Tumor tissue engineering: modeling cancer with biomaterial-based platforms

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Abstract

Tissue engineering technologies have produced innovative tools for cancer research. 3D cancer models based on molecularly designed biomaterials aim to harness the dimensionality, biomechanical and biochemical properties of tumor tissues. However, to date, in spite of the critical role that the extracellular matrix plays in cancer, only the minority of 3D cancer models is built on biomaterial-based matrices. Major reasons for avoiding this critical design feature are the difficulty to recreate the inherent complexity of the tumor microenvironment and the limited availability of practical analytical and validation techniques. Recent advances emerging at the interface of supramolecular chemistry, materials science and tumor biology are generating new approaches to overcome these boundaries and enable the design of physiologically relevant 3D models. Here, we discuss how these 3D systems are applied to deconstruct and engineer the tumor microenvironment, opening opportunities to model primary tumors, metastasis and responses to anti-cancer treatment.

Keywords: tissue engineering, biomaterials, tumor biology, 3D cancer models, tumor microenvironment, extracellular matrix

[H1] Introduction

Cancer is a leading cause of death worldwide, but the development of treatments is hampered by inherent tumor heterogeneity/patient-to-patient variability, resistance to therapy and disease progression. Therefore, many efforts have been made to model cancer for pre-clinical research. 3D cancer models are interdisciplinary platforms to conduct pre-clinical research. In tumor tissue engineering, biomaterials are key ingredients in 3D cancer models **(Table 1)** that can mimic tissue dimensionality, organization and function of solid tumors[1,2]. A wide range of hydrogel[3] **[G]** and scaffold[3] **[G]** materials based on building blocks such as synthetic polymers[4], biopolymers[5] and peptides[6,7] are used for tissue engineering and regenerative medicine applications and serve as base matrices for 3D cancer models. New engineering approaches enable the rational design of hydrogel and scaffold materials with multiple structural and signaling components to more accurately recreate the heterogeneity of the tumor microenvironment **[G]** (TME), a complex entity that homes malignant, or cancer, and non-malignant, or stromal, cells[8].

One of the first 3D cancer models using a tissue engineering approach was made of porous poly(lactide-co-glycolide) (PLG) scaffolds seeded with different cancer cells. These scaffolds promoted typical cancer cell growth, integrin and extracellular matrix (ECM) expression profiles, and effectively recreated oxygen gradients seen in tumor tissues[2]. Cancer cell-mediated angiogenesis, invasion and resistance to cytotoxic drugs were enhanced in the PLG scaffolds compared to cell monolayer and Matrigel **(Box 1)** controls. Furthermore, the implantation of these cancer cell-seeded PLG scaffolds into immunodeficient mice significantly increased tumor size, blood vessel density and angiogenic factors compared to controls[2]. This landmark 3D approach[2] highlighted the enormous potential of tissue-engineered technologies to study molecular interactions integral to tumor biology and drug responses, and was followed by the development of many other biomaterial-based 3D oncology approaches[4,9-13].

Initially, simple 3D cancer models were limited to the culture of cancer cells or to their co-culture with only one other cell type, which did not adequately capture the complex cellular interactions within tumor tissues. More advanced multicellular 3D cancer models have evolved using triple, tetra or even penta-cultures[6-8,14,15]. 3D cell biology can also integrate a fourth

dimension (4D), enabling temporal analyses[16], such as in real-time live-cell imaging of proteolytic or signaling cascades[17].

Leveraging biology to enhance biomaterial design offers unique advantages to increase the design space for biomaterials[18,19]. The combination of materials science and tumor biology has produced sophisticated approaches to capture parameters of the TME in a controllable, versatile and reproducible manner. Consequently, the number of cross-disciplinary groups combining biomaterials and 3D oncology for tumor tissue engineering has been steadily increasing[20-24]. In February 2017, the US National Institutes of Health and National Cancer Institute announced a global Cancer Tissue Engineering Collaborative promoting tissue-engineered technologies for cancer research. This research program facilitates the development and characterization of state-of-the-art bioengineering approaches as 3D cancer models to address questions related to cancer prevention, detection, diagnosis and therapy. Biomimetic **[G]** tissue-engineered technology platforms are now used to model cancer cell migration, invasion and metastasis, tumor vasculature, hypoxia and metabolic stress, mechano-transduction, inflammatory processes and the cellular TME to assay responses to immunotherapy and combination therapies.

In this Review, we focus on the interplay between bioengineering and biology to design improved 3D cancer models. We analyze how biomaterial-based 3D cancer models **(Table 1)** are being designed as physiologically relevant assays that recreate the characteristics of tumor tissues. We exclude cell monolayer cultures on plastic substrata or 3D techniques that are not based on biomaterials, such as hanging drop or non-adherent cell culture methods, which have been reviewed elsewhere. In the first part of this Review, we introduce key parameters in tumor biology and present existing tools that are employed to resemble the cellular composition and the ECM of tumor tissues **(Figure 1)**. Then, we discuss contemporary tumor-engineered models and summarize the progress in modeling the primary tumor and metastatic niches. We present the challenges and opportunities of using these tissue model approaches to test novel treatments and our perspectives on the future applications of biomaterial-based platforms.

Figure 1. Concept of tumor tissue engineering. Key parameters in tumor biology include the extracellular matrix, cancer and stromal cells. These parameters are resembled using existing tissue engineering tools, such as biomaterials, hydrogels or scaffolds, to build tumor-engineered models.

[H1] Key parameters in tumor biology

The development of a tumor is regulated by the surrounding niche, in which multiple cell types exist in a permissive and dynamic milieu that modulates tumor growth, invasion and spread[25,26]. Most solid tumors comprise stromal cells including cancer-associated fibroblasts (CAFs), immune cells, endothelial cells, adipocytes, ECM proteins and soluble molecules (such as cytokines, chemokines or growth factors), which form the TME[1,25-27].

The role of the TME in driving tumorigenesis **[G]**, which is the formation of a tumor, across various cancer types has become evident, and a tremendous amount of research has shed light on understanding the heterogeneity and evolution of the TME during tumorigenesis[28-31], which also dictates response to therapy[1,26,28,32]. Cancer and stromal cells crosstalk through the exchange of soluble molecules[26,28] and through the ECM, which is dysregulated during disease progression[33], losing its original architecture[27]. Solid tumors are often stiffer than the healthy tissues they arise from, leading to resistance to therapy and epithelial-to-mesenchymal transitions **[G]**, which allow cancer cells to become motile and spread[34,35].

During disease progression, cancer cells secret and remodel their own ECM[36], altering its biomechanical properties and creating a TME that permits cell invasion[37]. Post-translational modification, proteolytic degradation and force-mediated matrix modification promote matrix remodeling and cell migration[36]. Studying matrix dynamics and biomechanics (for example matrix stiffening during disease progression[37]) is restricted, partially owing to the lack of 3D cancer models that mimic essential aspects of the evolution of the TME. Assigning a clear function to individual components remains difficult. Thus, strategies mimicking individual components of the human TME using 3D approaches to tumor tissue engineering have attracted attention **(Figure 1)**. In the following subsections, we highlight extracellular and cellular elements of the TME of solid tumors, including the ECM, CAFs, the immune landscape, endothelial cells and adipocytes. Although these key parameters in tumor biology are characteristic for most solid malignancies, here we mainly focus on breast, ovarian and pancreatic cancers because they are widely researched.

[H2] ECM

The ECM is a non-cellular component in all tissues that enables structural support to cells. The ECM provides biomechanical and biochemical cues that control normal cell behavior, including cell proliferation, survival and migration[27]. Defined as the matrisome, the ECM is

composed of a combination of core proteins (such as collagen, glycoproteins and proteoglycans) and ECM-associated proteins (such as matrix remodeling proteases, inhibitors, cross-linkers, chemokines and growth factors)[27]. During disease progression, however, the ECM of most solid tumors, including breast[38], ovarian[28] and pancreatic cancers[1,33], is dysregulated and reorganized. Excessive amounts of ECM molecules, including collagen, hyaluronan, laminin and fibronectin, accumulate and form a desmoplastic tumor stroma **[G]**[7,33]. The accumulated ECM molecules are crosslinked into a dense network[36], which leads to matrix stiffening, altered mechanosensing pathways, and a more aggressive disease[1,30,34]. Integrins, the primary mediators of the cell-matrix communication, are activated in response to progressive matrix stiffening[27], acting on cell signaling cascades that mediate desmoplasia, tumor growth, invasion and resistance to therapy[27,30,35].

The ECM is also a reservoir for growth factors, cytokines and chemokines, which are released upon the cleavage of matrix molecules and activate various signaling pathways. In cancer, however, the secretion of matrix-degrading proteases, including matrix metalloproteinases (MMPs)[36,39] and kallikrein-related peptidases (KLKs)[40-42], is dysregulated and results in extensive matrix cleavage, the loss of the original matrix architecture[27] and the release of tumor growth-promoting signaling molecules[40]. This diseased matrix is central during cancer cell spread[36] and colonization, or homing[35,37].

[H2] CAFs

CAFs, a dominant type of stromal cells acting on other cells, form a major part of the TME[26,29]. During tumor development, tumor-stroma crosstalk reprogram resident or recruited fibroblasts into highly proliferative, secretory CAFs[27]. CAFs deposit many fibrotic matrix molecules, including collagen, glycoproteins, and proteoglycans, which form a dense network[1,27]. This dense network is stiff and poorly perfused, promoting tumorigenesis and impairing drug delivery[34]. CAFs are very complex, and multiple CAF populations within defined areas in the TME have been identified in pancreatic cancer, showing distinct differentiation states and cell behaviors that lead to matrix deposition and stiffening, disease progression and inflammatory responses[29].

[H2] Immune landscape

In some malignancies, prolonged inflammation is linked to tumor development and progression[25]. In that case, immune cells infiltrate and populate the TME[31,43], establishing a

distinctive niche to promote tumorigenesis[32]. Immune cells secrete soluble molecules (such as cytokines, chemokines or growth factors) that sustain cancer cell proliferation[25,26], as well as ECM-degrading proteases that promote cancer cell migration and metastatic spread. However, the cellular composition and heterogeneity of the immune landscape across cancer types and patients are dynamic and complex[31,44]. The thorough characterization of infiltrating immune cell populations and subtypes is critical for the prognosis of cancer patients[31]. Immunosuppressive cells, including myeloid cells and regulatory T cells, populate pancreatic[44] and other cancers[26,31], where they establish an immunosuppressive TME that protects tumors from immune destruction[31,45]. In these immunological 'cold' tumors, natural killer[46] and cytotoxic T cells (the primary immune mediators of tumor rejection) are exhausted and dysfunctional[44,46-48]. The expression of markers for T cell activation is low[45], and cells have increased levels of co-inhibitory receptors, or immune checkpoints[49], a hallmark of a dysfunctional adaptive immunity indicating resistance to immunotherapy[45]. In contrast, immunological 'hot' tumors are distinguished by infiltration of cytotoxic T-cells and increased inflammation, correlating with a success of immunotherapy[32]. In the pancreatic TME, M1 macrophages are associated with reduced tumor growth, whereas M2 macrophages promote tumor growth and correlate negatively with patient prognosis[44]. Immunomodulatory treatment decreases the amount of anti-inflammatory, tumor-promoting M2 macrophages, while the expression of M1-associated genes increases, suggesting their trans-differentiation into pro-inflammatory M1 macrophages[50].

[H2] Endothelial cells

The imbalance of pro-angiogenic factors (such as VEGF, IL-8 or TGF-β1) in the TME drives the proliferation of endothelial cells and tumor vascularization. This imbalance is to provide nutrient and oxygen for the metastatic spread to distant organs[2,51]. In glioblastoma, therapies harnessing the tumor vascularization, using the enhanced permeation and retention effect, have been unsuccessful, partially because of tumor-infiltrating macrophages, immunosuppressive cytokines and proteases that promote blood vessel growth and cancer cell invasion[51]. Other solid tumors, for example pancreatic cancer, are poorly vascularized and exhibit increased interstitial pressure, which hinders drug delivery and correlates with poor prognosis[1].

[H2] Adipocytes

The link between obesity and cancer is not well understood. However, cancer-associated adipocytes, or fat cells, sequester abundant pro-inflammatory cytokines and growth factors into

the TME, thereby establishing an inflammatory milieu[26]. Fibroblasts infiltrate adipose tumors, which results in extensive matrix deposition and stiffening, and activation of mechanosensing pathways[52]. Adipocytes promote tumor cell invasion and metastatic spread in several malignancies, including breast[39] and ovarian cancers[14,53], partially driven by the reprogramming of the cancer cell metabolism[53] and adipocyte-induced matrix remodeling[52].

[H1] Tools for rebuilding the TME

Because of the heterogeneous nature of solid tumors, tumor tissues cannot be engineered following a 'one-size-fits-all' approach. The biomechanical properties vary between different cancer types and regulate various cell functions, including cell proliferation, migration and differentiation[28]. Biomaterials can be used as tumor-engineered matrix to recreate the biomechanics of the TME. In that case, they must permit modulation of stiffness **[G]** and viscoelasticity **[G]** to resemble the spectrum of biomechanical properties exhibited by tumor tissues at different stages. Techniques based on multicomponent self-assembly[54-56], host-guest interactions[57,58] and expression of endogenous components[59] are being used to engineer matrices with structural hierarchy[60], dynamic signaling[61] and tunable properties[62]. However, despite these advances, the capacity to recreate the inherent complexity of the ECM solely based on materials science approaches remains limited[18]. In particular, the tumor-stroma crosstalk within the TME cannot be effectively recreated. Hence, the incorporation of diverse cell types that recapitulate the cellular heterogeneity of the TME is essential to build tumor-engineered models, underlining the importance of a multidisciplinary approach to tumor tissue engineering.

[H2] Natural biomaterials

Hydrogels can be made from both natural[63-65] and synthetic[4,9] building blocks. Among the biomaterials of natural origin, collagen hydrogels are extensively used because this protein is a key component of the ECM, providing mechanical support and guiding cell behavior[27,66]. In many solid tumors, including pancreatic and breast cancers, aberrant deposition and remodeling of collagen fibers result in increased tissue stiffness, affecting disease progression and prognosis[28,30,38]. However, collagen has poor mechanical properties, limiting its ability to reproduce stiff tumor tissues[64,66]. The structural stability of collagen can be enhanced using various modification strategies. For example, physical crosslinking strategies, including reduced gelation temperatures and elevated fiber densities, improve the mechanical properties

of collagen hydrogels[66,67]. Although biomaterials of natural origin have inherent benefits in modeling the ECM, they are limited in terms of their varying molecular composition and little control over their mechanical properties, leading to irreproducible results and a narrow range of applications in tumor tissue engineering. Among the biomaterials of natural origin, Matrigel, a reconstituted basement membrane extract from murine sarcoma cells, stands as the most popular matrix of choice for 3D cancer models and organoids **[G]** because of its mixed protein composition and ease of use, but also faces several limitations **(Box 1)**[68].

BOX 1 | Matrigel - a matrix with limitations for tumor tissue engineering. Despite the advances in materials science leading to the development of novel biomaterials, commercially available animal-derived matrices are still primarily used in tumor tissue engineering. Among those, Matrigel, a reconstituted basement membrane extract from murine sarcoma cells, is the traditional material applied to spheroid and organoid cultures[69]. The use of Matrigel is justified by its origin, which naturally contains many extracellular matrix elements and residual growth factors[70]. It is easy to use, and a growth factor-reduced formulation is commercially available. However, Matrigel has a high batch-to-batch variation and undefined composition[71]. This limits the reproducibility of experiments and comparability between studies in different laboratories. In addition, Matrigel has poor mechanical properties (about 100 Pa)[72], making it unsuitable to mimic the biomechanics of stiff tumor tissues, such as pancreatic cancer tissues (5.5 ± 3.2 kPa)[73]. Its viscoelastic properties hinder its application as a biomaterial for 3D printing and lead to reduced printing accuracy[74,75]. Undoubtedly, Matrigel-based organoids have broadened our understanding of tumor biology but these 3D models lack the microenvironmental context and control over individual extracellular components and multiple cell populations[69], limiting their biological relevance and potential clinical application.

[H2] Composite biomaterials

To improve the properties of biomaterials of natural origin, composite biomaterials have been developed by combining natural and synthetic polymer building blocks. Composite hydrogels display cell-instructive features capable of modulating cell functions[76-78]. From a biochemistry perspective, hydrogel matrices must be proteolytic-degradable but must also sustain their 3D network when undergoing extensive matrix remodeling[79]. Composite biomaterials are suited to partially reproduce the complex mix of proteins and sugar-based components of the tumor-associated ECM[41]. For example, the decoration of the polymer surface with ECM-derived proteins or short cell-adhesive peptide sequences **(Table 2)** is crucial for maintaining or

regulating cell behavior and functions[30]. Therefore, natural matrices, such as collagen and gelatin[80], are combined with synthetic polymers, or inert polymeric chains, such as PEG, are functionalized with peptide sequences[13]. Composite biomaterials also include for example Matrigel that can be mixed with collagen or alginate, a polysaccharide extracted from brown algae, to develop a 3D metastasis assay that mimics the basement membrane of tumor tissues[81].

[H2] Synthetic biomaterials

To achieve a better control over the properties of the rebuilt TME, synthetic biomaterials have also been investigated. Synthetic biomaterials are engineered with precise control over individual proteins and bioactive signals by functionalization with cell-instructive peptides at a desired concentration (**Table 2**). They also have tunable mechanical properties and superior sensitivity and reproducibility in drug screening assays[4,82-84]. Approaches based on polymers such as PEG, PEG diacrylate, polycaprolactone or poly(n-isopropylacrylamide) are facilitated by different crosslinking mechanisms (for example chemical, thermal, enzymatic or photo-initiated) to enhance mechanical properties and to incorporate bioactive signals such as integrin-binding RGD and proteolytic-degradable MMP motifs, soluble factors, such as FGF-2 and TGF-β, or polysaccharide fragments[85]. The addition cell-instructive sites **[G]** to the polymer network provides RGD motifs for integrin binding and proteolytic-degradable sequences for local cell-induced matrix remodeling[9]. PEG can also be decorated with bioactive signals to guide cell behavior, but controlling their local availability is challenging[86]. Control over distribution of bioactive signals and cell activity can be achieved by varying the concentrations of glycosaminoglycans and sulfate groups[86]. In particular, starPEG-heparin hydrogels decorated with RGD and MMP motifs and pro-angiogenic factors allow the in-depth analysis of cancer cell proliferation, invasion, angiogenesis[13] and drug responses[8,12]. Bioactive signals are also incorporated by directly introducing naturally occurring ECM components, such as type-I collagen (Col-I), fibronectin and laminin to promote specific cell functions. Taking advantage of multicomponent self-assembly, a peptide-protein co-assembling matrix was used to recreate multiple ECM components present in organ-specific TMEs[6,7].

[H2] Multicellular systems

Approaches based on biomaterials alone cannot effectively capture the cellular complexity of tumors and the numerous parameters of the TME. Therefore, cell mono-cultures have evolved into co-cultures containing cancer cells and various stromal cell types as well as organoids, which are mini-replica of tissues. This approach has greatly improved the accuracy for

mimicking in vivo tumor biology[7,87]. After many attempts to establish 3D primary (or patient-derived) cultures from different types of tissues, the importance of simulating the tissue microenvironment to grow the cells was evidenced. In 2009, two studies showed, for the first time, how to maintain a 3D mouse mini-gut culture in vitro[88,89]. These mini-gut organoids contained all the differentiated cell types, which are also present in the actual organ, and cells were supported by the growth factors required to regulate various signaling pathways. The main advantages of organoids are that they can be passaged and grown long-term, which is important for tumor tissue engineering to conduct functional analysis and drug screening over several weeks or even months mirroring the clinical scenario of patients receiving anti-cancer treatment. The organoid field has increased exponentially since then and, currently, organoids are being successfully derived from most healthy and diseased organs[18,22,68,69,90].

Although animal-derived matrices are by far the most widely used 3D cell culture approach, tumor-engineered models offer crucial advantages over these standard matrices, including a defined molecular composition, reproducibility and suitability for quantitative analysis, which are essential for drug discovery and pre-clinical research directed to develop personalized medicines **[G]**. Overall, the state-of-the-art in tumor tissue engineering allows scientists to rebuild the TME to model particular events that take place within the primary tumor and metastatic niches and support the development of more effective treatments **(Figure 2)**.

Figure 2. Applications of tumor-engineered models to investigate primary tumors, metastasis and anti-cancer treatment. Cellular interactions at the primary tumor site, extracellular matrix remodeling and the metabolic and inflammatory signatures in tumor tissues are modeled with biomaterial-based platforms. Tumor-engineered approaches recreate the migratory and invasive behavior of cancer cells, the pre-metastatic niche and colonization, or homing, of cancer cells. Analyzing the cell response to treatment in 3D cancer models allows drug screening and discovery, the development of personalized medicines and assessing immunotherapies.

[H1] Bioengineering the primary tumor niche

Recreating the primary tumor niche using tissue modeling requires that the cellular and extracellular elements of tumor tissues be rebuilt. Important components include the altered ECM and cancer and stromal cells incorporated with soluble factors that recreate the metabolic

and inflammatory profiles of cancer cells. Tissue engineering approaches help to mimic these biomechanical, biochemical and physiological properties by combining hydrogel matrices and multicellular 3D cultures that support cell-cell and cell-matrix interactions at the primary tumor site.

[H2] Cellular interactions

The process of cancer initiation is multifaceted and cannot be reduced to a single event. The critical capabilities that cells must acquire to form a tumor were first described in the 'hallmarks of cancer' more than two decades ago. Biomechanical and biochemical signals within the heterogeneous cell populations in tissues alter their genetic profile and lead to cell transformation or abnormal cell behavior[25]. Cells can then overcome the quality control mechanism that monitors cell fitness, which leads to cell competition[91]. The progression into a tumor-permissive microenvironment is caused by the crosstalk between transformed, or abnormal, cells and fibroblasts, immune cells, endothelial cells, adipocytes and many other stromal cell types[25].

The transduction of mechanical signals from the cellular microenvironment into biochemical pathways, or mechano-transduction, occurs via mechano-responsive proteins. Changes in this cascade lead to altered cell functions and signalling, promoting cancer development and progression. Mechanical cell competition eliminates, or extrudes, transformed cells by mechanical pressure that is applied by neighboring normal epithelial cells[91]. Because the ECM has an important role in this type of competition, its contribution was studied using a biomaterial-based 3D model[92]. Transformed kidney cells were co-cultured with normal cells using a polyacrylamide hydrogel coated with Col-I, laminin or Matrigel[92]. The extrusion of transformed cells by normal cells was observed in soft matrices (1.2-11 kPa) but inhibited in their stiff counterparts (23-90 kPa). Normal cells continuously express the cytoskeletal protein filamin to preserve their extrusion behavior. In soft matrices, filamin was found at the interface between the normal and transformed cells, preventing the elimination of the later. However, with increased stiffness, filamin moved to the region surrounding the nucleus[91]. Because many other stromal cell types mediate cancer development, the role of cytokines and metabolites produced by these stromal cells should be further studied.

Harnessing the patient's own immune system to treat cancer has become a promising therapeutic option. Thus, recreating the cancer-immune cell interactions seen in established

tumors is particularly interesting. For example, lung cancer tissues were rebuilt using an alginate-based multicellular 3D model of non-small lung cancer, in which lung cancer cells were co-cultured with CAFs and monocytes[50]. In this 3D model, cells interacted directly with each other and induced a tumor-stroma crosstalk. The cells secreted ECM proteins (including collagen and fibronectin) and MMPs, allowing for matrix remodeling and cell migration, thereby establishing the desmoplastic tumor stroma of lung cancer tissues. Monocytes infiltrated the tumor area and differentiated into tumor-associated macrophages. Secretion of cytokines by these macrophages was then associated with an immunosuppressive TME.

Understanding malignant growth, biochemical cues and response to therapy also requires thorough matrix characterization and an understanding of cell-matrix interactions. To mimic cell-matrix interactions, hydrogel cultures based on biopolymeric alginate are particularly interesting because of their biocompatibility, spatiotemporal regulation of cell distribution and viscoelasticity[63,93-95]. However, alginate does not support cell adhesion, matrix remodeling and cell migration[95,96], and its chemical modifications is needed to support cell functions and direct cell behaviors[94,95]. Modeling cellular interactions go far beyond studying the biomaterial impact of cell function and behavior. The multicellular microenvironment needs to be engineered by considering the tissue-specific ECM and ECM remodeling.

[H2] Remodeled ECM

Solid tumors are fibrous tissues with an ECM rich in collagens, laminins, fibronectin and glycosaminoglycans[27]. Hydrogels and scaffolds have been engineered to replicate the composition of tumor-specific ECMs and their biomechanical and biochemical properties[4,12,18,30]. Cells are either seeded on top of or embedded within these bioengineered matrices and grown for several days, weeks or even months to form cancer spheroids **[G]** or organoids[90]. The analysis of the matrisome of tumor tissues can guide the design of new bioengineered matrices. This reverse engineering of the ECM ensures that critical features of cancer and stromal cells are represented. This concept was applied to design a biomimetic hydrogel to model the pancreatic TME[30]. By analyzing the matrisome of pancreatic cancer tissues and studying the laminin-integrin α3/α6 signaling pathway, the minimal matrix-related cues needed for cell adhesion and proliferation were identified. To artificially mimic these cues, PEG-based hydrogels functionalized with laminin-511, collagen, fibronectin and MMP peptides (**Table 2**) were developed and used to grow pancreatic cancer organoids and stromal cells[30]. Despite the encouraging outcomes using this biomimetic hydrogel, cancer organoids

were first established in Matrigel and then passaged and grown in the PEG-based hydrogels. The initial growth stimulus provided by the Matrigel components (**Box 1**) may have not only affected cell behavior but also been carried over into the PEG-based hydrogels, masking the advantages of the biomimetic hydrogel. Other important laminin isoforms, such as laminin-322, which is associated with poor prognosis for pancreatic cancer patients[33], were also detected in the matrisome[30] but neglected in the biomaterial formulation, and thus remain to be addressed in further studies.

In a similar approach[97], the prostate-specific TME was profiled by RNA sequencing and proteome analysis, evidencing the presence of fibronectin- or collagen-derived and MMP-cleavable peptide sequences. Based on these results, PEG-based hydrogels were functionalized with fibronectin, collagen and proteolytic-degradable MMP peptides (**Table 2**). Prostate cancer cells seeded in hydrogels that contained collagen-derived peptides proliferated more compared to hydrogels containing fibronectin-derived peptides and Matrigel controls, thereby mimicking the cell behavior seen in tumor tissues. The relevance of collagen-derived peptides for prostate cancer cells contrasted the wide use of fibronectin-derived peptides as the cell-adhesive sequence in many synthetic hydrogel matrices[98]. Again, organoids were first established in Matrigel and then passaged and encapsulated in the biomimetic hydrogel. The impact of mechanical forces and immune cells were not investigated, paving the way for further analysis on this aspect. Apart from synthetic hydrogels, composites of biopolymers and natural ECM elements have been used to reconstruct the extracellular TME of solid tumors[64,99,100]. These biomaterials are easily modified to rebuild the composition and properties of organ-specific TMEs, adding to our tumor tissue engineering toolbox.

[H2] Metabolic transformation and inflammation

The metabolism of cancer cells is programmed to sustain their abnormal behavior[101]. This is exemplified by a high uptake of glucose and glutamine, which are sources of metabolites. These metabolic products are intermediates to activate signaling pathways that induce cancer cell proliferation[102,103] and invasion[104]. Nutrients and their derivatives are also essential for the biosynthesis of ECM elements by cancer and stromal cells, hence reshaping the TME[105].

Despite the importance of the cancer-associated ECM, the metabolism of tumors has been poorly explored using biomaterial-based 3D cancer models. The few studies addressing this issue used mostly Matrigel to model the ECM. For instance, to recreate the metabolic

vulnerabilities **[G]** of pancreatic cancer, cells were grown in Matrigel, and the expression of metabolism-related genes was compared to cell monolayer cultures on plastic substrata and animal models[106]. 3D cell cultures closely replicated the tumor metabolism of pancreatic cancer grown in animal models. The presence of *Fdft1*, a factor in cholesterol synthesis, was critical for cell proliferation in 3D cell cultures but unnecessary in monolayer cultures. When *Fdft1* was deleted, or when the Fdft1 inhibitor TAK-475 was present, the tumor metabolism-cell signaling axis was disrupted, suggesting that *Fdft1* may be a target for metabolic therapies. In another study, cancer cells were grown together with CAFs in Matrigel to investigate the association between the metabolic signature of pancreatic cancer and its resistance to treatment[107]. 3D co-cultures had an increased redox state and higher levels of oxidative stress-induced survival proteins compared to 3D mono-cultures. Treatment with metformin, a mitochondrial inhibitor, sustained the level of lactate excretion, indicating that alternative metabolic pathways, such as glutaminolysis, were activated to produce energy. Metformin enhanced the anti-cancer effect of the chemotherapeutic oxaliplatin and reduced cancer cell viability. These tumor-engineered models may be further explored, for example, by incorporating immune cells to decipher their impact on the cancer metabolism[108].

Similar to metabolic reprogramming, inflammation is another important feature of solid tumors[109]. Immune cells recruited to the TME produce various cytokines and chemokines that alter the behavior of cancer and stromal cells[110-112]. The ECM-mediated differentiation of macrophages in the TME was investigated using a biomaterial-based 3D model. Healthy and tumor-derived colon tissues were decellularized by means of removing all cellular components and used as bioscaffolds **[G]** for the 3D culture of myeloid cells. Both bioscaffolds retained major ECM components, including collagen, fibronectin, laminin and hyaluronic acid. However, tumor-derived bioscaffolds were denser and stiffer than their healthy counterpart. When seeded in tumor-derived bioscaffolds, myeloid cells differentiated into anti-inflammatory, tumor-promoting M2 macrophages and expressed lower levels of the pro-inflammatory proteins CCR7 and TNF, whereas the anti-inflammatory CCL18 and cytokines IL-10 and TGF-β were upregulated[113]. Macrophage-derived CCL18 enhanced the invasive behavior of cancer cells, similar to what is observed in clinical samples. The incorporation of other cell types, such as CAFs and endothelial cells, allows the investigation of the role of cytokines in angiogenesis. This aspect was explored using a microfluidic 3D model of glioblastoma[51], where Col-I was functionalized with RGD peptides and used as the hydrogel matrix. Endothelial cells were seeded in the microchannel adjacent to the matrix, which

contained glioblastoma and myeloid cells. 3D co-cultures induced the differentiation of most myeloid cells into immunosuppressive M2 macrophages, leading to increased levels of macrophage-derived cytokines TGF-β and IL-10. TGF-β induced a pro-angiogenic phenotype in endothelial cells and the formation of microcapillaries. Cell-cell interactions between endothelial cells and M2 macrophages through integrin αvβ3 also had a pro-angiogenic effect. The pro-angiogenic response was blocked by TGF-β and integrin inhibitors, which increased the efficacy of treatment with the anti-angiogenic drug cediranib[51]. These tumor-engineered approaches may be used to model other cancer types and decipher inflammatory processes leading to metastasis and responses to treatment.

[H1] Bioengineering the pre- and metastatic niche

Metastasis is a multi-step process where cells migrate from the primary tumor, invade adjacent tissues, intravasate into blood vessels, survive until they reach a distant organ and colonize the new organ. The niche where the cells initiate a secondary tumor provides stromal signals that are crucial to this expansion process. Tremendous efforts have been made to model the pre- and metastatic niche as more than 90% of patients die of metastatic lesions. To model this process, biomaterials combined with microfluidic platforms and high-throughput technologies have been used **(Figure 3)**.

Figure 3. Bioengineering the pre- and metastatic niche. Tissue engineering technologies like microfluidic platforms recreate the main steps in the formation and spread of metastatic cells. Using biomaterial-based 3D models, invasive cancer cells escape from the hydrogel matrix that mimics the primary tumor site to migrate and home to distant organs, forming metastatic lesions. Microfluidic platforms model these dynamic processes and cell functions by providing a vascular network and capillary-like structures.

[H2] Migration and invasion

Cells direct their migratory behavior toward external stimuli, changing their position or the microenvironment. This behavior is fundamental in many physiological processes, such as wound healing or immune cell trafficking[114]. However, cell migration and invasion lead to metastatic spread and are challenging for cancer treatment[114]. Advances in 3D disease modeling have provided critical insights into the ECM characteristics in metastatic spread and

responses to treatment, which helps to identify new drug targets and alternative treatment strategies.

Patients diagnosed with pancreatic cancer have minimal therapeutic options, partially due to the dense tumor stroma that is made of excessive amounts of ECM molecules[7,33]. KLKs, proteolytic enzymes that mediate matrix degradation and remodeling[40], have been considered as attractive targets for treatment of pancreatic[41] and other cancers[42]. They are cancer-associated factors, as their abnormal expression plays a role within the TME, acting on cancer cell proliferation, migration and invasion. A pre-clinical 3D model was used to investigate, the therapeutic potential of KLK6, the starting protease of the proteolytic network in pancreatic cancer[41]. Pancreatic cancer cells were grown encapsulated in RGD-functionalized, proteolytic-degradable PEG-based hydrogels. Over two weeks of 3D culture, cells formed cancer spheroids, which were treated with a specific, proteolysis-resistant KLK6 inhibitor. In the 3D cancer model, KLK6 inhibition reduced KLK6 mRNA levels, metabolic activity and secretion by pancreatic cancer cells. This result highlights the potential of bioengineered 3D disease models to study cancer cell responses to therapeutics[41].

Tumor growth and progression can also be promoted through structural and mechanical changes induced by extensive stromal matrix deposition and remodeling in adipose tissue[39,52,115]. Whether adipose stromal cells promote breast cancer cell migration and invasion in patients with obesity remains unclear[39]. To study the role of adipose stromal cells in breast cancer, non-invasive cancer cells were grown together with adipose stromal cells from patients with or without obesity under non-adherent conditions using a rotating shaker to form spheroids. The spheroids were then encapsulated in a collagen matrix to mimic the ECM components of adipose breast tissues and cell-matrix dynamics[39]. Cancer cells grown alongside adipose stromal cells from patients with obesity had increased migration compared to cancer cells from patients without obesity, implicating obesity-associated changes in the stromal cell fraction. MMP-dependent degradation of collagen and changes in matrix contraction, all key events in disease progression[36,39], also increased the invasive behavior of breast cancer cells due to cell-cell contacts in this multicellular 3D model.

Invasive breast cancer cells predominantly spread to bone tissues. Bone metastases are widespread in late-stage breast cancer[116,117]. Breast cancer-induced bone tumors are aggressive, and the underlying biology is complex[118]. A dynamic network of multiple cell types, ECM

molecules and signaling molecules direct bone remodeling[119]. Bone-infiltrating breast cancer cells disturb this network and disrupt the bone TME[35]. To model bone collagen fibrils, Col-I was mineralized on polyethylenimine and glutaraldehyde-treated polydimethylsiloxane microwells using polymer-induced liquid-precursor processing, forming hydrogels with bone-like collagen fibrils[35]. When breast cancer cells were cultured on top, they interacted with mineralized collagen through integrins, were less spread and showed a round morphology. This round cell morphology was linked to reduced cell adhesions and altered integrin-associated signaling pathways. The cells' ability to remodel the mineralized collagen matrix was reduced, and cell migration was enhanced, suggesting a role for collagen mineralization in cell migration and cell-matrix interactions[35].

Mineralized collagen hydrogels, however, do not effectively recreate the physiological microenvironment and mechanical strength of the native bone matrix, which are critical for studying disease progression[28,120]. To address these limitations, bone scaffolds that closely recapitulate the stiffness and pore size of bone tissues were 3D-printed using biocompatible liquid photopolymers and coated with fibronectin[120] (**Box 2**). When mesenchymal stem cells were seeded on these bone scaffolds, they differentiated into osteoblast-like cells, depositing collagen and mineralized calcium. 3D co-culture with bone-infiltrating breast cancer cells led to bone colonization and cancer cell growth[120]. Overall, these cancer-induced metastasis model offer great promise to study the metastatic TME and associated cell functions. Engineering tumor niches that allow cells to disseminate and spread to secondary sites offer new insights into cancer cell behavior.

BOX 2 | 3D and 4D bioprinting of the tumor microenvironment. Reconstructing the tumor microenvironment is a challenging task because of the inherent complexity of tumor tissues. Protocols for cell seeding are time-consuming and the handling of certain biomaterials is laborious, which limit the reproducibility of 3D cancer models[1]. Progress in additive manufacturing, or biofabrication, techniques, whereby polymeric fibers are 3D-printed in a layer-by-layer fashion onto a surface, have led to 3D structures suitable for 3D cell cultures. 3D bioprinting enables precise control over the structure of tissue-like constructs, while assuring accuracy of the volume, number and deposition of cells[23]. Cell-laden biomaterials, or bioinks[121], are extruded through a nozzle as droplets or continuous filaments to form the desired structure[122]. Inkjet printing is a 3D bioprinting technique of droplets of small volumes of liquid biomaterial through micro-scaled nozzles, whereas extrusion printing produces a continuous filament of biomaterial. 4D bioprinting has emerged to integrate conformational changes in

3D-printed structures in a predetermined manner using stimuli-responsive biomaterials with applications in robotics, biomedical engineering or drug delivery[123] or as shape-morphing hydrogels using multi-material systems[124]. To model the tumor microenvironment, 4D approaches capture the cell cycle status of cancer spheroids[125] and chemotactic behavior of metastatic cells[126].

[H2] Pre-metastatic niche

The pre-metastatic niche refers to the tissue landscape that undergoes several cellular, molecular and architectural transformations to establish secondary tumors. Primary tumor-derived factors, non-resident recruited cells and the stroma of the host organ are key elements of the pre-metastatic niche. Animal models revealed that pro-inflammatory cytokines (such as TGF-β[127]), chemokines (such as CCL2[128] and CXCL1[129]), growth factors (such as VEGF[130]) and bone marrow-derived cells interact with cancer cells and promote the formation of metastatic lesions. In addition, the stiffness of secondary tumor tissues has been associated with early metastatic spread, growth and resistance to treatment[131]. Tumor-engineered models recreate more accurately the characteristic components of the pre-metastatic niche, shedding light on the cell-cell and cell-matrix interactions[132].

Ovarian cancer cells often spread and form metastasis within the omentum, an adipose tissue lining the abdominal organs[53]. To recapitulate the cellular features of the pre-metastatic niche of the omentum, a 3D cancer model was engineered using a four-cell, tetra-culture system[14]. Human adipocytes, fibroblasts, mesothelial cells and ovarian cancer cells were seeded in a Col-I matrix[133], which revealed gene expression patterns similar to patient-derived tissues. The addition of human platelets, resulting in a five-cell, penta-culture, was used to investigate whether platelets drive ECM deposition and cell invasion. The expression of fibronectin, versican, cartilage oligomeric matrix protein, cathepsin and collagen, all ECM proteins associated with poor prognosis of ovarian cancer, were enhanced in the penta-culture. Platelets also induced the secretion of mesothelial cell-derived cytokines, which in turn increased cancer cell invasion. This multicellular 3D model evidenced the role of platelets in the metastasis of ovarian cancer and paved the way for more sophisticated approaches to rebuilt pre-metastatic niches.

Similarly, the formation of the bone pre-metastatic niche was modeled with a penta-culture approach[15], where breast cancer cells, osteoblasts, osteoclasts, endothelial cells and

macrophages were embedded in fibrin hydrogels. The bone tissue's microvascular architecture and oxygen gradients were recreated using a 3D-printed poly-methyl-methacrylate scaffold. Osteoblasts were surrounded by an endothelial cell network, while cancer cells formed small clusters co-localized with osteoclasts. The enhanced secretion of IL-10 by M2 macrophages was associated with an anti-inflammatory profile. Treatment with the anti-cancer drugs doxycycline and rapamycin reduced the length of the vascular structures and cancer cell viability[15]. This multicellular 3D model can be used in devising more appropriate treatments.

Although multicellular 3D cancer models can demonstrate the crosstalk between multiple cell populations within the pre-metastatic niche, the important stromal parameter stiffness or biomechanics was neglected. Quiescent cancer cells, which are inactive cells that do not replicate, and the efficacy of chemotherapeutics are directly linked to the stiffness of the TME of metastatic tissues[131]. To study this effect, hydrogels, such as methacrylated gelatin or glycol chitosan-montmorillonite, have been used to model bone tissues of different stiffness[134,135]. These 3D models evidenced the role of mechanobiology in bone marrow or bone tissues. To address the biomechanics, estrogen receptor-alpha-positive breast cancer cells were grown on either soft or stiff fibronectin-coated polyacrylamide gels. The cancer cells proliferated in the soft matrices but remained quiescent or inactive in the stiff matrices. Treatment with the anti-cancer drug tamoxifen revealed that cells became chemo-resistant in soft matrices and upregulated autophagy-related signaling pathways to prolong survival[131]. However, in contrast to studies using penta-cultures[15], this 3D model[131] did not account for the diverse cellular and molecular elements that may regulate the stiffness-dependent behavior of cancer cells. For example, the chemokine CXCL5 is commonly found in the early metastatic niche and is sufficient to trigger the colonization of bone tissue by initially dormant breast cancer cells[136]. Overall, these 3D studies exemplify the fragmented nature of approaches to deconstruct tissues of the pre-metastatic niche. They neglect the role of fundamental elements of the TME and result in findings that arise from biomaterials with varying stiffness, and thus, are non-comparable between different studies. The specific challenge is to establish unified approaches to the TME of metastatic tissues.

[H2] Colonization

The colonization of pre-metastatic landscapes by infiltrating cancer cells is the final step in forming secondary tumors. To proliferate and colonize distant organs, cancer cells suppress the local immune surveillance and settle in the ECM that supports their uncontrolled

proliferation[137]. Biomaterial-based 3D systems combined with microfluidic platforms or cell culture bioreactors mimic the dynamic biochemical process that cancer cells experience while moving to and colonizing distant organs[138,139] (**Figure 3**). A tumor-engineered model was used to study the metastatic colonization of lung and liver tissues by breast and pancreatic cancer cells, respectively[139]. Lung and liver tissues were decellularized but preserved their original structure and composition, allowing the visualization of cell-matrix interactions. Bioscaffolds were perfused with cancer cells and culture medium using a bioreactor. Breast cancer cells invaded the lung bioscaffold and migrated along the collagen fibers, forming colonies and remodeling the matrix, which increased crosslinking points and promoted an irregular porosity. Co-cultures of pancreatic cancer cells and macrophages also colonized the liver bioscaffold without remodeling the matrix. This bioengineered 3D system may be further explored by pre-seeding bioscaffolds with immune cells and perfusing pro-inflammatory factors found in the pre-metastatic niche.

Some of these aspects were explored in another study that modeled the colonization of liver tissues by cancer cells[138]. Kidney cancer cells and hepatocytes were co-cultured in decellularized liver extracts blended with methacrylated gelatin. The composite material formed hydrogels that represented the stiffness of metastatic liver tissues. Cell-seeded bioscaffolds were perfused with culture medium to recreate the fluid shear stress **[G]**. Treatment with the chemotherapeutic 5-fluorouracil encapsulated in PEG-based nanoparticles reduced cell viability. The incorporation of immune cells may advance this 3D model for the screening of immunotherapies[140].

To further investigate the colonization of bone tissue by breast cancer cells, a 3D cancer model was used to mimic the perivascular niche[141]. Bone tissues were decellularized and seeded with endothelial and mesenchymal stem cells. The cell-containing bioscaffolds were combined with a microfluidic platform (**Box 3**) to recreate the interstitial flow **[G]**, inducing cell proliferation, and the formation of a vascular network and capillary-like structures. To model the metastatic colonization, the vascularized bioscaffolds were perfused with cancer cells. The interstitial flow diminished cancer cell proliferation and the number of cancer spheroids and triggered their resistance to treatment with the tyrosine kinase inhibitor sunitinib. Conversely, static conditions promoted the colonization of cancer cells and responses to sunitinib. This multicellular 3D model may be useful to explore the regulatory effects of cytokines that are known for regulating bone colonization[136,142,143]. In addition, the use of high-throughput

'omics' technologies such as genomic, proteomic, transcriptomic or metabolomic profiling, may reveal whether these 3D approaches truly recreate the gene, protein, mRNA and metabolite profile of metastatic tissues, potentially allowing their application as platforms for anti-metastatic drug screening.

BOX 3 | Engineering the tumor vasculature. The induced formation of new blood vessels and capillaries is a classical 'hallmark of cancer'[25]. Despite the importance of the vasculature for disease progression, static 3D cancer models, which only rebuild a tissue-specific matrix for cancer cell, lack vessel-like or capillary-like structures[144]. Advanced tumor-engineered models combined with microfluidic platforms, or tumor-on-a-chip models[46,145-147], mimic the fluid shear stress and hydrodynamic pressure derived from vascular perfusion[144]. Microfluidic platforms continuously remove metabolic products whilst delivering nutrients, soluble factors or drugs to cancer and stromal cells[141]. Progress in biofabrication techniques led to vasculature-inspired microchannels with defined geometries using 3D printing[126], 3D bioprinting[145] or photolithography[146]. Patterned microchannels are cast with hydrogels that mimic the matrix that surrounds endothelial cells and vascular networks to promote cell growth and capillary-like structures[148]. For example, proteolytic-degradable matrices support the formation of permeable vessel-like structures to model tumor metastasis[126]. Blood and lymphatic vessels with tuneable diffusion profiles are 3D-printed to recreate the tumor vasculature[145]. Microfluidic 3D cancer models are used to predict the sensitivity of tumor cells toward chemotherapeutics in high-throughput drug screenings[147]. However, tumor-on-a-chip models are still limited by the use of patient-derived cells, and the level and standardization of analytical techniques[144].

[H1] Bioengineering cell responses to treatment

A major challenge in the treatment of cancer is the limited capacity to use 3D in vitro models that are not sufficiently advanced thus far to test drug responses. At present, less than 5% of oncology drugs that have been tested in pre-clinical studies are successful in clinical trials[1]. Consequently, there is a need for improved 3D cancer models that recapitulate the clinical scenario to study the patient-specific responses to anti-cancer drugs. Tumor-engineered models combined with microfluidic platforms and high-throughput 'omics' technologies provide an innovative approach to cancer research.

[H2] Drug screening and personalized medicine

Drug testing using 3D cancer models may help to increase a patient's response to treatment, reduce tumor relapse and prolong patient survival. In particular, patient-derived organoids (PDOs) have the potential to guide personalized medicines as they maintain the genetic composition of a patient's tumor[149-151]. To develop more effective treatments tailored to individual patients, platforms that incorporate tumor-engineered models may be beneficial, as biomaterials have the potential to standardize drug responses at the industrial scale. Tornado-sequencing, a non-expensive high-throughput platform that adapts targeted RNA-sequencing for evaluating and distinguishing complex mixtures of cell phenotypes and differentiation programs in organoids[152], was optimized to study the drug responses of colorectal cancer organoids. This technology, combined with bioengineering approaches, may be applied for drug discovery in PDOs and eventually for clinical applications. In another study, colorectal cancer organoids trapped in bioengineered microwell arrays using PEG-based hydrogels were used to screen anti-cancer drug candidates that are either clinically used or in clinical trials[153]. Organoid viability was determined by high-throughput imaging and automated high-content analyses. This organoid array technology may be expanded to other cancer types and patient-derived samples, yielding clear benefits in terms of robotized high-content screening for translational research. Novel platforms, such as hydro-organoids arrays, whereby colorectal cancer cells are grown in Matrigel using a hydroponic culture method based on growing plants in water, enabling a fluidic culture without a solid matrix, may be useful for high-throughput drug screening[154]. Advantageously, these hydro-organoids arrays can form uniform organoids.

The drug sensitivity of osteosarcoma cells toward the chemotherapeutic doxorubicin and the interactions of the TME with different stromal cell populations were studied using methacryloyl platelet lysate-based hydrogels[155]. The 3D co-culture of osteoblasts[156] and prostate cancer cells also presented a promising pre-clinical drug discovery platform as it integrates the multicellular components of the bone metastatic TME. Other biomimetic 3D systems were also used to test the effects of different drugs on neuroblastoma cells. For example, layer-by-layer films based on the chondroitin sulfate A and poly-L-lysine were crosslinked with genipin, a natural biocompatible crosslinker, using a wide range of stiffness (30-160 kPa)[157]. This platform highlights that the biomechanical properties of the TME impact drug responses and thus, need to be carefully considered for each organ-specific TME.

Microfluidic platforms have enormous potential for clinical applications, in particular for drug screening and the development of personalized medicines (**Box 3**). For example, breast cancer spheroids were used in a microfluidic array with a biomimetic hydrogel matrix that combined gelatin with cellulose nanocrystals to test their drug sensitivity[158]. Using this spheroid-on-a-chip model, the differential responses of PDOs derived from different molecular subtypes of breast cancer toward eribulin, an anti-microtubule agent, were determined and correlated with patient-specific responses. This example demonstrates that microfluidic 3D models can capture the cell responses toward anti-cancer treatment observed in patients.

[H2] Immunotherapies

Harnessing the patient's immune system to treat cancer has become an increasingly investigated therapeutic option. Although immunotherapies, such as immune checkpoint inhibition (**Box 4**), have shown remarkable success in various cancers, including melanoma[159], breast[160] and lung cancer[161], clinical trials for other solid tumors, including ovarian[49] or pancreatic cancers[162], have been thus far unsuccessful.

Biomaterial-based 3D cancer models can be used to study immune cell trafficking[163], immunologically defined tumors[83] and patient-specific responses to immunotherapies[47]. In addition, PDOs have emerged as a powerful 3D platform as they represent the architecture of the TME, tumor-specific immune cells and the tumor-stroma crosstalk[47,150]. To model the response to immunotherapeutics in 3D, PDOs from various solid tumors, including kidney, lung, colon and pancreatic cancer, were grown using an air-liquid interface approach[47]. Tumor tissue fragments were mixed with Col-I and placed on pre-solidified collagen matrices on a permeable membrane and surrounded by culture medium to facilitate the growth of tumor organoids[47]. The inhibition of immune checkpoints resulted in a robust immune response against tumor cells, highlighting the potential of a combined biomaterial-PDO approach to assess patient-specific responses to immunotherapy[47].

Microfluidic platforms are also well-suited to study dynamic processes relevant to immunotherapy[144], such as tumor-immune cell infiltration, mechanisms of immune suppression, and TME interactions in 3D[163,164]. They are used to engineer selective components of the tumor vasculature (**Box 3**), including vascular perfusion, fluid shear stress, immune cell trafficking and suppression[163,164]. For example, a tumor-on-a-chip device was used to uncover the effects of environmental stress on natural killer cells, a critical type of immune cell acting

against the tumor[46]. The device contained a central polydimethylsiloxane chamber in which breast cancer and natural killer cells were grown encapsulated in a collagen matrix. Alternatively, natural killer cells were perfused through the lumen, which is lined by endothelial cells, to model immune cell trafficking[46]. The natural killer cells lost their ability to destroy cancer cells in the area furthest away from the lumen, implicating that cancer cells established immunosuppressive gradients. The natural killer cells also showed signs of exhaustion for example by dysregulating exhaustion markers (such as cytokines, inhibitory receptors and metabolic pathways) and phenotypic and functional changes, which were only partially weakened by immune checkpoint inhibition. The stress exerted by the TME is crucial for onco-immunological therapeutic approaches aimed at reversing immune cell exhaustion in cancer patients[46].

Lack of T cell infiltration in tumors remains a significant barrier, deeming patients refractory to immunotherapies[45]. A microfluidic pancreatic cancer model helped to explore T cell infiltration across the vasculature[163]. The microfluidic platform was equipped with two lateral channels and a central region, allowing physiological and molecular interactions between the different cell compartments. Pancreatic cancer cells were grown embedded in a collagen matrix in the central region. One lateral fibronectin-coated channel was seeded with endothelial cells to mimic the vasculature, while the other channel was populated with pancreatic stellate cells to model the tumor stroma. T cells were perfused through the vascular channel to allow migration and invasion of the tumor region. Endothelial cells prevented T cell infiltration to the tumor region, which is in line with the literature[163,165]. Pancreatic stellate cells dampened the inflammatory response mediated by T cells, highlighting their role in establishing an immunosuppressive TME, favoring tumor cell growth and resistance to immunotherapy[163]. This comprehensive study emphasizes the importance and potential of the use of multicellular and microfluidic 3D models to study the mechanism of immunosuppression and assess therapeutic success. The immune landscape in cancer is complex, and using tumor-engineered models to study responses to immunotherapy is a key step in understanding the heterogeneity of the tumor-immune landscape and resistance to treatment.

BOX 4 | Immunotherapy. Immunotherapy has improved treatment of some cancers, but underlying mechanisms are poorly understood. Various immune cell subtypes exist and differ in their composition, response to immunotherapy and prognostic value[31]. The tumor-immune landscape is intricate, containing large amounts of tumor-infiltrating immune cells[31,44] and

tumor-permissive CAFs[166,167], all contributing to disease progression and resistance to therapy. Immune checkpoint inhibition is a type of immunotherapy that targets pathways of T cell inhibition, or checkpoint molecules, and their corresponding ligands using blocking antibodies[168]. Checkpoint molecules, including CTL-4 and PD-1, are gatekeepers of our immune response and limit the effector function of T cells to kill cancer cells[169]. Ipilimumab, a monoclonal antibody blocking CTL-4, was first approved in 2011[168] and enables co-stimulatory signaling and subsequent T cell activation[168]. The approval of Pembrolizumab and Nivolumab followed, both monoclonal antibodies acting on PD-1[168], promoting T cell activation, proliferation and differentiation into memory T cells[169] and tumor-killing cytotoxic T cells[168]. Other immunotherapies include adoptive transfer of tumor-fighting T cells (CAR-T cell therapy), the transformation of the TME from immunological 'cold' to 'hot'[45], soluble targets (such as cytokines and chemokines)[26] and oncolytic viruses[168]. However, their clinical relevance across different cancer types needs to be carefully investigated.

[H1] Perspectives

Tumor-engineered models enable cancer cell growth, migration and invasion, as well as the study of drug responses. Steps were also taken toward integrating tumor-stroma interactions and other elements of the TME during cancer tissue engineering[3,6,7,14,50]. To effectively represent a particular cancer type surrounded by an organ-specific TME[4] **(Figure 4)**, strategies such as biomaterial-based matrices with modifiable mechanical properties have been leveraged. Furthermore, using contemporary 'omics' methodologies from other disciplines can lead to improved treatment and clinical outcomes for cancer patients. The integration of tissue engineering technologies with cancer research began over two decades ago, with 3D cancer models and 3D scaffolds engineering breast tumor tissues. The synergy between both areas is critical to accelerate our research progress in the 21st century.

Advanced 3D oncology approaches, based on multi-material and/or 3D-printed scaffolds, are becoming powerful pre-clinical platforms to evaluate the efficacy and safety of novel therapeutics, the potential of combination therapies and for drug discovery in high-throughput mode. Pre-clinical 3D platforms integrate patient-derived cells to screen targeted therapies, personalized medicines or immunotherapies. For example, a co-clinical trial showed that responses of PDOs to chemoradiation matched the responses of patients with nearly 84% accuracy[149].

Figure 4. Convergence of tumor biology and engineering. Multi-disciplinary research efforts between engineers and biologists are needed to advance our current technologies for tumor tissue engineering. Pre-clinical 3D platforms promote the growth of patient-derived tumor organoids that can be used for drug screening to identify more effective and personalized, next generation therapies. Multi-level biological analysis of the different cell populations and secreted factors sheds light on their role in disease progression. Stimuli-responsive biomaterials and additive manufacturing may be used for biomimetic 4D systems, leading to the development of assays that help discover next generation therapies.

Despite these advances, several limitations impose barriers and delays to the synergistic research efforts between biologists and engineers. The terminology used to communicate collaborative research data is often misused or misleading, for example the meaning of 'biomimetic' or 'bioscaffold'. At present, advanced user training is required to operate, for example, custom-made melt electrospinning machines for multilayered scaffolds for biomedical applications[170] and commercially available 3D bioprinters. The implementation of user-friendly biofabrication processes or facilities is a stepping-stone for non-trained users, enabling them to apply 3D bioprinting and microfluidic approaches to design and establish new 3D cancer models.

A major challenge for current 3D cancer models is that the platforms used to study the process and complexity of metastatic colonization are currently mostly microfabricated or based on microfluidics. This limits the accessibility for researchers not familiar with these techniques and the performance of large-scale and long-term 3D studies. Circulating tumor cells have intrinsic mechanisms to survive in blood, find supportive tissue microenvironments and invade distant organs. The number of different pre-metastatic pathways, patterns of metastatic lesions, mechano-environment and therapy-resistant cancer cells makes it extremely difficult to design tissue-specific metastasis models[132]. However, tissue-engineered bone tumor models represent a translational approach to how cancer cells colonize the bone niche that may be further pursued for pre-clinical drug testing[137]. Humanized 3D disease models will help us understand the fundamental, molecular and mechanical mechanisms of disease progression and resistance to therapy and will undoubtedly deliver breakthroughs in cancer and translational medicine.

Another limitation is the increasing complexity associated with designing realistic or physiologically relevant 3D cancer models, and the related analytical difficulties. The inclusion of multiple cell populations and biomaterials complicates the determination of the behavior and function of individual cell types and TME components. Techniques to degrade or dissociate hydrogels and scaffolds for cell recovery and separation with limited cell loss are just now being refined. Multi-level biological analyses are required to determine cell responses to changing model parameters, TME factors and drug treatment. Questions need to be asked about the level of complexity needed for effectively recreating the TME or cancer tissue (for example stroma-rich versus stroma-low, high immune-infiltrate or proliferative tumors) and which quantitative measures need to be implemented to analyze small cell numbers and 3D co-cultures. To date, the validation of findings from 3D models is limited, for example by incorporating patient-derived cells, association with clinical outcomes, use for multi-omics technologies, and the predictive or regulatory requirements hinder their widespread use.
Two research areas particularly in need of novel 3D oncology approaches are cancers of unmet clinical need, such as pancreatic tumors[7], and rare pediatric cancers, such as osteosarcoma[171], neuroblastoma[172] or Ewing's sarcoma[173]. The strategies reviewed herein emphasize the necessity for collaborative research between polymer chemists, bioengineers and cell and molecular biologists to fully exploit the ever-expanding biomaterial-based 3D technologies. We anticipate that this will foster cross-disciplinary thinking and research endeavors.

Although several challenges remain, the future looks bright for tissue tumor model engineering. This approach will help improve our understanding of fundamental processes and develop more effective treatments for different types of cancer. 3D cell biology has now entered the fourth dimension, and tissue engineering continues to deliver tools to manipulate biomolecules and cells. Advanced biofabrication techniques, such as additive manufacturing[174], molecular self-assembly[175] or their combination[176], have improved capacity to build biologically relevant structures. These capabilities enable the design of 3D objects and microenvironments on which spatial and temporal control is possible, and that can respond and adapt to external stimuli like water, light or temperature. For example, ultrasound-responsive microenvironments allow dynamic control over the biomechanics and mechano-transduction in a 3D breast cancer model[177]. Biomimetic 4D printing leads to dynamic constructs for different applications, such as robotics, biomedical engineering or drug delivery[123]. Self-assembling materials based on different types of peptides or biomolecules are another kind of 4D biofabrication, with the capacity to generate responsive multi-material systems[124]. In the future, these new biologically-

inspired materials may lead to even more sophisticated 3D cancer models, tailored biosensors and drug release systems.

Glossary box

Biomimetic: is a replica of the properties or elements of natural tissues or systems[4,6,99,157,178,179].

Bioscaffold: is a decellularized tissue that preserves the structural and molecular integrity of the organ-specific native ECM[87,139].

Cell-instructive sites: are short peptide sequences in biomaterials that present insoluble adhesion ligands and proteolytic-degradable motifs and enable binding or release of soluble factors.

Desmoplastic tumor stroma: is the deposition of a dense and crosslinked extracellular matrix resulting in a fibrotic tumor tissue[1,33,180].

Epithelial-mesenchymal-transition: is when cells become more motile, migratory and invasive through the loss of epithelial features and the gain of mesenchymal ones[34].

Fluid shear stress: is the force created by the fluid flow parallel to a surface of a material or tissue[140,144].

Hydrogel: is a water-swollen polymer network[79].

Interstitial flow: is the movement of fluid that is transported through the extracellular matrix[141].

Metabolic vulnerabilities: are the abnormal metabolism of cancer cells and targeted by anti-tumor therapies[106].

Personalized medicines: are therapies designed for a specific patient based on its genetic/individual signature[149].

Organoid: is a self-organizing and self-renewing cluster of cells derived from tissue fragments or stem cells that mimic the functionality and behavior of tissues[22,68,69,90].

Scaffold: is a 3D structure with a defined geometry and mechanical properties[85].

Spheroid: is a cell aggregate or cluster that resembles some features of tissues[1].

Stiffness: is a material's, or tissue's, resistance to deform and alter its original shape when a force is applied[181].

Tumorigenesis: is the formation of a tumor whereby normal cells transform into cancer cells following a disrupted differentiation[25].

Tumor microenvironment: describes a dynamic network of malignant and non-malignant cells, extracellular and cell-secreted elements and soluble factors that promote tumor development, growth and metastasis[23,28].

Viscoelasticity: is time-dependent mechanical property of materials, or tissues, that exhibit viscous and elastic responses when forces are applied, causing temporary or permanent deformation, respectively[73,79].

Abbreviations

CAFs, cancer-associated fibroblasts; Col-I, type-I collagen; ECM, extracellular matrix; KLK, kallikrein-related peptidase; MMP, matrix metalloproteinase; PDO, patient-derived organoids; PEG, polyethylene glycol; PLG, poly(lactide-co-glycolide), TME, tumor microenvironment

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Author contributions

D.L. conceived the article. All authors researched the data and drafted the article. R.C., V.K. and D.L. conceived and illustrated the figures and tables. All authors made substantial contributions to the discussion of content, and reviewed and edited the article.

Table 1. Examples of different biomaterials used as 3D culture models for cancer, TME-associated, and stromal cells. AFM, atomic force microscopy; CAFs, cancer-associated fibroblasts; Col-I, type-I collagen; FG, fibrinogen; HUVECs, human umbilical vein endothelial cells; MMP, matrix metalloproteinase; MSCs, mesenchymal stem cells; PDOs, patient-derived organoids; PEG, poly(ethylene glycol); PEGDA, poly(ethylene glycol) diacrylate; PSCs, pancreatic stellate cells; TME, tumor microenvironment.

Biomaterial (concentration)	**Cell types**	**Cell density**	**Analyses**	**Mechanical properties (if tested)**	**Possibility for 3D bioprinting**	**Reference**
Col-I (1 mg/ml)	adipocytes, omental fibroblasts, mesothelial cells, ovarian cancer cells, platelets	$2x10^6$/ml, $1x10^6$/ml, $2x10^6$/ml, $2x10^8$/ml	viability, invasion, gene and protein expression, morphology			[14]
Col-I (10 mg/ml), mineralized	breast cancer cells		protein expression, collagen structure and mineralization, migration	Young's modulus, ~4 kPa, AFM		[35]
PEG, functionalized with GFOFGER, RGD, PHSRN and MMP peptides	fibroblasts, myeloid cells, pancreatic cancer cells	$1x10^6$/ml, $5x10^6$/ml (1:5 cancer:stromal cells)	viability, proliferation, survival, gene expression, morphology, protein expression	Young's modulus ~1.4-20.5 kPa, AFM		[30]
PEG (functionalized with RGD and MMP peptides)	pancreatic cancer cells	$3.5x10^5$/ml	metabolic activity, cell proliferation, protein secretion	Young's modulus ~1.5-2.5 kPa, unconfined compression testing (indentation)		[41]
Col-I (4 mg/ml)	breast cancer, natural killer cells, HUVECs	$1.5x10^6$/ml, $0.5x10^6$/ml	viability, metabolic imaging, gene expression, migration			[46]
Col-I (8:1:1 in NaOH and HEPES)	PDOs of varying origin with native stromal cells including T, B, natural killer cells, macrophages		gene and protein expression, mutation analysis, immune checkpoint inhibition and immune cell activation, cytotoxicity, (single-cell)			[47]

			RNA sequencing			
Col-I (3.47 mg/ml), functionalized with RGD peptides	glioblastoma cells, endothelial cells, macrophages	$1x10^7$/ml	cell differentiation, angiogenesis, gene and protein expression, cytokine secretion			[51]
Col-I (1, 3, 6 mg/ml)	adipose-derived stromal cells, breast cancer	$5x10^3$ cancer cells/spheroid, increasing numbers of adipose-derived stem cells (50, 500, 5000)	protein expression, collagen pore size and intensity distribution, collagen contraction, cell-matrix interactions, migration	elastic modulus 2.2 ± 0.2 kPa, dynamic mechanical thermal testing		[39]
alginate (1.1% wt/vol)	non-small cell lung cancer cells, CAFs, monocytes	$3x10^5$/ml (1:1:1 tumor:CAFs: monocytes)	viability, apoptosis, proliferation, protein expression and secretion, gene expression			[50]
fibronectin-coated resin (DS-3000) scaffolds, (10 µg/ml)	breast cancer cells, MSCs	$1x10^6$/ml, $0.5x10^6$/ml	gene and protein expression, osteogenic differentiation mineralization, proliferation, colonization, viability	Young's modulus ~2.45 GPa ± 125 MPa, AFM	X	[120]
polyacrylamide (12.5%) coated with fibronectin (0.2 mg/ml)	breast cancer cells		viability, morphology, protein expression, mechanical testing, gene expression	elastic modulus 0.1-100 kPa, rheology		[131]
fibrin (2.5 mg/ml)	breast cancer cells, HUVECs, monocytes, myoblasts, fibroblasts, osteoblasts, osteoclasts	$0.15x10^6$/ml, $1.5x10^6$/ml, $3x10^6$/ml	morphology, protein expression, cell cycle, vessel length and area fraction			[15]
gelatin (porcine, type A, 3-8 wt/vol%)	adipocytes, MSCs	$1.5x10^6$/ml	viability, proliferation, gene and protein expression	compressive modulus 0.98-32.96 kPa, unconfined compression testing		[134]

glycol chitosan (2% wt/vol)	MSCs	$2x10^6$/ml	viability, invasion, gene expression, osteogenic differentiation	Young's modulus 10->60 kPa, compression testing		[135]
Col-I (3 mg/ml)	pancreatic cancer cells, PSCs, HUVECs, T cells	$1x10^6$/ml, $3x10^6$/ml, $8x10^6$/ml	permeability assay, protein expression, cytokine expression			[163]
alginate (3% wt/vol), alginate / Col-I (3 mg/ml), alginate / gelatin (porcine, type A, 10% wt/vol)	breast cancer cells, adipose-derived stem cells	$2x10^6$/ml, $5x10^5$/ml	viability, invasion, proliferation, adipogenic differentiation	elastic modulus 1.7-2.7 kPa, atomic force indentation	X	[179]
Col-I (2.5 mg/ml)	fibroblasts, CAFs, pancreatic cancer cells	$8.4x10^4$/matrix, $2x10^5$/matrix, $4x10^4$/matrix	contraction, invasion, proliferation, survival	Young's modulus ~3-4 kPa, AFM		[182]
agarose (13-15 mg/ml) / Col-I (15 µg/ml) / droplets	embryonic kidney cells, MSCs	0.5-$1.5x10^7$/ml	viability, proliferation, chondrogenic differentiation		X	[183]
PEGDA / FG (3.1% wt/vol) microspheres	breast, prostate and colon cancer cells	$2x10^7$/ml, $6x10^7$/ml	viability, proliferation, morphology	Young's modulus 4.7 kPa, compression testing		[184]
PEG (5% wt/vol)	endometrial cancer cells, endometrial stromal cells, colon cancer cells, hepatocytes	$8x10^6$/ml, 5-$5.3x10^6$/ml (1:1 epithelial:stromal cells)	morphology, actin polarization, protein expression and secretion			[185]
gelatin (type B, 8% wt/vol)	fibroblasts, CAFs, pancreatic cancer cells	$7.5x10^5$/50 mg microbeads (1:3 epithelial:stromal)	proliferation, gene and protein expression			[180]
gelatin (porcine, type A, 1 g/10 ml)	ovarian follicles	3-4 follicles à 150-180 µm, 40-50 follicles à ≤180 µm	survival, steroidogenesis, in vivo organ function	elastic modulus 16.84 kPa, compression testing	X	[186]

Table 2. Cell-instructive peptides incorporated into tumor-engineered models. PEG, poly(ethylene glycol).

Extracellular protein	Peptide sequences	Binding receptors	Biomaterials	Cancer types	References
collagen	GFOGER	integrins α1β1, α2β1, α10β1, α11β1	PEG	breast, pancreatic and prostate cancer	13,30,97,187-189
fibronectin, vitronectin	RGD	integrins αvβ3, αvβ1, α5β1	PEG	breast, ovarian, pancreatic and prostate cancer	6,13,30,38,41,83,187,190-192
fibronectin	REDV	integrin α4β1	PEG	prostate cancer	97
	PHSRN	integrin $\alpha_5\beta_1$	PEG	endometrial and pancreatic cancer	30,185
			self-assembling peptides	breast cancer	193
laminin	YIGSR	integrins α3β1, α6β1	PEG	breast cancer	194
	IKVAV	integrins α3β1, α6β1	bacterial cellulose	melanoma	195
			PEG	breast and prostate cancer	13,187,189
	AG73	syndecan 1	self-assembling peptides	breast cancer	193

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