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Engineered *In Vitro* Tumor Models for Cell-Based Immunotherapy

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Abstract

Tumor immunotherapy is rapidly evolving as one of the major pillars of cancer treatment. Cell-based immunotherapies, which utilize patient's own immune cells to eliminate cancer cells, have shown great promise in treating a range of malignancies, especially those of hematopoietic origins. However, their performance on a broader spectrum of solid tumor types still fall short of expectations in the clinical stage despite of the promising preclinical assessments. In this review, we briefly introduce cell-based immunotherapies and the inhibitory mechanisms in tumor microenvironments that may have contributed to this discrepancy. Specifically, a major obstacle to the clinical translation of cell-based immunotherapies is in the lack of preclinical models that can accurately assess the efficacies and mechanisms of these therapies in a (patho-)physiologically relevant manner. Lately, tissue engineering and organ-on-a-chip tools and microphysiological models have allowed for more faithful recapitulation of the tumor microenvironments, by incorporating crucial tumor tissue features such as cellular phenotypes, tissue architecture, extracellular matrix, physical parameters, and their dynamic interactions. This review summarizes the existing engineered tumor models with a focus on tumor immunology and cell-based immunotherapy. We also discuss some key considerations for the future development of engineered tumor models for immunotherapeutics.

Graphical abstract

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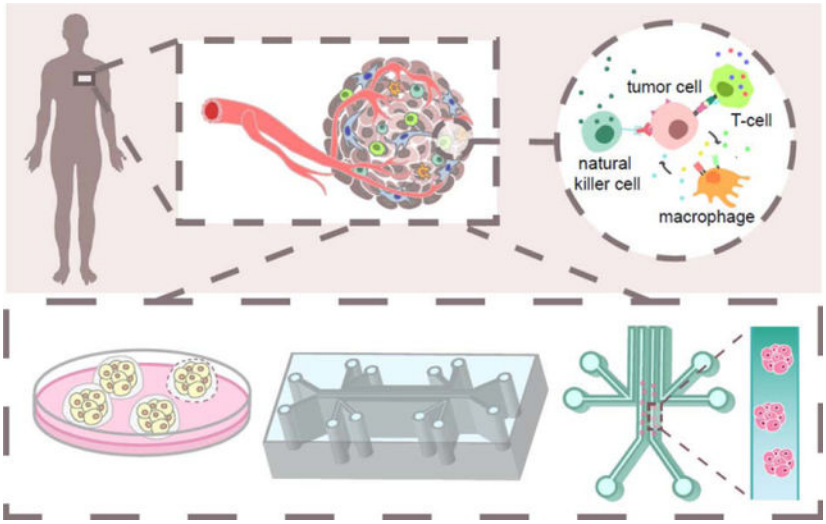
Declaration of interests

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



Introduction

Immunotherapy is arguably the most rapidly evolving and promising cancer therapeutics in the past decade [1] and regarded as the fourth pillar of cancer treatment [2]. Unlike conventional therapeutic strategies, immunotherapy aims to boost the functions of a patient's own immune system to recognize and attack the cancer cells, which can generate durable remission even in advanced tumors with relatively few side effects [3]. Immune checkpoint inhibitors, which target the negative regulators of the immune system, have been integrated in the standard anti-cancer therapies as single agents or in combination with chemotherapies [4] The emerging cancer viruses and personalized cancer vaccines are also showing promising results in clinical trials [5, 6]. Most notably, cell-based immunotherapies are taking the center stage of immunotherapy in recent years. For example, Sipuleucel-T (Provenge) and three chimeric antigen receptor (CAR) T cell therapies, Tisagenlecleucel (Kymriah), Axicabtagene ciloleucel (Yescarta), and Brexucabtagene autoleucel (Tecartus), were approved by the US Food and Drug Administration (FDA). Many others including CAR and T cell receptor (TCR)-engineered T cells are in the preclinical and clinical pipelines [7, 8]. However, the success of these therapies has largely been limited to a few types of malignancies, particularly those of hematopoietic origin, and their efficacies still fall short of expectations in most solid tumors [9], which account for over 90% of all cancer types [10]. There is also tremendous variability in the efficacy of immunotherapy across patients, therapies, and cancer types [11].

Part of this disappointment is attributed to the staggering complexity and heterogeneity of the tissue microenvironment in solid tumors, or the tumor microenvironment (TME). The TME impose physical, chemical, molecular, and cellular barriers to infiltrating therapeutic immune cells [12, 13]. Preclinical *in vitro* and *in vivo* models are crucial for understanding the working mechanisms and evaluating the efficacy of cell-based immunotherapies in the context of the TME. However, it remains challenging to recapitulate the immune TME in most existing *in vivo* models, which typically involve immunodeficient mice and/or TME components with little/limited resemblance to human pathophysiology [14]. Thus,

the mechanistic insights derived from these models often fail to predict the outcomes in clinical trials. Engineered *in vitro* models have emerged to mimic the essential elements of the TME using human cell lines or patient-derived cells. With the assistance of modern techniques such as micro-fabrication, organoid culture, and bioprinting, a new generation of *in vitro* tumor models are emerging that recapitulate the human TME with unprecedented control. They allow for precise mechanistic evaluation of individual TME components in immunotherapy, while also enabling assessment of therapeutic efficacy in a more (patho)physiologically relevant manner, and potentially on an individual patient basis for personalized medicine. In this review, we discuss the unique aspects of cell-based immunotherapy, the major challenges for their efficacy, and recent advances in engineered tumor models in the context of cellular immunotherapies. We also examine the additional crucial aspects of TME that need to be incorporated in future models to further accelerate immunotherapeutic discoveries at the molecular scale and the systems level and to enable faithful clinical translation.

Cell-Based Cancer Immunotherapy

Cell-based cancer immunotherapy focuses on using living immune cells as a therapeutic agent to kill or aid the killing of tumor cells. In most cases, immune cells are extracted and isolated from patient's body, expanded *ex vivo* with or without modifications, and reinfused back into the patient in a process known as adoptive cell transfer (ACT) [15] (Figure 1). In addition to increasing their number and immune potency, adoptively transferred immune cells can be modified to selectively target tumor-specific or tumor-associated antigens.

Among the many cell types involved in tumor immunity (Figure 1), dendritic cells and T cells have been the most successful and extensively used for cell-based immunotherapies. Dendritic cells (DCs) are specialized antigen-presenting cells (APCs) that mediate the innate immune response and induction of the adaptive immune responses. Adoptively transferred DCs are used for cancer vaccination: DCs loaded with cancer-specific antigens recruit and activate endogenous antigen-specific cytotoxic T cells to induce anti-cancer immunity [16, 17]. Clinical trials against cervical, ovarian, and colorectal cancer have shown DC vaccine-induced recruitment of CD4+ and CD8+ T cells in tumors [18–20]. Provenge (Sipuleucel-T) was the first FDA-approved cell-based immunotherapeutic vaccine against advanced prostate cancer [21, 22]. It utilizes APCs isolated from patient's white blood cells (consisting of mainly DCs) that are cultured *ex vivo* with a recombinant fusion protein containing a prostate tumor antigen and granulocyte-macrophage colony stimulating factor (GM-CSF) [17]. T cells play a central role in anti-tumor immunity. Tumor infiltrating lymphocytes (TILs), which contain T cell clones that recognize tumor antigens, have been expanded *ex vivo* and reinfused into patients with metastatic melanoma [23–25]. T cells without tumor-specific TCRs can also be endowed with anti-tumor immunity by cloning the TCR gene from TILs (also known as the TCR-engineered T cells), which overcomes the dependence of TIL therapy on the preexistence or identification of tumor-reactive lymphocytes [26].

Another form of genetically engineered T cell is CAR engineered T cells. CARs are synthetic receptors that recognize tumor-associated antigens (TAAs) expressed on target cell surfaces, most often composed of an extracellular single chain variable fragment (scFv) from

a clinical antibody and intracellular signaling domains that activate T cells [27, 28]. Three CAR T cell therapies have been approved by the FDA. Novatis' Tisagenlecleucel (Kymriah) was the first approved CAR T therapy against B-cell precursor acute lymphoblastic leukemia (B-ALL) in 2017. Strikingly, up to 90% complete remission was observed in B-ALL patients in earlier clinical trials [29]. In the same year, Kite's Axicabtagene ciloleucel (Yescarta) was approved for treating adult patients with relapsed or refractory large B-cell lymphoma. Most recently, in July 2020, the FDA approved Kite's Brexucabtagene autoleucel (Tecartus) for treating adults with relapsed or refractory mantle cell lymphoma. All three CAR T cell therapies are based on CD19, which is a target for B cell malignancies [30], despite reports of on-target, off-tumor effects and neurotoxicity [31]. In August 2019, the FDA granted breakthrough therapy designation to an experimental CAR T cell therapy targeting surface molecule CD22 in children and young adults with relapsed of CD19-resistant B-ALL [32]. Following these incredible clinical developments, CAR T cells targeting multiple tumor antigens have been actively investigated for treatment of several solid cancer types. Some identified targets include EGFRvIII for glioblastoma, HER2 for sarcomas, MET for melanoma and breast cancer, PSMA for prostate cancer, and mesothelin for metastatic breast cancer. Nevertheless, the preclinical and clinical results have been disappointing so far [33–36].

Several other immune cell types are also gaining attention in cell-based immunotherapies. Macrophages are highly plastic immune cells that regulate tissue homeostasis, wound healing, and innate immune responses [37]. Tumor-associated macrophages (TAMs) are often correlated with poor patient prognosis, and exhibit bipolar effects promoting and inhibiting tumor immunosuppression [38]. Modifying macrophages to express CARs that specifically target tumor antigens resulted in phagocytosis of antigen-expressing cancer cells [39]. On the other hand, TAM depletion is a common approach to lower the immunosuppressive TME and synergistically enhance immune checkpoint blockade, DC vaccines, and ACT of cytotoxic T cells [40, 41]. Natural killer (NK) cells are another innate immune cell capable of eradicating tumor cells. Adoptive transfer of alloreactive NK cells has shown promising results in treating a range of malignancies including acute myeloid leukemia and non-small cell lung cancer [42–45]. Engineering NK cells with CAR further enables their tumor targeting through both the native receptors and the CAR, thus reducing the chance of immune escape by cancer cells [46].

TME as a Barrier to Cell-based Immunotherapy

Cell-based cancer immunotherapy shows tremendous promise for targeted and durable remission of cancer. However, much of the exciting success in preclinical studies and clinical applications with certain cancers have not translated well to a broader range of cancer types, especially in solid tumors [47, 48]. While the reason is most likely multifactorial, the immunosuppressive TME has been recognized as a major player [49]. The TME consists of intricate interactions between its cellular and acellular components. The cellular players include myofibroblasts, immune cells, endothelial cells, adipocytes, etc.; the acellular players include the extracellular matrix (ECM), cell-secreted soluble factors, metabolites, nutrients, oxygen, as well as physicochemical parameters such as pH, ECM stiffness, and mechanical forces [50].

The architecture of solid cancer consists of the parenchyma (neoplastic cells) and the stroma (all other cellular and extracellular components) [51]. Nests of parenchyma are in complex interplay with the stroma, molding phenotypic heterogeneity and immunosuppressive factors [52, 53]. Elevated ECM production and stiffness/density impose physical barrier to therapeutic immune cell infiltration [54]. Recruitment or differentiation of regulatory T cells (Tregs), TAMs, myeloid-derived suppressor cells (MDSCs) and cancer-associated fibroblasts (CAFs) can establish an immunosuppressive TME that compromise cytotoxic T cell cytokine secretion, growth, and cytotoxicity against tumor cells [55]. Increased expression of suppressive ligands and soluble factors/cytokines by cancer and stromal cells in TME, such as PD-L1/L2 [56, 57], prostaglandin E2 (PGE2), adenosine, and TGF-β function against anti-tumor immune cells [58–60].

The metabolic landscape in TME has lately emerged as one of the crucial regulators of immunotherapeutic resistance. Nearly all cancers exhibit shifted energy metabolism from oxidative phosphorylation (OXPHOS) to aerobic glycolysis (also known as Warburg effect) [61]. Meanwhile, hypoxia is a common feature of most solid tumors, especially in those with high malignancy [62]. Hypoxia signaling pathways, particularly that of the hypoxia inducible factor (HIF), overlap strongly with those implicated in the altered cancer and immune cell metabolism, where the action of HIF is involved in almost every step of glycolysis [63]. In cell-based immunotherapies, the competition for glucose between cancer cells and the glycolytic anti-tumor leukocytes has been proposed to hinder the penetration and functionality of these therapeutic cells, and to activate the stress responses that lead to their exhaustion or death [64]. On the other hand, hypoxia has been shown to impair CAR T cell expansion *in vitro* and their differentiation into effector memory cells [65]. Hypoxia can also upregulate the expression of some antigens in tumors such as carbonic anhydrase IX [66]. These evidences suggest opposing roles of hypoxia in cell-based immunotherapy which require further investigation.

Major Limitations with Conventional Tumor Models

The integrated, heterogeneous facets of the TME leads to immune evasion and immunotherapeutic failures [67]. Currently, no tumor model has fully addressed the complexity of the TME. In this section, we will briefly review the challenges of conventional approaches to model the immune microenvironment in the TME.

Traditional *in vitro* cell culture still serve as mainstream screening platforms for drug discovery and therapy evaluation [68]. In cell-based immunotherapies, random mixture of therapeutic immune cells with target cancer cells is often used to evaluate the efficacy of targeted cell killing. For epithelial cancers, this usually involves culturing immune cells on a two-dimensional (2-D) monolayer of cancer cells in a dish. The advantages of the random mixture or 2-D studies include high turnover rate, relatively cheap costs, and facile maintenance. However, clinical translation remains a challenge, which is largely due to the lack of tissue-level characteristics like tissue morphology, vasculature, cell-cell/cell-matrix interactions, and diffusion-limited processes in the TME [69, 70].

In vivo models allow investigation of whole-body systemic response to treatment [14]. Mouse syngeneic tumor models involve implantation of MHC-matched cancer cell lines

into subcutaneous or orthotopic sites of recipient mice. Since the implanted cancer cells are compatible with the host immune system, these models have been widely used to assess anti-tumor immunotherapies [71]. Yet, such tumors usually lack genetic heterogeneity and do not truly capture the morphology and microenvironment of the tumors they represent from the original organs. Genetically engineered mouse models (GEMMs) carry oncogenes that are induced/activated in specific organs, which then develop *de novo* tumors with a natural immune microenvironment [72]. Some advanced GEMMs display genetic heterogeneity, mimic the histopathological and molecular features of the corresponding human tumors, and can spontaneously progress into metastatic disease [73]. GEMMs are thus of great importance in understanding the biological mechanisms underlying tumor immunology. However, both mouse syngeneic models and GEMMs come with high costs, slow turnover rate, inability to control selected processes of the TME [74], and most importantly, lack of consistency with human immune system [75]. Thus, these models have limited utility in predicting the immunotherapeutic outcomes in human tumors.

Xenograft models have been developed to improve human relevance of mouse tumor models, by implanting human cell lines or patient-derived tissues in immunodeficient mice [76]. Notably, cell-line and patient-derived xenograft (PDX) models have been utilized for preclinical evaluation of CAR T cell therapies [77, 78]. Nonetheless, cell-line xenografts often do not accurately model the true behaviors of the human tumors due to the adaptation of cell lines to *in vitro* growth conditions and a lack of human stroma. Similarly, the preservation of the TME in PDX models is also incomplete and temporary, and tend to deviate from the patient's original tumor [79], such as with murine stroma replacing human stroma over time [80, 81]. Furthermore, the hosts of cell-line xenografts and PDX models are immunocompromised to prevent rejection of human tumor tissue. The lack immune infiltrate renders the platform inadequate to assess immunotherapy in the context of human immune system, particularly those associated with the adaptive immune functions. Even in humanized mouse models that allow investigation of xenografts with an intact human immune system (i.e. through transplantation of human hematopoietic stem cells, HSCs [82]), there are significant hurdles such as obtaining HSCs from the same patient to avoid immune rejection [83]. Existing models, therefore, are insufficient for efficient clinical translation. The advantages and disadvantages of these models are outlined in Table 1.

Engineered Models for Tumor Immunology

Engineered *in vitro* tumor models can recapitulate specific features of the TME while maintaining relatively low costs and medium to high throughput for rapid investigation. These models enable unprecedented spatiotemporal control of microenvironment factors that play major roles in tumor biology [84, 85]. A major advancement in the *in vitro* tumor modeling has come from replicating the 3-D tumor architecture *in vivo*. Compared to traditional 2-D assays, cancer cells in the 3-D spatial architecture exhibit distinct behaviors [86–88] that mimic signaling pathways in primary tumor samples [89] or those from *in vivo* models [90]. These include decreased tumor proliferation [91, 92], loss of cell polarity [93], up-regulation of angiogenic factors such as hypoxia-inducible protein 2 and VEGF 1/2 [86, 94], and increased reliance on glycolysis [95–97]. Such features directly influence the assessment of various therapies at the preclinical stage [98]. For example,

cells in 3-D exhibit decreased sensitivity to radiation therapy, death receptor ligation, interferons, or chemotherapeutic reagents [91, 94, 99]. For cell-based immunotherapy, 3-D microenvironment also poses physical, chemical, and cellular barriers to the infiltration of therapeutic immune cells that 2-D cultures cannot recapitulate.

Another major feature of the TME is the heterogeneity of cellular types that interact with each other. Co-culture models that interrogate the interactions of different cell types are crucial to provide a systemic study of the complex interchange of cellular communication during immunotherapeutic regimens. Therapeutic outcome has been associated with the existence of certain immune cell types such as macrophages inhibiting cytotoxic T cell activity through engagement of PD-L1/PD-L2 [100] or through immunosuppressive molecules such as IL-10, TGF-β, and prostaglandins [101]. Therefore, the inclusion of innate and adaptive immune cells in preclinical tumor models can provide a more wholesome outlook on the TME and its potential impact on cell-based immunotherapy.

In addition, like the other organs-on-a-chip models [85], engineered tumor models have exploited self-assembly and/or microfabrication to facilitate mass production of highly customizable and tunable platforms. Miniaturization further improves the high-through capacity to screen various therapies and developing novel pharmaceuticals. Here we divide the section into reviews of tumor spheroids, organoids, and microfluidic models, with a focus on their adaptation in evaluating immunotherapies.

Tumor spheroids

The first generation of bio-inspired cell culture involved cancer cells that spontaneously formed cell-cell interactions under non-adherent conditions [102]. These structures, or tumor spheroids, depict dense cancer cells in 3-D (Figure 2a) [102]. The spatial configuration limits passive diffusion of oxygen, nutrients, metabolites, waste products, and other signaling molecules, resulting in a heterogeneous landscape of soluble factors much like the TME (Figure 2b) [103, 104]. Tumor heterogeneity in the 3-D space have provided important insight into mechanisms of immunotherapy. Tumor spheroids down-regulated human leukocyte antigen (HLA) expression and antigen presentation, effectively evading T cell treatment as compared to monolayer studies [105, 106]. Glioblastoma spheroids, in drastic contrast to cell monolayers, resembled patient phenotypes (Figure 2c) and significantly impaired anti-cancer IFN-γ production from DCs and T cells [107]. Spheroids produced similar results as in a mice xenograft model when assessing combinatorial synergy of bispecific antibodies and PD-1 inhibition in activating T cells [108]. Examples like these suggest that spheroids are representative of how *in vivo* tumors evade current immunotherapies.

In addition, spheroids provide visual confirmation of immune cell infiltration and the resulting cytotoxicity; for example, CAR T cell infiltration and cytotoxicity were quantified in spheroids of HCT 116 colorectal cancer cells [109]. Courau et al. recently reported an interesting comparison of the infiltration efficiency of activated T cells and NK cells in HT29 colon cancer spheroids. NK cells infiltrated better than T cells, while $CD8^+$ $CD45RO^+$ memory T cells were up-regulated within the infiltrated T cell population (Figure 2d) [110], which have been implied to correlate with better patient prognosis [111, 112]. On the other

hand, NK cells consistently show efficient infiltration despite the dense cell-cell barriers of tumor spheroids [113, 114], while antibodies failed to penetrate spheroids in 72 hours [114]. Further, immune stimulation with IL-15 or anti-MHC class I chain-related protein A and B (anti-MICA/B) antibodies induced an almost 3-fold increase in infiltration by both T and NK cells, and the effect was reversed upon the addition of IFN-γ or anti-natural killer group 2D (anti-NKG2D) antibodies [110]. SDF-1α has been confirmed as NK cell attractants in a lung carcinoma spheroid model [115]. Interestingly, inhibition of CXCL12 with NOX-A12 (Olaptesed pegol) was found to enhance infiltration of T and NK cells (B cells to a lesser extent, and no enhancement of monocyte infiltration) into spheroids of colorectal carcinoma, pancreatic adenocarcinoma, glioblastoma, and non-small cell lung cancers (mixed with murine stromal MS-5 cells). Activation of T cells was enhanced through PD-1 inhibition as well, exemplifying the ability for spheroids to assess combinatorial effects from multiple immunotherapeutic agents [116].

The relevance of immunotherapy infiltration studies further depends on the depiction of a dense stroma and ECM network in patient tumors. Spheroids, however, often lack scaffolds that resemble *in vivo* ECM architecture. To remedy this, 3-D culture of spheroids embedded in ECM materials like Matrigel have been explored [117]. In spheroids cocultured with stromal cells, bispecific antibodies targeting the tumor (carcinoembryonic antigen, or CEA) or fibroblasts (fibroblast activation protein, or FAP) increased T cell infiltration and tumor cell lysis, especially when coupled with T cell activation the interleukin-2 variant (IgG-IL2v) which selectively activates CD8+ cytotoxic, CD 4+ helper T cells, and NK cells [118]. The addition of human SV80 stromal cell line into human A549 and Calu-6 tumor spheroids confined lymphocyte infiltration to the boundaries as compared to spheroids consisting of only cancer cells, but influenced chemokine secretion and increased the portion of activated T cells into the tumor bulk [119]. Nevertheless, spheroid models rarely depict clonal and genetic heterogeneity, as they are often derived from immortalized cancer cell lines. It is thus difficult to evaluate immunotherapeutic efficacies associated with intratumor heterogeneity and tumor mutational burden [120].

Tumor organoids

Organoids are 3-D *in vitro* tissue cultures embedded in an ECM hydrogel with organ-specific cell types derived from patient tissues, embryonic stem cells, or induced pluripotent stem cells [121, 122]. In their seminal work in 2009, Sato et al. first demonstrated a crypt-villus organoid formed through differentiation and self-organization of intestinal stem cells [121], which has since transformed the *in vitro* studies of human tissues and organs. Organoids offer unique advantages over spheroids due to the more complex, heterogeneous, and 3-D architectural and physiological functions [123]. Tumor organoid cultures have been established from cancer biopsy samples from various cancer types, which resemble the tumor epithelium they were derived from both phenotypically and genetically [124]. Compared to PDX models, organoids can be more efficiently cultured and expanded [83]. A major advantage of tumor organoids is its ability to capture self-organization of cells and cell-ECM interactions in the 3-D space (Figure 3a,b). Further, tissue-derived organoids have recently been shown to preserve the complex immune profile of the TME, allowing *ex vivo* evaluation of a variety of anticancer agents [125–129]. *Ex vivo* analysis has

been carried out in multiple functional readouts, including histology staining, high-content imaging after immunostaining, gene expression and sequencing, immune profiling with flow cytometry, and cytokine profiling with immunoassays. Interestingly, patient-derived tumor organoids were used as a platform to obtain tumor-reactive T cells from the same patient's peripheral blood lymphocyte population (Figure 3c) [130, 131]. Tumor organoids have also been propagated using an air-liquid interface (ALI) method, which successfully modeled infiltrated immune cell species as well as response to immune checkpoint blockade with anti-PD-1 and/or anti-PD-L1 [127]. The Hedgehog pathway inducing PD-L1 expression in organoids of gastric cancer also led to assessment of anti-PD-L1 therapy in combination with $CD8^+$ CTLs [132]. In a similar manner, effector immune cells and CAR-targeting capabilities were assessed against colorectal cancer organoids as a proof-of-concept preclinical model that simultaneously allows studies of CAR-targeting and safety assessment [133]. In a more recent case study, organoids of patient-derived non-small cell lung cancer (NSCLC) were studied *ex vivo* in parallel to clinical evaluation of chemotherapy (cisplatin and vinorelbine) and immunotherapy (pembrolizumab) in the patient [134]. In addition to immunofluorescence of immunosuppression markers, the authors quantified mutational burden with next-generation sequencing and tumor proliferation rate with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. While the organoids were cultured in Matrigel (derived from a non-human source), the study demonstrated the potential for personalized clinical translation of immunotherapy using organoids [134]. Synthetic designer matrices have also been explored as an alternative to animal-derived matrices, potentially increasing human-relevance in organoid cultures [135]. However, organoids are less common as a tool to evaluate immunotherapy due to its difficulty in logistics (establishing a clinical collaboration and a need for consent to access patient tumor and blood) and costs [134]. Although there is an improvement of architectural complexity from spheroids, organoids still incompletely capture genotypic and phenotypic heterogeneity [136].

It is worth noting that another intermediate culture type, namely the organotypic tumor slice culture, has been developed to examine tumor response to therapeutics. In contrast to the self-assembled tumor organoids, these cultures are derived from sliced primary tumor tissue in varying thicknesses, which can capture the phenotypic and genetic heterogeneity as well as maintain TME conditions *in vitro* for a short period of time. For instance, organotypic tumor slices closely maintain architecture and cell composition of a primary tumor [137, 138]. Immunophenotyping has been conducted in tissue slices using microscopy techniques based on immunofluorescent staining or immunohistochemistry [139–141], or with multicolor flow cytometry after tissue dissociation [138]. Tracking tumor slices over time has revealed the importance of ECM components in T cell migration [142] and macrophage in the efficacy of anti-PD-1 therapy [41]. In a separate study, organotypic tumor slices also reliably predicted tumor response to immunotherapeutic agents like IFN-γ, an anti-PD-L1 antibody, and an anti-CTLA4 antibody, although cytotoxic effects from pharmacological agents exhibited some variability compared to those observed in organoids [138]. While still unknown, such organotypic cultures offer exciting potential to the assessment of cell-based immunotherapies in a patient-specific manner.

Microphysiological models

Microfabrication and microfluidics are a popular approach to study the interaction of multiple cell types from tissues and organs in the same system. This is often achieved by connecting different cell compartments with microfluidics with physiological flow, or interfacing them through medium/ECM-filled conduits, mechanical structures, or direct physical contact. Such models are also known/referred to as the organ-on-a-chip or microphysiological models [143, 144]. In most microfluidic designs, cancer cells are confined to a compartment connected to a channel that serves to create flow, establish gradients of soluble factors, and/or deliver immunotherapeutic agents (Figure 4a). These models are often fabricated in polydimethylsiloxane (PDMS), a transparent polymer material; the transparency allows real time imaging to study cell migration and interactions [145]. Immune cell response and motility can be studied in microfluidics because of its ability to establish gradients of soluble factors and chemokines, allowing for *in vitro* observations of cytokine-dependent immune cell recruitment [146]. The ability to optically track immune cells in microfabricated devices is an important advantage in resolving temporal and spatial dynamics. For example, live-cell microscopy based cell tracking revealed a heavy dependence of dendritic cell migration on CXCR4 [147]. Chemotactic gradients were also shown to guide directional NK cell recruitment and penetration into breast cancer through the CXCL12-CXCR4, CCL28-CCR3, and CXCL8-CXCR1/2 axes [114]. Conversely, the effects of immune cells on tumor behavior can also be visualized; a three-compartment microfluidics with pneumatic control over compartment connectivity revealed that myofibroblasts enhanced breast cancer cell migration, while the effect was reversed through TNF-α production from macrophages [148]. Bai et al. showed a contact-dependent mechanism (through ICAM-1 and β2 integrins) of M2a macrophage stimulating lung adenocarcinoma dispersion [149]. Yet another microfluidics device incorporated cancer, PBMCs, endothelial cells, and fibroblasts, to reveal the systemic effects, including enhanced cancer-immune interactions, when treated with an anti-HER2 receptor antibody, trastuzumab [150]. Microfluidic models have also reported on the differential cytotoxic effects of TCR-engineered T cells that have been prepared by viral transduction or mRNA electroporation against tumor compartments (Figure 4b) [151, 152], as well as the effect of anti-PD-1 therapy on TILs infiltration into murine and human tumor fragments cultured *ex vivo* [153]. Moreover, microfluidics have the added advantage of investigating delivery of immunotherapeutics through channels mimicking the vasculature [143, 154–156]. For instance, macrophages increased the permeability of endothelial-lined fluidics channels, resulting in enhanced tumor intravasation (Figure 4c) [143], which corroborates with *in vivo* data on M2 macrophages stimulating tumor relapse resulting from revascularization and increased likelihood of metastasis [157]. Vascularized microfluidics have the potential to provide insight into the delivery mechanisms of a variety of immunotherapeutic agents. As such, tumor modeling in microfluidics platforms offer approaches to study underlying mechanisms of tumor immunotherapy that are difficult to examine in animal models [158].

Towards capturing the increasingly complex TME landscape

Novel platforms that combine components from different engineering strategies provide a more complex microenvironmental landscape. Scaffolding approaches, where cancer cells are embedded in a matrix of biological and/or synthetic materials, is one approach to

controlling 3-D cell-ECM architecture. For example, CAR T cell infiltration and cytotoxicity was assessed in lung and breast cancer cells cultured in a 3-D matrix of the basement membrane from decellularized jejunum [159]. Another popular approach is hybrid modeling of spheroids and microfluidics consist of a fluidic chamber that embeds spheroids in a hydrogel material, reminiscent of parenchyma nests in a dense matrix of ECM (Figure 5a) [160, 161]. Organoids derived from mouse and patient tumors were embedded in a microfluidics compartment and successfully demonstrated recapitulation and ability to predict outcome to PD-1 blockade therapy [125, 162]. The same group identified CDK4/6 inhibition and PD-1 blockade as a potentially promising combinatorial approach to augment anti-cancer T cell activation [163]. In a similar manner, the antitumor activity of TCR-engineered T cells were assessed in a microfluidics device that compartmentalized spheroids of liver carcinoma cells embedded in a collagen gel (Figure 5b) [151]. A higher throughput approach of cytotoxicity assay using an injection molded plastic array culture (CACI-IMPACT) device has been explored to assess NK cell-induced cytotoxicity in cancer cells embedded in a 3-D ECM matrix [164]. We have previously engineered a unique hypoxic tumor model that integrated micromilling, micropatterning, and fluidics technology. Using this model, we assessed the infiltration of Her2-targeting CAR T cells, and their induced cytotoxicity against cancer cells in a solid tumor model with a gradient of oxygen and metabolism (Figure 5c) [165]. Bioprinting, or extrusion of cells embedded in bioinks such as hydrogels, ECM proteins, and various polymers, offer precision over the spatial architecture of multiple cell types [166]. Co-extrusion of cancer cell-embedded alginate and $CaCl_2$ solution created a hollow lumen, with geometrical control of the lumen. Macrophages were then introduced into the lumen, where it was observed that cancer-macrophage interactions increased with serpentine lumens compared to straight lumens, implying the impact of geometry-mediated gradients of biochemical factors [167]. These tools evaluate mechanisms of immunotherapy efficacy while considering multiple influential TME factors, potentially improving clinical translatability.

While this review does not focus on computational and mathematical studies of tumor immunology, it is worth mentioning that system biology approaches provide powerful methodologies that elucidate the complex interactions of TME players and predict emergent behaviors that arise from the complex, interconnected network. Mathematical models of tumor-immune microenvironments have contributed meaningful advanced in understanding cancer immunotherapy, especially by enabling predictions based on situations that are difficult to test experimentally or by integrating different time and spatial complexities [168, 169].

Important Considerations

Engineered models, or microphysiological systems, are rapidly emerging as novel tools for biology and medicine [170]. These models allow for controlled recreation of key aspects of the TME for better predictability and translatability of therapeutic regimens. As the field of tissue engineering advances, microphysiological systems have shown great promise in addressing the complexity and multi-dimensionality of biology. For further progress, multiple considerations must be addressed in the design of engineered models for cell-based immunotherapies (Figure 6).

ECM in a 3-D arrangement is an important factor that regulates accurate tumor biology [171, 172]. Specifically, different biomaterials have been exploited to modulate the 3-D architecture [173]. Biomaterials range from natural, synthetic, or a hybrid of the two, with various methods of modulating cell-ECM interactions as well as mechanical tunability [174]. Scaffolds and matrices derived from biomaterials provide complex biochemical and mechanical cues, further increasing the relevance and translatability of preclinical tumor models.

Another crucial parameter is the spatial organization of various cell types. Solid tumors comprise of two interdependent compartments: the parenchyma and the stroma. The parenchyma, or neoplastic cells organized into nests, while stroma surround these nests. The spatial architecture of different cell players give rise to complex cell-cell interplay and environmental cues (e.g. mechanical and chemical). Endothelial cells and fibroblastic cells (such as CAFs) can form conduits or physical barriers to immune cell infiltration. Additionally, the incorporation of CAFs and bone marrow stromal cells (BMSCs) in tumor models show spatially and temporally altered cancer phenotype and response to therapy [175–177]. Moreover, systemic integration of engineered tumor models with microphysiological models of other healthy tissues/organs can potentially reveal unexpected life-threatening side effects before therapies are introduced in clinical studies.

Recently, the effects of soluble factors in giving rise to differential therapeutic outcomes is increasingly appreciated. In addition to cytokines and chemokines, soluble factors from external sources include oxygen and nutrients can also play significant roles in immunotherapy [178]. As a hallmark of solid tumors, tumor hypoxia enhance chemoresistance [179] and immunosuppression [57, 180], and correlate with poor patient outcomes [181]. Most hypoxia models induce a uniform degree of hypoxia with enclosed chambers or through chemical induction [182–184]. For a true bioinspired approach, soluble factors must be controlled spatially and temporally, with similar methods of induction (most often driven by a combination of cellular production/consumption and limitation to diffusion through solid tumor bulks). Our group has engineered platforms with a diffusion-limited gradient of oxygen in a 2-D [185] and 3-D tumor models with cell-cell and cell-ECM communications in order to elucidate CAR T cell function against solid tumors [165]. Cellular response against hypoxia is reflected in cell proliferation, metabolic state, and survival [165, 185]. As a result, the metabolic demands of tumor cells exist in a spectrum, creating a complex landscape of nutrients such as glucose, lactate, and amino acids [178]. The pH of the TME also share similarities with lactate production, correlating with the metabolic status of cancer. Fluidics technology enables controlled gradients of soluble factors. It also adds further dimensions of complexity such as shear stress from fluid flow, and allows for connectivity between different tissue compartments in the engineered models [186].

While integrating multiple TME parameters, engineered tumor models must retain a smart design that allows for isolation of individual components for effective readout and accurate assessment. A common approach is to compartmentalize each cell type, with the flexibility to add or remove each compartment. Stackable tissue blocks of tumors, monocytes, ECM, and vasculature, for instance, allowed spatiotemporal investigation of monocyte

activation and macrophage-endothelial interactions that resulted in distinct morphological characteristics [187]. Engineered models that allow heterotypic cell co-cultures may predict unwanted side effects in immunotherapies. Future models should also integrate sensors/biosensors to monitor soluble factors such as glucose [188, 189], lactate [190, 191], and pH levels [192], e.g. like microparticle-based oxygen sensors [165, 185, 193]. On the other hand, the entire complexity of the TME cannot, and probably should not, be incorporated in a single model [84, 85]. Just as organ-on-chips aim to recapitulate the basic functional units of functional tissues/organs, engineered tumor models should focus on evaluating the crucial interactions in TME with well-designed controls. Moreover, the design of engineered models should facilitate the observation and quantification of the interested cells, factors, or interactions [194].

Today, various groups are developing state-of-the-art engineered models through rapid iterations and validations for their functional and biological relevance. For translational purposes, however, additional consideration should be taken to standardize critical engineering techniques and analytical tools across these platforms. They should also be simplified to ensure adoption across fields and disciplines, to supplement existing *in vitro* and *in vivo* studies and integrate in the pipeline of immunotherapeutic discoveries.

Conclusion and Future Outlook

Despite its promising outlook, cell-based immunotherapy still faces multiple difficulties. Like the organ-on-a-chip systems, engineered *in vitro* tumor models allow rapid assessment of novel cell-based immunotherapeutic strategies for solid tumors. They are powerful tools capable of delineating therapy efficacy through multidimensional incorporation of TME components. Strategically combining insight from multiple engineered tumor models may also provide more accurate mechanistic insights and therapeutic predictions. At present, it is important to note that most existing engineered models incorporate only a few key TME parameters at best, while tumor progression and immunotherapeutic outcomes depend on a milieu of local and systemic factors that interconnect in a complex manner. By integrating key cellular and molecular components, physical and chemical factors, spatiotemporal biosensing and analysis, as well as next-generation single-cell and omics technologies, future engineered tumor models will have the potential to provide more powerful insights and accurate therapeutic predictions for cell-based immunotherapies and personalized medicine.

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statement of significance

Cell-based immunotherapies have shown great promise in treating hematological malignancies and some epithelial tumors. However, their performance on a broader spectrum of solid tumor types still fall short of expectations. Major obstacles include the inhibitory mechanisms in tumor microenvironments (TME) and the lack of preclinical models that can accurately assess the efficacies and mechanisms of cellular therapies in a (patho-)physiologically relevant manner. In this review, we introduce recent progress in tissue engineering and microphysiological models for more faithful recapitulation of TME for cell-based immunotherapies, and some key considerations for the future development of engineered tumor models. This overview will provide a better understanding on the role of engineered models in accelerating immunotherapeutic discoveries and clinical translations.

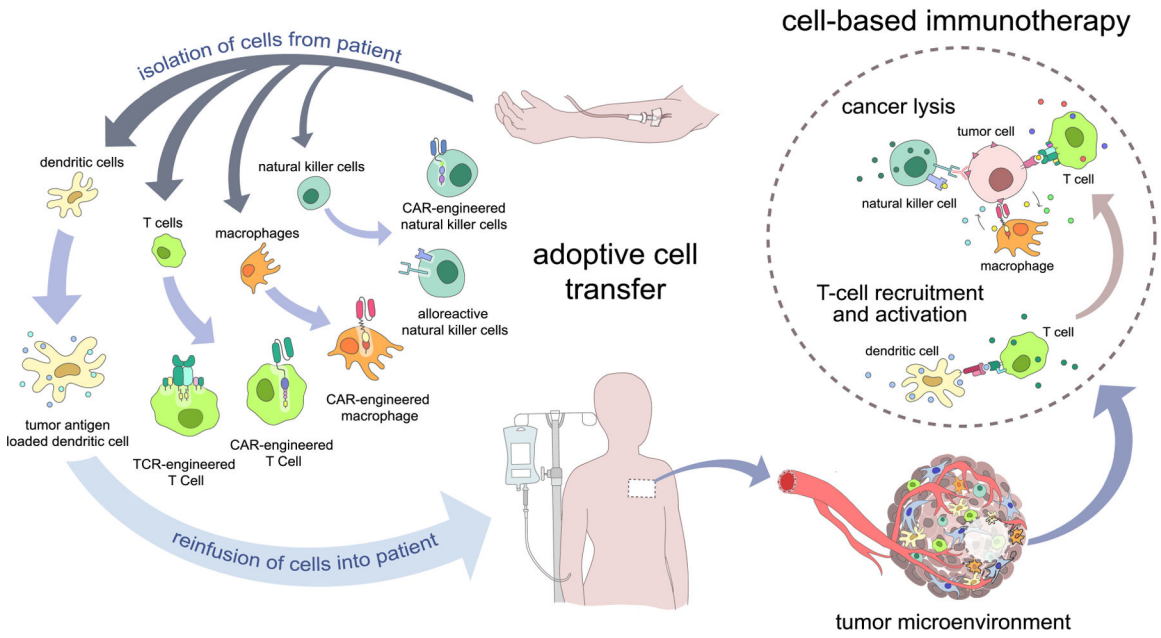

Figure 1.

A brief overview of cell-based immunotherapy. In a process known as adoptive cell transfer, cells are extracted from patient blood, engineered, and reinfused into the patient. Engineered immune cells circulate in the bloodstream until they eventually arrive in the tumor site, resulting in cancer killing or T cell recruitment and activation.

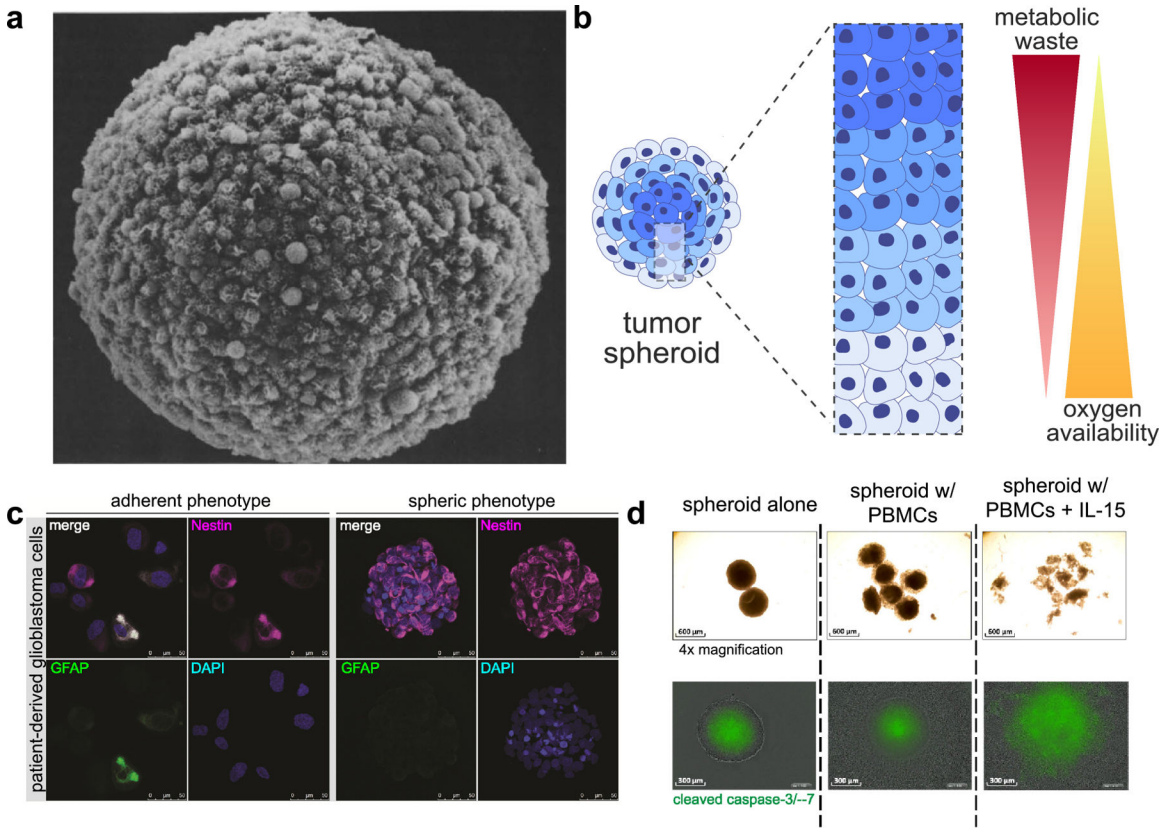

Figure 2.

(a) A representation of a multicellular tumor spheroid (ref [102]). (b) Tumor spheroids naturally form a gradient of soluble factors, with a core that is oxygen-deficient and rich in metabolic waste products. (c) Culture conditions (2-D versus spheroid culture) change phenotypic expression of patient-derived glioblastoma cells (ref [107]). (d) PBMC-induced tumor apoptosis was monitored by evaluating spheroid volume (top) and expression of cleaved caspase-3/−7 (bottom) (ref [110]).

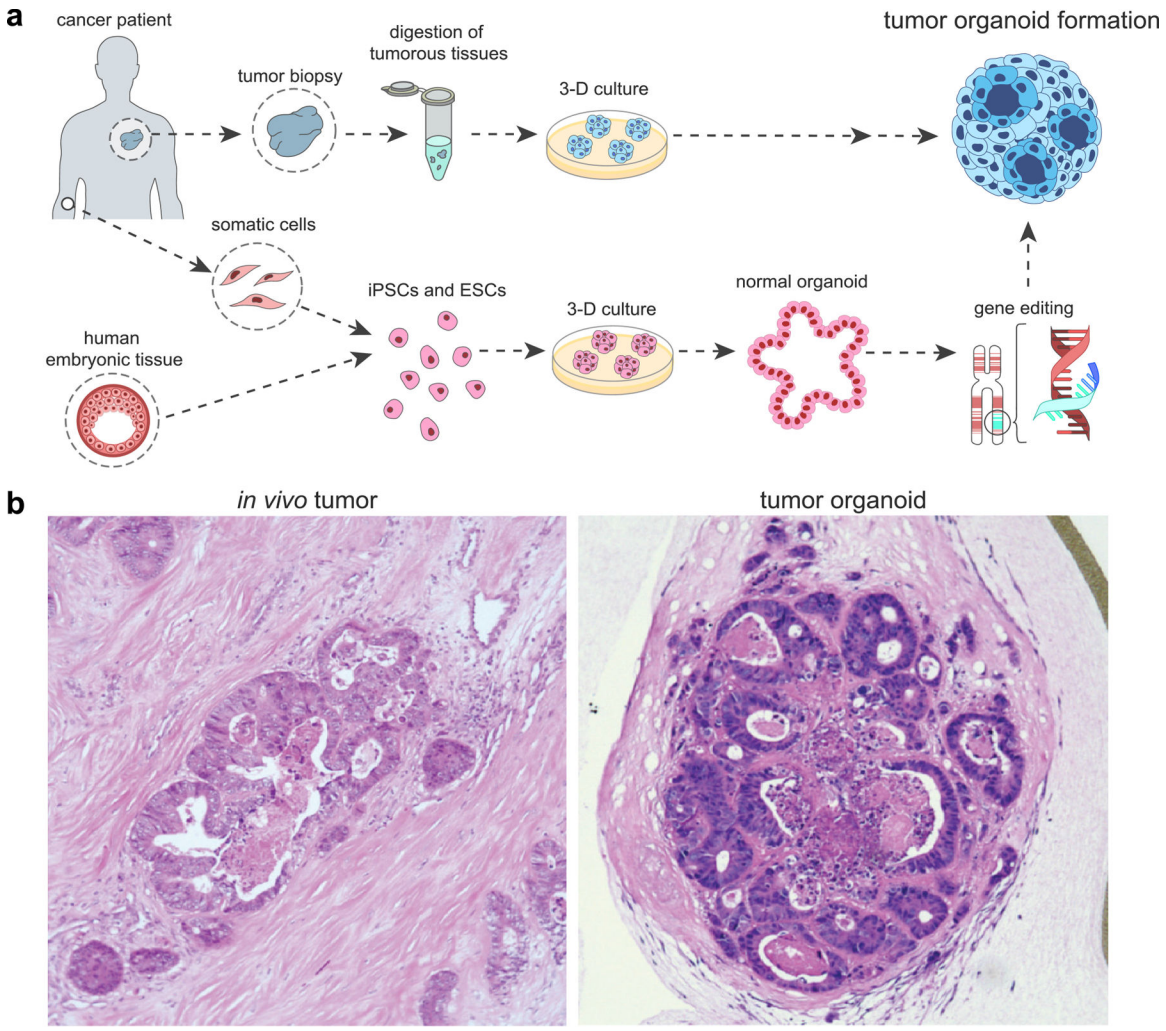

Figure 3.

(a) A schematic of tumor organoid formation. Organoids can be formed from digested tumor tissue or through self-assembly of differentiated stem cells. (b) Tumor organoids recapitulate in vivo histology (ref [195]).

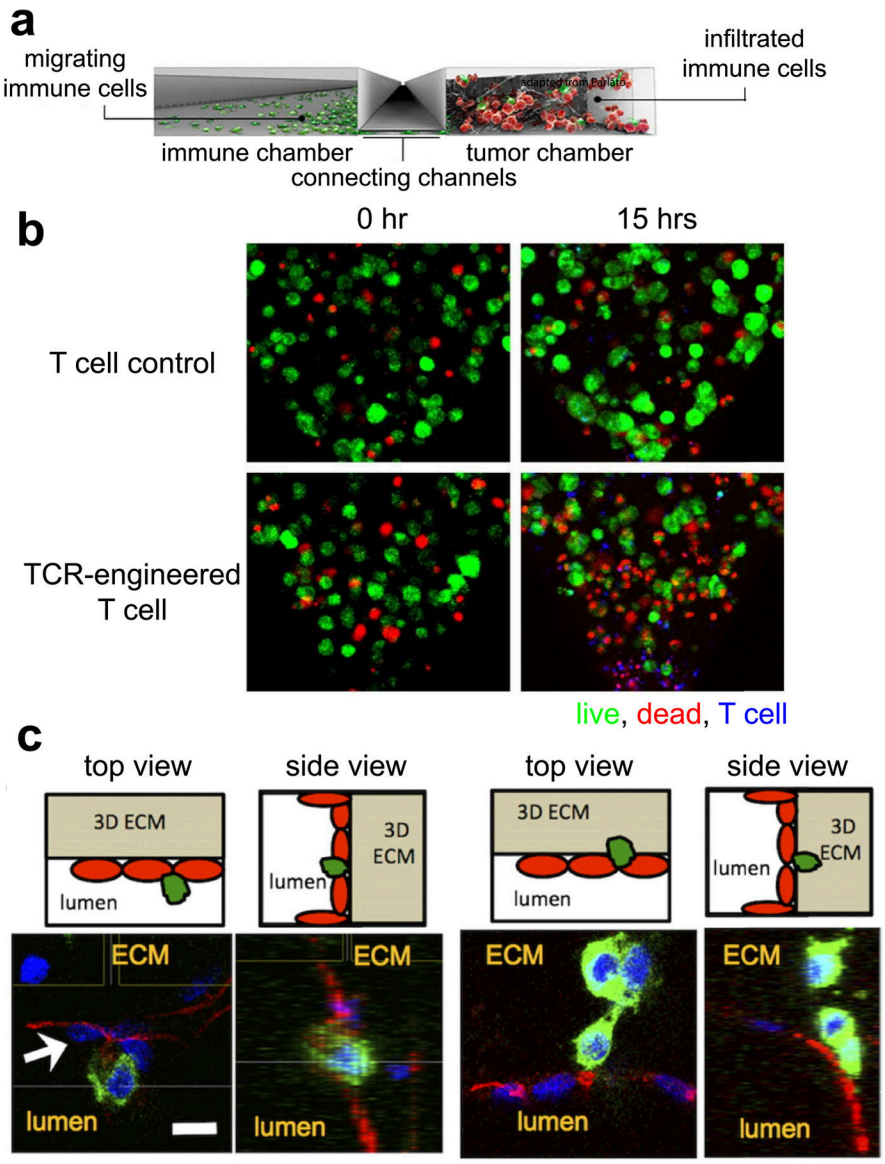

Figure 4.

(a) A side section of a typical microfluidics device. A channel connects the immune and tumor chamber, allowing for crosstalk between the two cell types. (b) TCR-engineered T cells (blue) showed tumor (green)-specific cytotoxicity (red) over time, compared to control T cells (ref [151]). (c) Endothelial cells (red) lining the surfaces of a microfluidics channel interacted with tumor cells (green) extravasating (ref [143]).

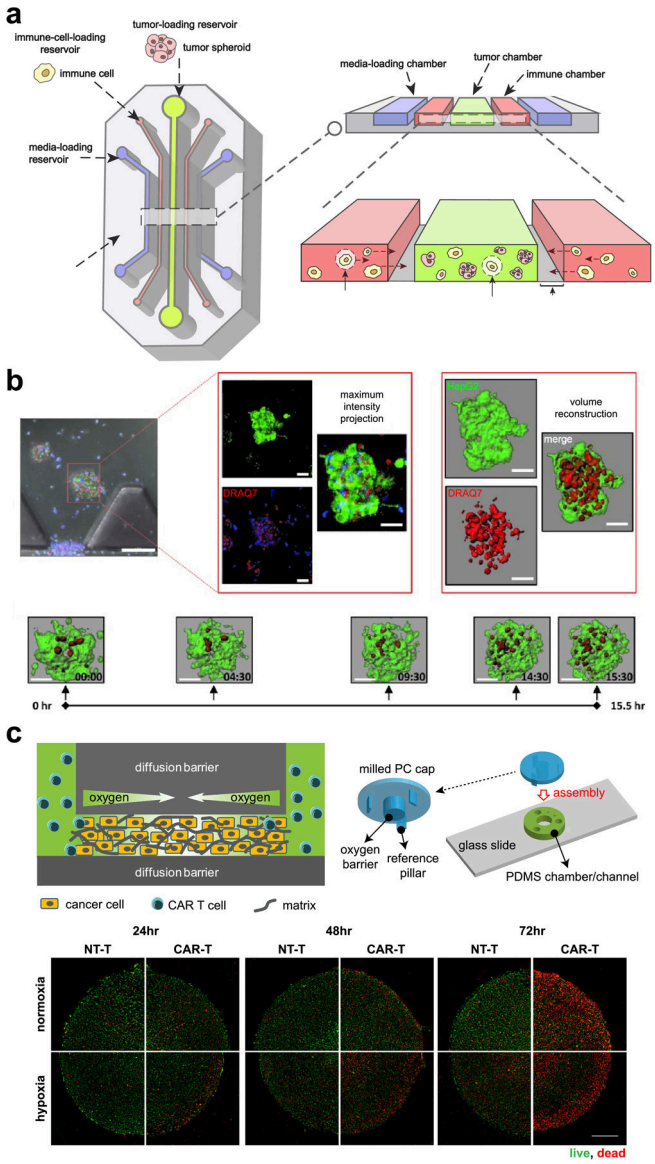

Figure 5.

(a) Multicellular structures like spheroids and organoids can be embedded in a hydrogel-filled chamber of a microfluidics device. (b) A microfluidics device with TCR-engineered T cells (blue) and tumor spheroids (green) was imaged over time to assess the degree of T cell-induced cytotoxicity (red) (ref [151]). (c) 3-D tumor micropatterns cultured under a gradient of hypoxia were treated with CAR T cells to resolve spatial and temporal cytotoxicity patterns (ref [165]).

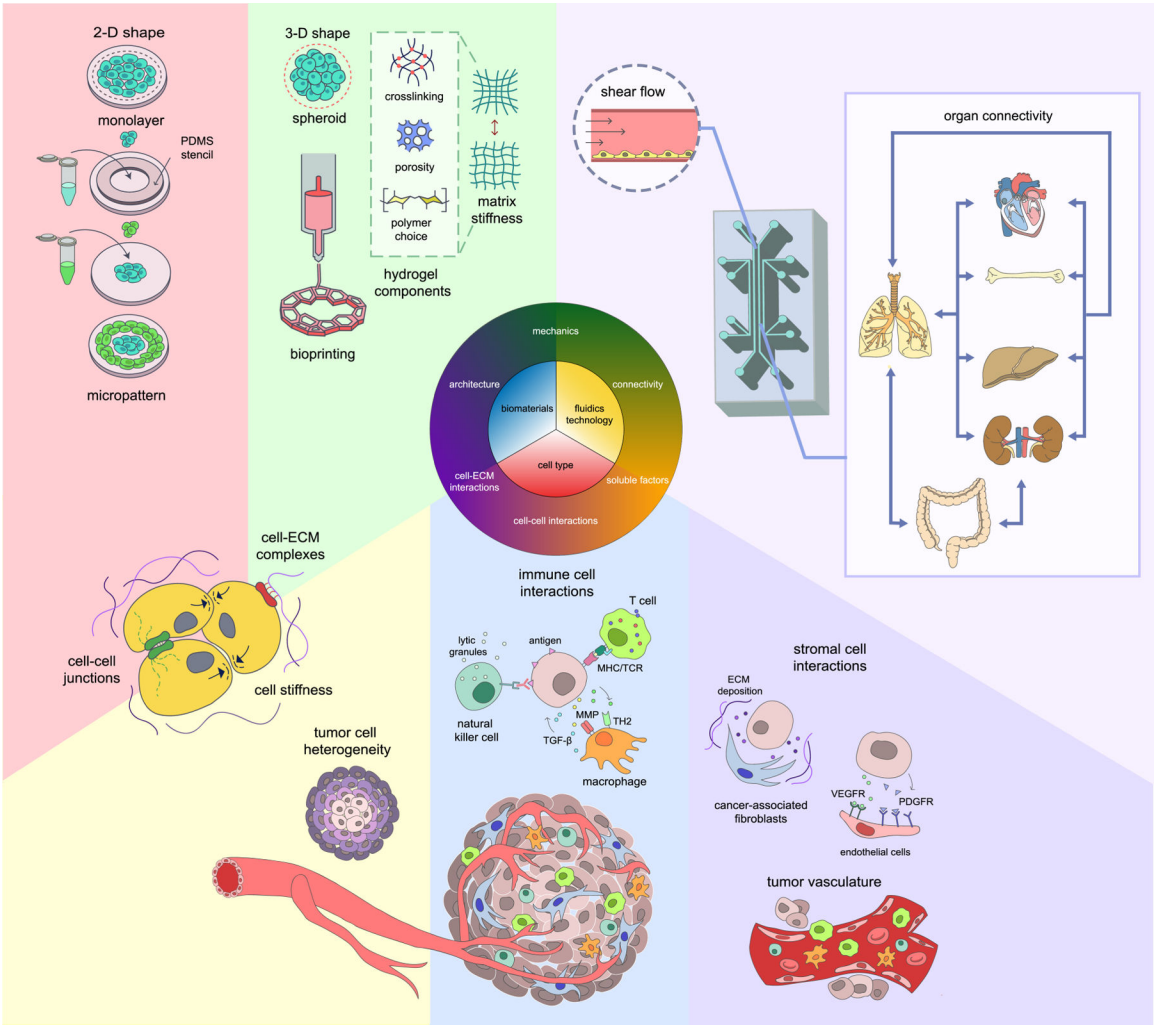

Figure 6.

Engineered tumor models assessing tumor immunology and immunotherapy require smart designs. Special considerations should be made in cell-cell interactions of different cell types, connectivity of cell-secreted factors, biomechanics such as fluid pressure and ECM stiffness, and accurate depiction of a 3-D architecture.

Table 1.

A brief summary of the advantages and disadvantages of existing tumor models.

Model Type		Advantages	Disadvantages
Cell monolayer		Uses human source of cancer cells; relatively cheap costs; high throughput	Ignores the TME and its effects; cancer cell lines fail to capture phenotypic and genotypic heterogeneity
Mouse models (syngeneic models and genetically engineered mouse models (GEMM))		Recapitulates the TME; allows systemic evaluation of therapies	Very limited human relevance; difficult to control selected aspects of the TME; high costs; low throughput
Cell-line xenografts and patient-derived xenografts (PDX)		Uses human source of cancer cells; recapitulates the TME (i.e. cancer-stromal interaction)	Limited human relevance when studying cancer-immune interactions; high costs; low throughput
Engineered	Spheroid	Uses human cancer cells; recapitulates selected aspects of the TME; easily controllable; relatively cheap costs; medium throughput	Lacks the extracellular architecture; usually lacks parenchyma-stroma organization; lack clonal and genetic heterogeneity
	Organoid	Uses human cell sources; recapitulates selected aspects of the TME	Difficulty with sourcing cells
	Organotypic models	Uses human source of cells; includes parenchyma and stroma cells	Difficulty with sourcing cells and long-term maintenance
	Microfluidics	Uses human source of cells; able to control local concentrations of soluble factors; includes connectivity	Requires expert handling