes or neurons (Zujovic and Taupin 2003; Nakagawa et al. 2009). As a result, a cell-cell interaction co-culture method is more structurally similar to in vivo condition (Malina et al. 2009). Accordingly, astrocytes and pericytes are assumed to partake a favorable influence on ECs on their own, but when combined as a tri-culture, they provide synergic stability of such a close junction configuration (Schiera et al. 2003).

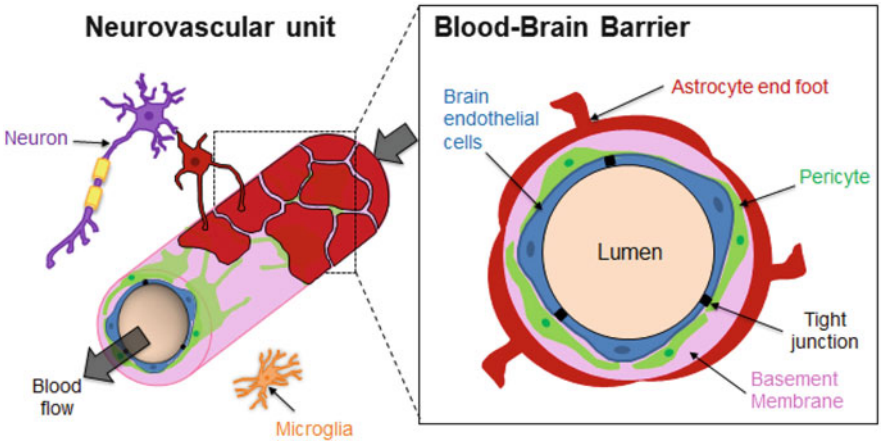

Fig. 14.1 The organizational structure of NVU and the BBB. NVU is a cellular and extracellular matrix-based structural and functional complex wherein ACs, microglia, BBB ECs, PCs, and neurons are all part of the NVU. The components of NVU cooperate synergistically to regulate the exchanges throughout the blood arteries and the brain

Tri-cultivation transwell model system encompasses the reconstruction of astrocytes, endothelial cells, and pericytes, the three BBB constituents. To generate a co-culture, ECs are full-fledged on the apical, and ACs are introduced to the basal surface of the membrane. A tri-culture can then be formed by adding pericytes to the in vitro system. Instead, the pericytes well developed on the basal membrane, while the astrocytes are produced on the culture plate (Nakagawa et al. 2007).

The highest TEER values were found in tri-culture models integrating pericytes. Tri-cultivation is the most accurate in vitro model that mimics in vivo scenario and should be investigated further.

Mono-cultivation Transwell Models

The development of cultured cell supplements with highly permeable membranes permitted in vitro permeability investigations with brain ECs (Grobstein 1953). Because of the favorable data from these models, transwell filters utilized as the benchmark modeling technique for in vitro BBB investigations ever since. To form a monolayer, ECs are cultured on a microporous membrane referred to as a monoculture. To improve cell adhesion, ECM such as fibronectin or collagen may be introduced to cover the membrane (Yue 2014). The simple monoculture transwell approach can simultaneously investigate a wide range of operations. For instance, drug permeation can be measured by an array of transwell filters over a wide range of concentrations. Additionally, BBB constituents like ACs and PCs have been demonstrated to affect the B-endothelial BB's cell characteristics. In comparison to ECs cultured alone, a syngeneic co-culture system comprising rat ECs and ACs has been shown to upsurge close junction confrontation amid ECs (Demeuse et al. 2002). Pericytes, that demonstrated to enhance ECs maturation and a thick barrier

development, are only used in a few transwell models. To improve the monoculture BBB model, astrocytes and pericytes must be explored.

14.2.2 Tumor Spheroids

Under non-adherent cell culture conditions, three-dimensional aggregations of cells can develop into tumor spheroid structures. The appearance, growth dynamics, nutrition transport, and connections amid cells and the matrix in the cancer spheroid mimics a minor tumor mass (Benien and Swami 2014). Due to its remarkable three-dimensional in vitro tumor model capabilities, the tumorospheres aids as an exceptional model. Disaggregated cells from primarily isolated tumorospheres and organotypic tissues, as well as sole- or numerous-cell suspensions from persistent cell lines, can all be used to create tumor spheroids. Patient- or murine-derived organotypic tumor spheroids (MDOTS/PDOTS) sustain the immunological milieu of the tumor microenvironment. To replicate ICB ex vivo, a 3D microfluidic system is used to develop MDOTS/PDOTS for a brief period. Multicellular organoids with corresponding immune cells were obtained after limited collagenase decomposition of fresh tumor tissues. P- or MDOTS been examined by flow cytometry (FC) or collagen injection into the device's central channel for anti-PD-1 or -CTLA-4 antibody exposure. They assessed the proportion of myeloid and lymphoid immune cells in a mass tumor with distinct spheroid cultures (S1 $>$ 100 m; S2 40–100 m; and S3 murine-derived organotypic tumor spheroids) after establishing that enzyme-mediated metabolism won't affect the expression of the surface antigen. In ex vivo 3D microfluidic culture, recapitulate sensitivity and resistance to PD-1 blockade. To assess the ex vivo response to PD-1 inhibition, anti-PD-1 antibody reacts with MDOTS for 3 or 6 days inside the device. Dual-labeling deconvolution fluorescence microscopy using AO (acridine orange), and PI (propidium iodide), was then used to see live and dead cells (Fig. 14.2a). The baseline survivability of MC38 MDOTS ($>$90 percent at Day 0) dose- and time-dependent death of MC38 MDOTS in response to anti-PD-1 using the MC38 syngeneic model (Woo et al. 2012) reacts to anti-PD-1 in vivo (Fig. 14.2b–c). Multiple independent replicates in different laboratories corroborated this result. Immune cell-derived MC38 spheroids without stromal cells had to be unresponsive to anti-PD-1 drugs for PD-1 (Fig. 14.2d). Despite comparable treatment, MDOTS produced from the PD-1-resistant B16F10 (Curran et al. 2010) (Fig. 14.2e–f) and Lewis lung carcinoma (LLC) models showed less cell death than MC38 MDOTS (Fig. 14.2f). The CT26 model (Duraiswamy et al. 2013), which is intermediately sensitive, showed little killing (Fig. 14.2g). Furthermore, co-treatment with an anti-CD8α Ab inhibited anti-PD-1-mediated death of CT26 MDOTS, suggesting a particular prerequisite for CD8+ T cells (Fig. 14.2h), which we also established with MC38 MDOTS. The MDOTS produced from explanted GL261 glioma tumors (Reardon et al. 2016) retained sensitivity to ex vivo PD-1 blockage in a CD8+ T cell-dependent manner. It is confirmed that CD45+ immune cells and CD8+ T cells survived ex vivo in the device after tumor cells died due to PD-1 inhibition. These findings show that using well-defined

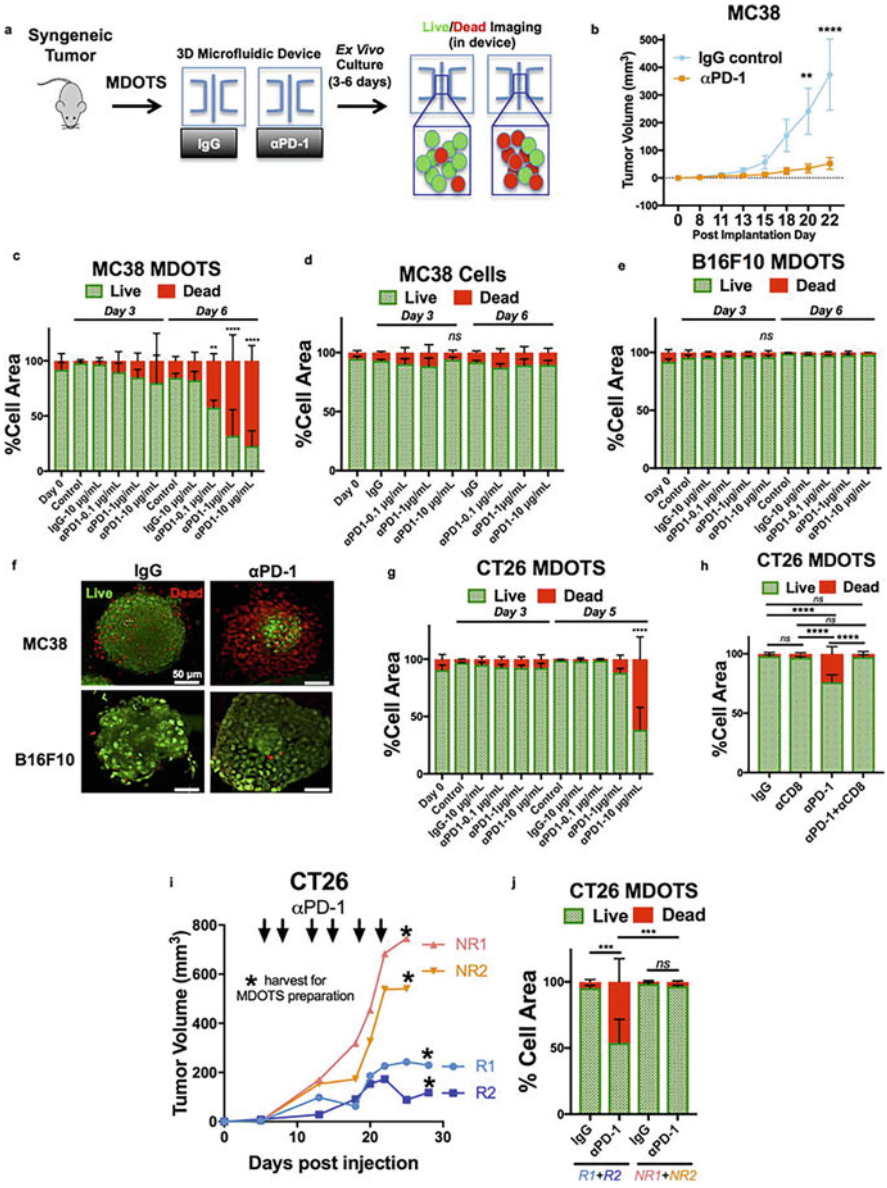

Fig. 14.2 Ex vivo PD-1 blockade MDOTS profiling. (**a**) Model for the MDOTS live/dead imaging process. (**b**) Rat anti-mouse anti-PD-1 antibody or isotype control IgG therapy on the MC38 tumor volume ($n = 10$). (**c**) Live (AO = green)/dead (PI = red) Day 3 and Day 6 quantification of MC38 MDOTS in response to IgG control or specified anti-PD-1 antibody dosages ($n = 4$, biological replicates, two-way ANOVA with Dunnett's multiple comparisons test). (**d**) Live/dead study of MC38 spheroids devoid immune cells ± anti-PD1. (**e**) B16F10 MDOTS ± anti-PD1 live/dead assay (**f**) MC38 and B16F10 MDOTS Day 6 ± anti-PD1 deconvolution fluorescent microscopy. (**g**) CT26 MDOTS ± anti-PD1 live/dead assay. (**h**) On Day 6, CT26 MDOTS conducted a live/dead assay in which isotype IgG controls (10 μg/mL) or anti-PD-1 (10 μg/mL) and anti-CD8 (10 μg/mL) were

mouse models, sensitivity and resistance to PD-1 blocking may be replicated ex vivo. Even when identical cell counts are implanted into syngeneic animals, stochastic tumor growth and response to ICB are common. Further, MDOTS from explanted CT26 tumors of various sizes was used to assess the influence of inter- and intra-tumor heterogeneity on PD-1 blockade sensitivity. When MDOTS from bigger CT26 tumors were compared to MDOTS from smaller tumors, there was less CD8 T cell infiltration and less ex vivo sensitivity to PD-1 inhibition. CT26 MDOTS were made from mice that responded (R) or did not respond (NR) to PD-1 inhibition in vivo and were then re-challenged with anti-PD-1 therapy in 3D microfluidic culture (Fig. 14.2i). CT26 MDOTS generated from PD-1-responsive tumors (R1 + R2) maintained PD-1 blockade sensitivity ex vivo, whereas MDOTS prepared from PD-1 non-responsive tumors (NR1 + NR2) maintained PD-1 blockade resistance (Fig. 14.2j). These findings show that tumor development and responsiveness to ICB heterogeneity found in vivo are conserved ex vivo in MDOTS.

MDOTS makes novel therapeutic combinations easier to test. The CT26 model was chosen due to its half sensitivity to anti-PD1, which could help to find new PD-1 blockade combinations that could overcome intrinsic resistance (Peng et al. 2016). The inhibition of CCL2 a chemokine alone did not improve PD-1-mediated CT26 MDOTS killing, indicating the need for alternate methods that decrease immune-suppressive signaling more extensively throughout the TME and reawaken T cells (Vanneman and Dranoff 2012; Benci et al. 2016). The impact of combining the MDOTS platform with Compound 1, a novel potent/selective TBK1/IKK inhibitor, showed the improved efficacy of PD-1 blocking. The kinases TBK1 and IKK promote autocrine/paracrine signaling (Zhu et al. 2014) as well as restrain T cell activation (Yu et al. 2015), suggesting that inhibiting TBK1/IKK could improve multiple strategies to overcome an immunosuppressive TME for tumor control in response to PD-1 inhibition. In contrast to the multi-targeted inhibitor momelotinib, they validated Cmpd1's efficacy and selectivity including the absence of JAK inhibitory action (CYT387). Cmpd1 boosted the production of IL-2 and IFN- from isolated CD4+ and CD8+ T cells from healthy adult patients as well as IL-2 from Jurkat human T-cell leukemia cells and decreased immune-suppressive cytokine synthesis by CT26 cell line spheroids with no cytotoxicity.

Ex vivo addition of Cmpd1 to PD-1 inhibition improved CT26 MDOTS death (Fig. 14.3a, b), which was related to lower CCL4, CCL3, and IL-1 levels and activation of cytokines implicated in activated innate immune responses (e.g., G-CSF) (Fig. 14.3c). Cmpd1 anti-PD-L1 was administered to Balb/c mice bearing CT26 tumors to see if MDOTS profiling predicted the in vivo response to combination TBK1/IKK inhibition (Fig. 14.3d–f). Cmpd1 + anti-PD-L1 had better tumor

Fig. 14.2 (continued) administered. (**i**) Treatment with anti-PD-1 to CT26 tumor volumes in Balb/c mice for the responder (R1 + R2) and non-responder (NR1 + NR2) indicating the moment of sample collection for MDOTS preparation. (**j**) Ex vivo isotype IgG control group or anti-PD-1 therapy and live/dead examination of CT26 MDOTS from R1 + R2 and NR1 + NR2 mice (Day 6)

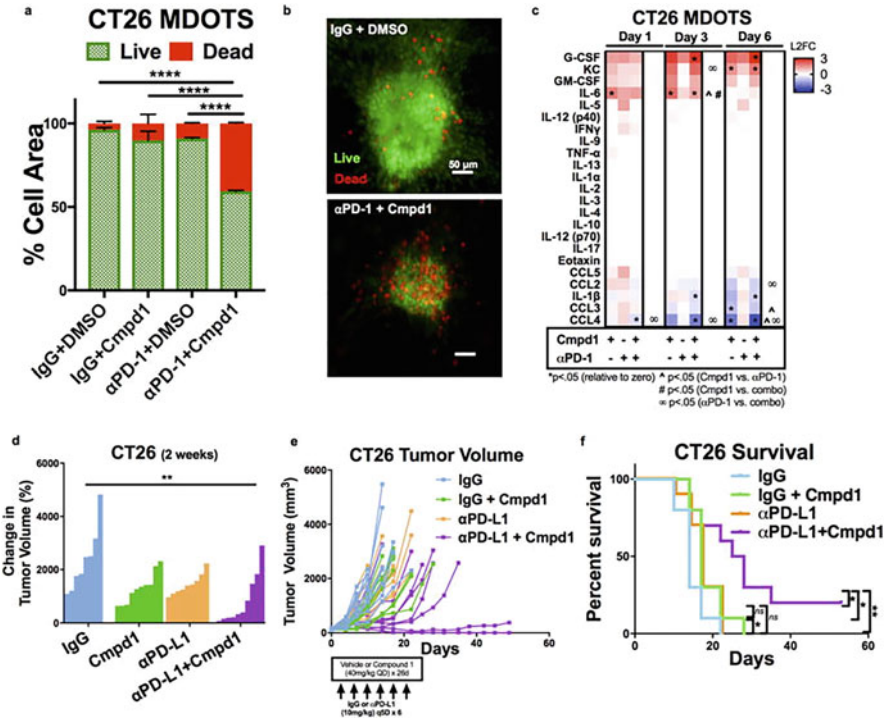

Fig. 14.3 Inhibition of TBK1/IKK improves PD-1 blockade responsiveness. (**a**–**b**) Following 6 days of treatment with IgG-DMSO, Cmpd1 (1 M), PD-1, and PD-1+ Cmpd1, the CT26 MDOTS was either alive (AO = green) or dead (PI = red). (**c**) Treatment of CT26 MDOTS with IgG + Cmpd1 (1 μM), αPD-1 (10 μg/mL), or αPD-1 + Cmpd1 (1 μM) cytokine heatmaps, with vehicle control, displayed as L2FC compared to isotype control IgG. (**d**–**e**) CT26 tumor volume implanted. (**f**) Percent survival following IgG + vehicle, IgG + Cmpd1, αPD-L1 + vehicle, and αPD-L1 + Cmpd1

control and longer survival than Cmpd1 or anti-PD-L1 alone (Fig. 14.3d–f), which was consistent with MDOTS profiling findings. Reimplantation of CT26 cells into animals with extraordinary responses to co-administered culminated in negligible proliferation, while EMT-6 implanted tumors grew properly, implying that Cmpd1 + anti-PD-L1 treatment induces immunologic memory of CT26 cells. As a result, MDOTS profiling accurately predicted the response to PD-1 inhibitor +/ TBK1/IKK blockade, demonstrating the use of ex vivo screening MDOTS for the development of combination immunotherapies.

14.2.3 Cancer Three-Dimensional Cell Culture in 3D Matrices

In contrast to 3D models that can imitate these conditions in vitro, 2D models cannot study cell-cell and cell-matrix interactions. Owing to the absence of preclinical

models that are appropriate for 2D cultures, 3D culture provides a practical pathophysiological TME and may contribute to drug discovery. Three-dimensional tumor models have been developed using tissue engineering techniques. A scaffold is an extracellular support structure that is biocompatible and chemically stable and functions as a cell adhesion model, growth, and tissue morphogenesis. Some biomaterials having ECM-like qualities can be used in inserts, and cells can also manufacture ECM proteins like collagen. Due to modifications in the physical and biological conditions of 2D and 3D cultures, 2D cells are more vulnerable to the effects of medications than 3D cultures because of their inability to retain normal morphology and variations in the arrangement of surface receptors on the cell. It's worth noting that there's mounting evidence showing cells cultivated in a 3D environment exhibit differences from cells cultured in a two-dimensional setting, retaining critical ECM signals. Consequently, suitable 3D culture provides a more appropriate physiological method of studying gene activity and cell phenotypic ex vivo. The preferred technique for succinct tissue construction of benign and malignant tumors in recent years is reconstructed 3D culture. As a result, the modifications, communications, and molecular and cellular signals that occur during tumor hypoxia-mediated malignant transformation can therefore be better understood using 3D culture.

Three-Dimensional Cell Culture Scales

Because of the unavailability of a single technique that can handle the requirements of all 3D cultured cells, many approaches have been created to meet the expanding need for cell culture. The influence of ECM molecules is ignored in 2D culture. Nonetheless, its density and packing play a crucial influence in the construction of a three-dimensional environment. The 3D model is inspired by the original microenvironment and is an in vitro recreation of the ECM. It maintains the ECM's geometric, mechanical, and biological qualities. Solid tumors grow by interacting with a variety of cell and non-cell components, using methods akin to those used in the initial phases of organogenesis. Several cells are combined in a unique setting and structured in such a way that they generate 3D tissues that resemble real tissue structures. The tumor microenvironment, which includes many cell types such as immune cells, stromal cells, and ECs, surrounds tumor cells in vivo. Other extracellular elements include the ECM, EVs, metabolites, growth factors, and cytokines (TME) (Riedl et al. 2017). The morphological and cellular structure formed by ECM interactions that are altered through oncogenesis can be studied using 3D culture models. As a result, in vitro 3D tumor models are a critical method for studying cancer growth and metastatic pathways. They are most advantageous because they permit tissue development derived from human cells and contain specific, physiologically significant components. Consequently, 3D culturing offers a more physiologically appropriate method of studying gene activity and cell phenotypic ex vivo. The characteristics of the selected cells show 3D artificial ECM imitation in which they grow; scaffold-based natural, synthetic, or hard biomaterials; signaling molecules; and cell culture bioreactors that support a biologically active environment. As a result, such variables should be assessed prior to selecting the best-suited

approach and methods. Scaffold-based culture (spheroids), scaffold-free (natural), or manufactured solid scaffolding (non-scaffold based) were exploited to study tumor hypoxia.

14.3 Tumor-Microvascular Model in Microfluidics

When it comes to tissue engineering and tumor biology, vasculature is indeed crucial. Additionally, the development of new vasculatures for nutrient delivery is essential for the proliferation and progression of the tumor. Chemical processes and evaluations carried out in microchannels and microstructures produced using semiconductor microfabrication approaches such as photo- and soft lithography (Betancourt and Brannon-Peppas 2006; Hacking et al. 2012). In the 1990s, an interdisciplinary branch of research named "Lab-on-a-Chip" or "Microfluidics" was formed by merging micro-/nano-device, chemical sensors, and analytical chemistry (Kimura et al. 2018). In general, in vitro-cultured cell lines are mostly inactive and lack physiological capabilities. This process also occurs in initially cultivated cells, and maintaining biological functions over long periods, even if they are regular right after harvest, is extremely challenging. Cells are cultivated in the semi-static condition in traditional methods, with the administration of experimental chemicals to cells relying solely on diffusion. However, in vivo, cells are exposed to chemical and physical stimulation from their ambiance, including stretching and shear stress, as well as oxygen and nutrients through blood flow. Variations in the environment and structure of cells in vivo and in vitro may lead to the loss or inactivation of cellular activities in culture. To bridge the gap between in vitro and in vivo environments, experts in the tissue engineering field, including those working in the mTAS and other domains, have been using microfluidic devices since the early 2000s (Gupta et al. 2016). Microfluidic approaches can manage spatial and temporal liquid conditions, cell adhesion, and mechanical stimulation of cells. To accurately recreate the TME in vitro, a functional microvasculature network must be developed in conjunction with a three-dimensional tumor model. It is possible to more effectively kinetically examine crucial cancer growth phases including intravasation, angiogenesis, and extravasation in a meticulous microenvironment by co-culturing vasculature and cancer cells on a microfluidic preferable platform (Pfisterer and Korff 2016). Organ-on-a-chip (OoC) which mimics organ functionalities using microfluidics has gotten a lot of press recently (Wu et al. 2020). To assist as models for cell-based assays during drug research, tissue and drug discovery disease models using OoC technology have been provided. This is especially true given the introduction of an iPS cell differentiation induction approach. Two early instances of organs-on-a-chip indicated enhancements in functional activity through the culture perfusion of 3D hepatocyte aggregations and evaluation of shear stress by EC vascular exposure and maintaining medium flow in a microchannel. This has evolved into a cutting-edge technology proficient in recreating normal cell behaviors in vitro thanks to recent developments in cell engineering, microfabrication, and imaging. Recent years have seen investments in OoC initiatives from the NIH

(National Institutes of Health), the FDA (Food and Drug Administration), the Defense Advanced Research Projects Agency (DARPA), the FP7 (Framework Program 7) in the European Union, and the AMED (Agency for Medical Research and Development) in Japan. This funding also demonstrates the size of the research aspirations for OoC technology.

Organ-on-a-Chip (OoC)

The mTAS researchers have proposed OoC devices that simulate a variety of organ and tissue processes. Although it is beyond the context of the study to give a comprehensive assessment of all the strategies, we do present an overview of in vitro models in this section.

(a) *Lung-tumor-on-a-chip*

Nearly all solid tumors have a characteristic microenvironment feature called hypoxia. The unchecked and rapid tumors growth which reduces the available oxygen due to insufficient blood flow. Intratumoral oxygen gradients facilitated by hypoxia contribute to the adaptability and heterogeneity of malignancies. Controlling the tumor parameters has been necessary throughout the entire 3D model-building process. By using light-sheet fluorescence microscopy, tumor spheroids, ECM buildup, or hypoxia may all be characterized phenotypically. Fluorescence imaging was used to track the 3D-3 model, which was made up of NSCLC cells, CAF, and monocytes, and is based on the alginate microencapsulation method. The improvements made in the creation of 3D models of NSCLC opened up several opportunities for investigations of hypoxia (Hsu et al. 2013). The "lung-on-a-chip," also identified as the "breathing lung," made by Harvard University's Ingber group is the most well-known OoC. A microporous membrane constructed of flexible polydimethylsiloxane, silicone, separates the two-layer channel structure vertically (PDMS). To mimic the form of the lung on a microfluidic device, they cultivated alveolar ECs on the top surface of the membrane and vascular ECs on the bottom side and employed flowing air and culture media through it. The internal pressure of the channel on each sides of the main channel was changed at a precise cycle to stretch and contract the membrane, simulating the expansion physiologically and contraction motions of the alveolus while breathing. Using this device, they mimicked inflammatory situations in which vascular ECs express a large amount of the integrin ligand (ICAM-1) following exposure to TNF-a and bacteria. Furthermore, following the development of ICAM-1, neutrophils flowing in the vascular side channel linked to vascular ECs; then traveled to the alveolar ECs surface side, through the vascular ECs and the membrane's pores; and engulfed the bacteria. Z. Xu et al. devised a framework for a successful drug sensitivity test using a microfluidic chip-based, three-dimensional co-culture (Xu et al. 2013). Fresh lung cancer tissues and a variety of stromal cell lines were produced in three dimensions (3D) under conditions that were similar to those found in vivo. To identify the most effective chemotherapy plans, various anticancer medications were administered to the cells using a gradient concentration generator within

the chips. To separate CTCs (circulating tumor cells) in NSCLC patients with high purity, J. Zhou et al. developed a unique multi-flow microfluidic (MFM) method (Zhou et al. 2019). Because of the specific oxygen regulator made possible by their minuscule size, investigations have conclusively shown that microfluidic devices offer a potential platform for the exploration of hypoxic TME.

(b) *Liver-tumor-on-a-chip*

Hepatocytes, the body's largest and primary organ for metabolism, a type of parenchyma cell make up 80% of all liver cells. Among the non-parenchyma cells are the ECs of sinusoid lumens, stellate cells, Kupffer macrophage cells, and pit cells (located in the same region as Kupffer to disrupt and kill CTC). High concentration of oxygen and particular shear stress are necessary for the normal survival of hepatocytes. To survive longer in vitro, hepatocarcinoma (HepG2) or its clonal derivation with significant contact-induced growth inhibitory (known as C3A) need more stringent environmental circumstances.

Because the liver is the primary organ involved in pharmaceutical synthesis, it's critical to estimate its metabolic capabilities and toxicity early on in the drug development process. Hepatocytes employed for in vitro testing, on the other hand, lose a lot of their natural capabilities and activities. The inversion of prodrugs like TF5 to metabolite drugs 5-FU, which causes hepatotoxicity, and doxorubicin to doxorubicinol and metabolism which led to hematological toxicity are two significant instances of the liver's contribution to drug metabolism. Accordingly, the liver must be co-cultured in microscale CCA (cell culture analogue) models to study the pharmacological properties of anticancer agents (Esch et al. 2010). Powers and colleagues suggested a microfluidic system that allows for the creation of 3D tissue architectures while in continuous perfusion (Fig. 14.4b) (Powers et al. 2002). In a cell culture chamber, 3D scaffolds were paired with structural support and a cell-retaining filter to allow culture media to flow across the top of the edge and through the 3D cell aggregates in every channel. A chamber for cell culture was created with flow rates that fulfill predicted cellular oxygen requirements while maintaining a healthy range of fluid shear stress. They revealed that this technology allows the development of hepatocellular aggregates resembling those found in hepatic acini and that they can uphold their shape and vitality for 2 weeks. Maintaining the polarized transportability of hepatocytes is also critical for studying drug reactions. Hepatocarcinoma is more sensitive to environmental factors than other cancer cells; hence it is essential to effectively recreate the tumor microenvironment. A significant challenge for 3D hepatocyte culture is inducing hypoxia and necrosis of surrounding inner cells in a functional realistic 3D structure. Hanging spheroid tissues were cultured in 3D spherical structures using hanging drop networks (HDNs) of media (without cell-surface adhesion) that have shown significant advancements toward biological and functional cancer microenvironments which include high gas exchange (O_2, CO_2) during incubation, the prevention of bubble formation in secretion, and cell injection (Frey et al. 2014). Additionally, oxygen-permeable chips induce central hepatocarcinoma cell death and

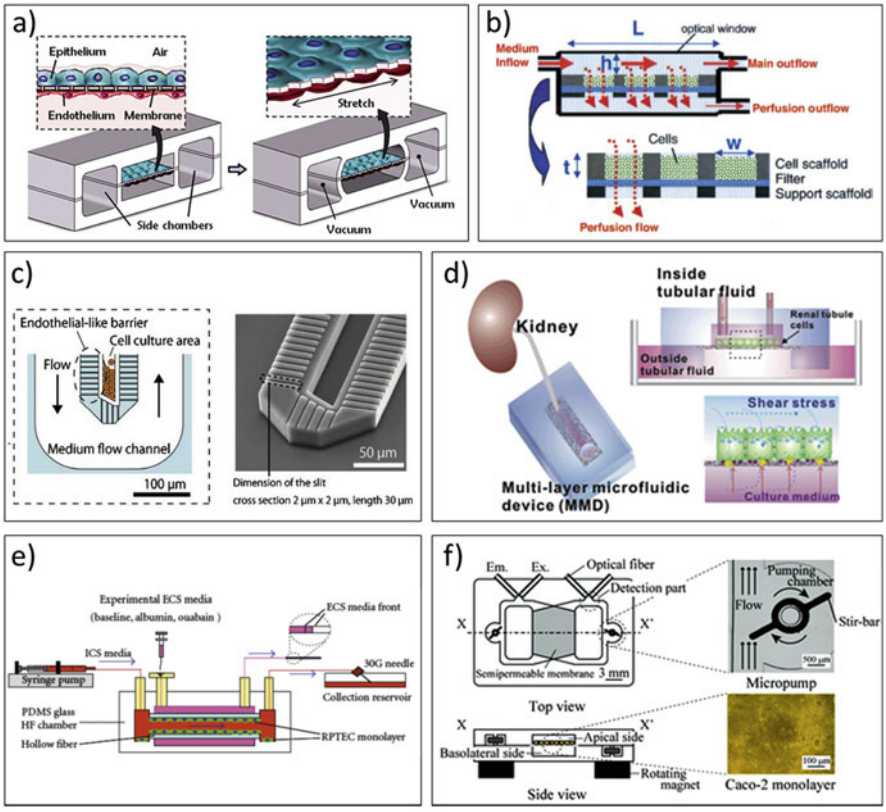

Fig. 14.4 Devices for OoCs: (**a**) by delivering a vacuum to the side chamber and mechanically stretching an elastic membrane creating the alveolar-capillary barrier, the lung-on-a-chip system mimics normal breathing motions. The American Association for the Advancement of Science (AAAS) owns the copyright to this image. (**b**) The perfused 3D liver culture microfluidic device. Wiley Periodicals, 2002. Copyright. (**c**) The liver-on-a-chip device has a hepatic cord-like shape. The American Institute of Physics (2011) owns the copyright to this image. (**d**) A porous membrane on the kidney-on-a-chip technology creates in vivo-like tubular habitats for collecting duct cells. The Royal Society of Chemistry (2009) owns the copyright to this image. (**e**) A tubular hollow fiber membrane is included in the kidney-on-a-chip device to mimic the tubular shape of the renal tubule. Hindawi Publishing Corporation (2013) owns the copyright to this work. (**f**) The gut-on-a-chip system comprises a two-compartment structure with stirrer-based micropumps and optical fiber inserts for each compartment, separated by a microporous membrane. The Royal Society of Chemistry (2008) owns the copyright to this image

hypoxia in 3D spheroid models (Anada et al. 2012). In the hepatic lobules, bile canaliculi are generated between consistently organized hepatocytes that spread radially and routinely from the central vein. Bile is discharged into the bile canaliculi and contains metabolic products biosynthesized in the cells. As a result, the bile canaliculi are mainly targeted for in vitro drug metabolism research. As an in vitro model for physiologic bile canaliculi development,

Nakao et al. (2011) designed a hepatic lobules device model that replicated the microstructure of the hepatic cord, a smallest unit hepatic lobule (Fig. 14.4c). The device's cell culture chamber was intended to arrange hepatocytes in two lines that resembled hepatic cords. Along with the hepatic cord-like structure, hepatocytes aligned progressively self-organize and create bile canaliculi. The drug response evaluation was then used to perform a drug metabolism test utilizing carboxydichlorofluorescein diacetate (CDFDA) as a model metabolite. The hepatocyte esterase hydrolyzes CDFDA to carboxydichlorofluorescein (CDF), a fluorescent substance that is discharged into the bile canaliculi. Herein, CDFDA-encompassing culture media was perfused into the blood vessel flow channel; CDF excretion into the bile canaliculi developed continually in the cells. The finding demonstrates how these processes keep tissues' form and polarity that may be replicated by simulating microstructures.

(c) *Kidney-tumor-on-a-chip*

Tubulointerstitial hypoxia has been identified as the ultimate pathway prominent to end-stage kidney disease (ESKD) as a result of studies conducted over the past two decades. CKD, or chronic kidney disease, is usually linked to a variety of varying levels of hypoxia damage in various tubular segments, based on the etiology and stage of the disease, which is a complex connection between oxidative stress, inflammation, and fibrosis. The kidney's resident cells are outfitted with defenses against hypoxia. Hypoxia-inducible factors (HIFs) play a key part in this process by transcriptionally activating genes. The kidney is an essential organ for both regulating metabolism and excretion. Because there are no adequate in vitro models, medication lead effectiveness and toxicity are investigated in the kidney entirely through animal testing throughout drug development. Drug dropouts in clinical trials, on the other hand, result from variations in metabolic pathways between humans and experimental animals, resulting in huge expenses in drug development. Preclinical testing that accurately identified nephrotoxic substances would result in considerable cost savings and the avoidance of nephrotoxic medications throughout development (Su et al. 2014). A study reported by Cho et al. stated that due to the nanoparticles' ability to agglutinate after being immunocaptured by released γ-glutamyl transpeptidase (GGT) and increasing the fluorescence measured in the outflow, 500 nm fluorescent polystyrene nanoparticles coupled with anti-GGT antibodies within the apical channel show the ability to track drug-induced nephrotoxicity (Cho et al. 2016). Additionally, it is anticipated that a highly accurate in vitro disease model would be employed as an effective drug screening method for potential therapies, providing fresh perspectives on the mechanisms underlying kidney disease. The most basic kidney-on-a-chip systems involved the attachment of human kidney-2 (HK-2) and Madin-Darby canine kidney (MDCK) epithelial cells to the lower surface of a microchannel and the application of biological shear stress (Frohlich et al. 2012). The shear stress caused by this device enhanced cell thickness, and expression of Na^+/K^+-ATPase, and promoted the production of cilia in the cells, according to the scientists. These findings imply that by employing a well-regulated shear stress

load, the physiological behavior of kidney-derived cells may be mimicked. To mimic the reabsorption function of renal tubules, two-layered kidney-on-a-chip devices having porous membranes have been developed (Fig. 14.4d) (Jang et al. 2013). The physiological reactions to variations in concentration of sodium and osmotic pressure of the apical channel were replicated in these investigations by injecting hormones like aldosterone and vasopressin into the device's basal channel. Further, they discovered that shear stress stimulates cilia, cell polarity, and P-glycoprotein expression and absorption of albumin/glucose in cells, in addition to changing cell orientation.

(d) *Gut-tumor-on-a-chip*

The gut is an organ that is primarily accountable for the absorption and digesting of nutrients; growing evidence indicates that it also plays a crucial part in the proper operation of other organs and the etiopathology of many disorders. Due to the existence of foldable microstructures called the intestinal villi and microvilli, the gut has a remarkably large surface area. Biological analysis-capable lab-on-a-chip devices have been made possible by recent developments in microfabrication methods and microfluidics. The intestines contain vital microbes that support digestion, immunological control, and defense against invading pathogens (Donaldson et al. 2015). The particular hypoxic environment in the intestines allows these mutualistic microbes to thrive. The most common material utilized for OoCs systems is polydimethylsiloxane (PDMS) which offers a non-toxic, gas-permeable, biologically inert, and surface with minimal adhesion to enable adequate O_2 and CO_2 exchange to their cells (Mata et al. 2005), but gas permeation is not ideal for creating the hypoxic environment seen in the intestines. The small intestine, in particular, acts as a barrier to orally delivered medications; thus it's crucial to know how it works during drug development. Kimura et al. (2008) developed a miniature intestine-on-a-chip device having an optical detecting mechanism to test this function (Fig. 14.4f). Small intestine model (Caco-2) cells were cultivated in two distinct compartments separated by a microporous membrane. Protracted monitoring and culture of the cells' polarization transport activities were used to assess the device's performance. The cells could be grown for more than 2 weeks, and online fluorescence studies of rhodamine 123 monolayer transport were successful; however, there was no physiological cell function. Kim and Ingber (2013) suggested a gut-on-a-chip system that allows Caco-2 cells to be subjected to stimuli like shear stress and cyclic mechanical strain to imitate peristalsis-like movements. Experts claim that under these physiological conditions, cells produced using the device are repurposed to naturally go through small intestinal cell differentiation and 3D villus morphogenesis. This apparatus was utilized to co-culture numerous commensal microorganisms in intestinal ECs and investigate the associated biological phenomena to duplicate more precise physiological circumstances. The outcomes concluded sensors in the system showed that hypoxic culture conditions can be sustained and the gut-on-chip device may be employed to examine intestinal pathophysiology and unravel ailment causes in vitro after demonstrating that this in vitro setting reproduced results from previous animal and human investigations.

(e) *Body-on-a-chip*

Humans are made up of tissues and organs that perform many biological functions and may be thought of as a complicated setting. As previously stated, it is challenging to anticipate interfaces amid tissues and organs via traditional in vitro cell culture methodologies; hence animal experiments are required to predict pharmacokinetics. Microfluidic systems comprising the activities of multi-tissues and multi-organs, dubbed "body- or human-on-a-chip," have been suggested in the OoC research field. Several tools may be employed to track ongoing or connected pharmacokinetic activities like ADME of multiple drug delivery pathways, and the information gathered could be utilized to build computational equations for anticipating the efficacy of medications. Since the 2000s, pioneers have led the globe in body-on-a-chip investigations. They created the micro cell culture analogue (μCCA), a device with numerous organ chambers, and co-cultured diverse organ-derived cells on it (Sung and Shuler 2009; Sung et al. 2014). These techniques were used to study organ interconnections utilizing cell-based tests employing tegafur, an anticancer medication. Their findings demonstrated that complicated biological reactions to a dosage scenario including oral or intravenous delivery, which had previously been explored using animal experiments, may be replicated using body-on-a-chip technology. Imura et al. (2010) created a device that includes absorption-related processes in the small intestine as well as metabolic functions in the liver (Fig. 14.5a). They demonstrated disparities in anticancer activity by employing medicines with varying therapeutic mechanisms and intestinal absorption rates using their technology. By incorporating a dialysis membrane into a system, they were also able to integrate renal excretion processes. Even though these systems were able to simulate various organ interactions in vitro, each biological parameter recorded that was not pathological (Kimura et al. 2015) built a device that replicates several physiological factors such as blood flow rate and organ volume ratios on the system (Fig. 14.5b) to achieve accurate pharmacokinetic predictions. This technology was utilized to investigate the efficacy of anticancer medications and proved that microfluidics devices may reliably restrict intestinal barrier absorption and provide pharmacological advantages owing to liver metabolism. We must keep in mind that these systems cannot replicate all biological reactions. According to Shuler's study, the importance of this device is not only to develop a smart minuscule human body but also to identify undiscovered reactions that can only be detected in instantaneous interfaces between organs. The information gathered by these sensors should help enhance the accuracy of mathematical models. To put it another way, pharmacokinetic models must be combined with this equipment to forecast unknown processes or events. By permitting the introduction of mixtures of cells and substances, the devices described here could be used in a variety of pharmacological toxicity tests, and a statistical centered on the testing data obtained with these devices could be in practical use as a pharmacokinetic prediction evaluation system. Certainly, Sung et al. (2010) presented a strategy for predicting cell behavior after anticancer drug exposure that combined data

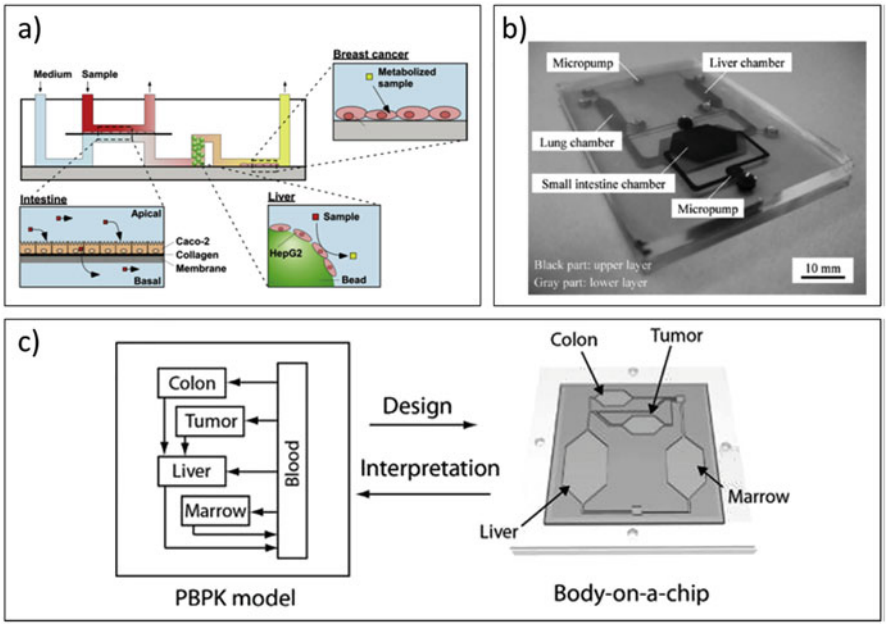

Fig. 14.5 Body-on-a-chip devices: (**a**) Small intestine and liver models are included in the body-on-a-chip system. The American Chemical Society (2010) owns the copyright to this image. (**b**) A device that measures a variety of physiological parameters. The Society for Laboratory Automation and Screening (2014) owns the copyright to this image (**c**) The PBPK modeling principle was used to design the body-on-a-chip technology. The Society for Experimental Biology and Medicine (2014) owns the copyright to this image

from mCCA with mathematical models to predict pharmacokinetics-pharmacodynamics behavior. As a consequence, study demonstrated that a body-on-a-chip technique may be combined with a statistics modeling method (Fig. 14.5c).

14.3.1 Mimicking TME Using Microfluidic Devices

Cancer is a multifaceted and diverse malignancy driven by the stroma's epigenetic, genetic, and cellular signaling. Three features of the TME are critical: (1) Cancer cells engage with stroma in the peri-necrotic niche to avoid detection by the immune system and to take on invasive and migrating characteristics that allow them to spread to distant organs. Due to a growth in tumor bulk, cancer cells' metabolic status is altered under hypoxia and ischemia (Brown and Wilson 2004; Byrne et al. 2014). Cancer cells are more likely to survive in harsh environments and develop metabolic resistance to many cancer treatments in a necrotic microenvironment that is significant in acidity, poor in oxygen and nutrients, and heterogeneous. (2) The tumor associated stroma in the perivascular forte cause new vasculature to develop in

response to nutrition (Charles and Holland 2010). As opposed to normal vasculature, tumor vasculatures are frequently immature and permeable. The perivascular and metastatic niches are both intertwined (Ren et al. 2015). Due to new vasculature, cancer cells detach toward circulating tumor cells and tumor-initiating initiating cells amid them might proliferate into secondary metastases when implanted in remote regions. (3) Cancerous cells engage in stromal interactions to avoid immune detection and take on invasive and migrating characteristics. A metastatic cascade is a complex sequential procedure that tumor cells go through. Recent research has suggested that tumor beginning cells, also known as cancer stem cells, are a small subset of CTCs. This suggests that the TMEs are essential for cancer stem cells to establish themselves in multiple organs and develop new metastases (Psaila and Lyden 2009).

14.3.2 Modeling Hypoxia and Necrosis

Hypoxia influences tumorigenesis and therapy tolerance (Abou Khouzam et al. 2021). A variation amid hyper-proliferative cancer cell proliferation, nourishment, and the vasculature gas supply induces ischemia in the surrounding tissue when the main tumor develops and its hyper-proliferating region grows (Lugano et al. 2020). The perivascular place, in part due to hypoxia, induces new vasculature to transport additional nutrients and gas. The new vasculature, on the other hand, is frequently aberrant and fails to compensate for the nutritional deficiency. The effects of chronic hypoxia in the tumor include the selection of survival cancer cell genotypes, metabolic shifts toward anaerobic glycolysis, an increase in pro-survival gene expressions, epithelial-mesenchymal transition (EMT), and treatment resilience (Martin et al. 2016). The chip material gas permeability in microfluidics allows for the creation of a hypoxic microenvironment to imitate the peri-necrotic area. For the fabrication of delicate lithographic microfluidic chips, polydimethylsiloxane (PDMS), a biocompatible silicone rubber having excellent gas permeability, has become a prominent material (Miranda et al. 2022). A low oxygen environment or an oxygen gradient can be induced by the flow of various gases, oxygen scavengers, or gas-equilibrated liquids in microfluidic networks. The gas environment can also be controlled by employing a poor gas-permeable thermoplastic for the microfluidic chip or by embedding a thin thermoplastic layer inside the chip (Chang et al. 2014). Zhang et al. (2015) employed SUM159 breast carcinoma cells on two-dimensional microfluidic devices to show enhanced mesenchymal migration and lactate generation amid hypoxia circumstances. Cell migration is aided by the acidic microenvironment created by metabolic reprogramming. Environmental acidification can slow cancer cell migration while also improving the efficacy of CSF-1R, HIF-1, and CCR4 therapies. Cell embedding hydrogel models may also be used to investigate the intervention of malignant cells to hypoxic conditions in 3D. Xu et al. (2015) found that hypoxia inhibited the growth and invasion of glioblastoma U87MG cells. Normoxia gas was pumped into one control channel close to the PANC-1, a pancreatic carcinoma cell, while hypoxia gas was pumped into other.

Microfluidic platforms were also employed to investigate the kinetic rate of a necrosis core formation in a 3D cell-embedded hydrogel tumor model. Over 6 days, Ayuso et al. (2016) used a 3D cell embedding hydrogel system to monitor the kinetic generation of necrosis cores in HCT-116 colon cancer and U-251MG glioma cells. Additionally, in situ on-chip studies of dynamic changes in glucose and oxygen concentrations, apoptosis, cell proliferation, formation of ROS, and therapeutic response are all possible. A significant strategic path is the development of more sophisticated microdevices to replicate various microenvironments for comprehensive kinetic studies of signaling cross talks among different microenvironments, such as elucidating the interrelatedness between necrosis and neo-angiogenesis in the cross talk of the peri-necrotic and perivascular area. Validating the in vitro necrotic tumor model to tumor lysis and including stroma to explore tumor-stromal cell interaction has indeed been difficult in the peri-necrotic niche. Tumor lysis is defined as the abrupt death of huge numbers of cells, resulting in metabolic abnormalities and the development of tumor lysis syndrome (TLS). To overcome these technological hurdles, new microdevice designs including (bio)sensors and active aerodynamics such as microvalves are required.

14.4 Application of Tumor-on-a-Chip

Tumor chips have been filled with patient-specific cells for targeted therapies, tailored for tumor metastases research such as tumor cell extravasation and micrometastasis formation, and arrayed for high-throughput screening applications (Bray et al. 2019). Organotypic tumor chips that can replicate complicated organ-level patterns of cancer development, spread, and therapy response seen in patients are rapidly becoming available. Multi-MPS were created to research drug pharmacokinetics (PK), pharmacodynamics (PD), and toxicity in more depth. Tumor chip models, in particular, enable innovative manipulation and closely managed real-time research of dynamic interactions between stromal, tumor cells, and TME, which is more difficult to achieve by traditional animal models and tissue culture.

14.4.1 Multiplexed Drug Screening

Among the most crucial steps for medication/lead candidates to undergo clinical trials that are based on pharmacological activity is drug screening. Preclinical testing is required after bio- and chemical synthesis to completely comprehend the efficacy, toxicity, and ADME (absorption, distribution, metabolism, and elimination) characteristics of the drug candidates. Organoids or organs-on-a-chip, which uses 3D cell culture methods to mimic human organ-specific microenvironments in vitro, have shown significant promise in overcoming the constraints of traditional animal models. Recent advancements in microfluidics technology have made high-throughput drug screening more economical and feasible. Microfluidic chips need a substantially smaller sample volume, in addition to having a cheaper cost and

higher processing speed. These chips may also be tailored to track the impact of anticancer medications on a variety of factors, such as cell migration. Organ-on-a-chip technologies and organoid models have arisen to better imitate the TME with human cells and enhance efficiency with higher throughputs for more effective translational cancer therapy research. Because tumor heterogeneity makes a "one-size-fits-all" approach to cancer treatment ineffective, substantial attempts have been undertaken to personalize cancer medicines for Individual or tiered healthcare organizations. Ex vivo screening on solid tumor samples and biopsies from patients has become a supplemental technique with the ability to accurately determine the most effective treatment plans. While next-generation sequencing (NGS) is employed to direct these strategies, ex vivo screening on solid patient tumor samples and biopsy has arisen as a complementing method, with the ability to establish ideal therapeutic regimens. To achieve this, several groups have been developing tumor-on-chip platforms that enable the cultivation of tumor samples obtained from patients as well as the assessment of their therapeutic response. Mazzocchi et al. (2018) exhibited abiding culturing of viable 3D spheroids in six chambers, followed by pharmacological testing. A peristaltic pump was employed to keep fluid or drug-infused media flowing continuously to the spheroids. The treatment reactions of patients were also replicated in the on-chip tumor spheroids, indicating that this method might be useful for precision medicine. Lim et al. (2018) cultivated size-controllable spheroids of breast tumor cells obtained from biopsies and treated them with the same chemotherapy medicines that the patients received. They also stated that their spheroids' medication responses were identical to the patients'.

14.4.2 Transport and Delivery of Nanoparticles

Because of tumor invasion, progression, and metastasis, the hypoxic TME is categorized by muddled vasculature and fast tumor growth. Hypoxic conditions reduce the effectiveness of cancer treatments such as chemotherapy, radiation, phototherapy, and immunotherapy, which has major consequences such as tumor recurrence and high mortality rate. Fabrication of efficient nanomaterials to alleviate hypoxic tumors has recently been the focus of research. The application of formulations with nanocarriers to administer precision medications and diagnostic agents to tumor locations has made tremendous progress in the burgeoning field of nanomedicines. By delivering enhanced coverage for the variables' bioactive components in the serum-rich setting, extending bloodstream circulation times, reducing side effects, increasing permeation and retainment effects, improving tumor-targeting effectiveness, increasing controlled release, and potentially integrating stimuli sensitivity for on-demand treatment, nanomedicines offer clear advantages over systemic therapy of free compounds (Blanco et al. 2015). Nanomaterials can be developed with characteristics that make administration smoother in a multitude range of settings such as enhancing oxygen-dependent tumor therapy that raises oxygen levels in tumors using oxygen-carrying nanomaterials (Khan et al. 2019), oxygen-generating nanomaterials (such as

nanozymes) (Liang and Yan 2019), and oxygen-economizing nanomaterials (Yu et al. 2019) and reduced oxygen dependence for tumor hypoxia treatment using gas-generating nanomaterials (Deng et al. 2018) and radical-generating nanomaterials (Lv et al. 2018). Due to the complexity of hypoxia tumors, a lot of research has also concentrated on hypoxic tumor treatment methods using nanomaterials that are less oxygen-dependent. Farokhzad and groups employed a simple microfluidic device with cancer cells monolayer on the bottom to explore the dynamic interactions of perfused particles with cancer cells (Farokhzad et al. 2005). PC3 and LNCaP a prostate cancer cell lines, grown in a microfluidic environment, revealed distinct uptake behaviors for polylactic acid (PLA) particles functionalized with and without aptamers recognizing PSA in a size- and flow-dependent way (PSMA). 3D configurations of interactions between NPs and malignant cells were established. Lee et al. (2009) employed inverse opal-structured hydrogel scaffolds to generate spheroids of HepG2 hepatocellular cancer cells. The vitality of the pristine spheroids was significant, and they had intact cell junctions. When these liver cancer organoids were treated with semiconductor CdTe NPs, their vitality was diminished, and the surface-located cell activities were compromised. Recent advancements in microfluidic on-chip tumor models have further integrated the benefits demonstrated in the first two examples. These advancements include the ability to replicate not only the systematic mobility of NPs with various parameters, such as size, shape, and surface characterizations but also the configuration of the pertinent frameworks.

14.4.3 Microfluidic Devices for the Analysis of Transcriptomic and Proteomic Factor

Droplets in a microfluidic device can be used to do transcription analysis at the single-cell level. Zhang et al. (2012) created a microfluidic system that used agarose droplets to do single-copy RT-PCR utilizing both sample and RT-PCR reagents. Significant variations in the expression of the EpCAM cancer diagnostic gene across different kinds of cancer cells were used to verify the platform. Hayes et al. (2016) employed microfluidic droplets in a separate investigation to assess ECM gene expression levels in colorectal cancer patient samples to see whether there was a link between metastatic potential and differential expression.

Quantitative characterization of cancer proteomics has the efficacy to revolutionize not just molecular diagnostics but also the discovery of new novel therapies. Using tissue samples and cultivated cell lines with as few as 1000 cells, Sun et al. (2010) designed a microfluidic cytometry imaging system that can do quantitative single-cell proteome analysis. Its therapeutic use was validated by examining four proteins involved in the mTOR signaling pathway in human brain tumor samples and contrasting the outcomes with those obtained via well-known clinical immunohistochemistry (IHC) procedures. On a broader scale, Xu et al. (2016) devised a biomimetic multi-organ microfluidic chip to examine changes in RANKL, CXCR4, and other markers' expression levels in the multiple "remote organs" after tumor cell invasion.

14.5 Challenges and Future Prospects

Tumor hypoxia emerges as a consequence of uncontrolled cell growth, altered metabolism, and aberrant tumor blood vessels, which diminishes the transfer of oxygen and nutrients. One of the key characteristics of solid tumors is hypoxia, which has been linked to cancer patients' poor prognoses. Three factors are significant inside the TME such as the following: (a) cancer cells' metabolic changes in the peri-necrotic niche are further driven by hypoxia in the necrotic core of initial tumor tissue, (b) the tumor and stroma in the perivascular area cause new vasculature to develop in response to nutrition, and (c) in the metastatic niche, cancer cells engage in interactions with stroma to circumvent the immune system and take on invasive and migrating behaviors to spread to distant regions. Apart from that, several crucial concerns should also be addressed to copiously exploit the efficacy of ToC techniques. For example, how to use such a system to model the mechanism of intra- and extravasation; how to enable tumor-associated tissues to sophisticated chip concerning self-organization; and if the construction of a tumor-on-a-chip system that enables a tumor to develop on a chip can be done with the bare minimum amount of components. To comprehend the augmented permeability and retention impact, along with the dormancy phenotype, alternative methodologies to statistically analyze tumor-matrix interactions along with matrix remodeling and growth factors must be established. Using a ToC approach, it's critical to depict the tumor's heterogeneity and progress. Depending upon biological and medical significance, ToC technologies possess varying unique designs and degrees of sophistication. Oversimplified or overcomplicated systems must be avoided, and proper complexity must be driven by demand. As a result, one or more of the following concerns may be included in a ToC system: (a) transport of culture media, cytotoxicity, elimination of waste, and cellular metabolism; (b) kinematic and physiological constraints, such as composite stiffness and inhomogeneity, adhesion molecules, and flow rates; (c) concentration gradients and physiological concentration levels of circulatory components; (d) structural features, such as 3D tumor constructs and microfluidics models; and (e) cell sources and types, such as culture, stromal, patient, stem, or progenitor cells. To precisely capture crucial characteristics of a tumor, it is essential to examine metastasis sites, recapitulate cancer-immune cell interactions, and conduct real-time, on-chip monitoring of key biochemical and biophysical variables. While premade tumor scaffolds offer certain benefits, self-organized tumor structures resulting from cell-cell interaction may be a superior paradigm for ToC platforms. For various tumors and/or to answer different questions, multiple tumor-on-a-chip models with varied characteristics and complexity are likely to be required.

The innovative ToC paradigm is effective in significantly altering oncology and cancer biology. Nevertheless, there are stumbling barriers in the creation of technology, such as design, optimization, analysis, and validation. Material selection determines the integrity of device qualities such as suitability, biocompatibility, and mechanical characteristics. The majority of the devices had been constructed on PDMS-based materials that have shown to be excellent for studying biological

phenomena but have serious drawbacks when employed with hydrophobic medicines. Alternative printable and moldable substitutes must be discovered to bypass this restriction, like off-stoichiometry epoxy resin, thiol-enes, and perfluorinated polymers. Integrating microdevices printing specialists with polymer and material scientists will be required for systematic modification and automation of chemical and physical parameters within the microfluidic device system. When it comes to device scalability, the creation of sound manufacturing practices, material selection, and user operability are the most important considerations. Furthermore, the shelf-life and sustainability of ToC devices must be determined. The ToC device has significant benefits of accurately influencing physio-chemical characteristics in the TME, co-culturing stromal cells with tumors, offering an optical screen for on-time microscopy assertion of molecular and subcellular mechanisms, and incorporating biosensors for detection and quantification.

14.6 Conclusion

The tumor-on-a-chip device might be more advantageous to animal xenograft models in terms of physio-chemical variances, biological diversity, affordability, and ease of statistical analysis. Altogether, to fully leverage the promise of ToC technology, material scientists, biophysicists, biomedical engineers, cell biologists, and oncologists must work together to build and optimize these systems for cancer research, drug discovery, and clinical application. Although proof-of-concept reports have demonstrated the efficiency of ToC systems for tumor research, major challenges remain in implementing the device in clinical settings, together with the authenticity of device implementations by correlating with traditional in vivo tumor models and data obtained via ToC technology and clinical tumor samples.

Acknowledgments The authors thank the Director, Council of Scientific and Industrial Research-Advanced Materials and Process Research Institute (CSIR-AMPRI) Bhopal, India, for his interest and encouragement in this work. The fellowship provided to Arpana Parihar under the DST-WoS-B scheme (DST/WOS-B/HN-4/2021) is duly acknowledged. Raju Khan would like to acknowledge Science and Engineering Research Board (SERB) for providing funds in the form of the IPA/2020/000130 project.

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