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Emerging Biomimetic Materials for Studying Tumor and Immune Cell Behavior

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Abstract

Cancer is one of the leading causes of death both in the United States and worldwide. The dynamic microenvironment in which tumors grow consists of fibroblasts, immune cells, extracellular matrix (ECM), and cytokines that enable progression and metastasis. Novel biomaterials that mimic these complex surroundings give insight into the biological, chemical, and physical environment that cause cancer cells to metastasize and invade in to other tissues. Two-dimensional (2D) cultures are useful for gaining limited information about cancer cell behavior; however, they do not accurately represent the environments that cells experience *in vivo*. Recent advances in the design and tunability of diverse three-dimensional (3D) biomaterials complement biological knowledge and allow for improved recapitulation of *in vivo* conditions. Understanding cell–ECM and cell–cell interactions that facilitate tumor survival will accelerate the design of more effective therapies. This review discusses innovative materials currently being used to study tumor and immune cell behavior and interactions, including materials that mimic the ECM composition, mechanical stiffness, and integrin binding sites of the tumor microenvironment.

Keywords

Biomimetic; Extracellular matrix; Epithelial-to-mesenchymal transition; Cell–ECM interactions

INTRODUCTION

The ability of tumor cells to metastasize beyond the primary tumor and invade into new tissues is one highly-studied hallmark of cancer.⁴⁴ To gain more information about this phenomenon, physicians and researchers use techniques such as incisional biopsies to

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extract tumor samples. These specimens are helpful for gaining information such as expression of genes and prevalent proteins that are associated with malignant phenotypes and cancer cell proliferation.⁹⁹ Although informative and extracted from patients, one disadvantage of direct evaluation of primary tissues is the large degree of variability in each sample. Another popular methodology in studying cancer *in vitro* is two-dimensional (2D) tissue culture. One key limitation of this method is that 2D culture does not recapitulate physiologically relevant three-dimensional (3D) microenvironments, which typically results in distinct outcomes from *in vivo* conditions.⁸⁰ Another drawback of this technique is primarily the lack of integrin binding sites present in the tumor microenvironment. In addition, the complexity and tunability of 2D environments are severely limited.¹⁴

While much information has been gained from monolayer systems, there has been a recent push to develop tunable, 3D cell-culturing systems to better mimic the tumor microenvironment, which includes fibroblasts, immune cells, soluble factors, and extracellular matrix (ECM) proteins (Fig. 1). One of the most sought-after approaches to address these issues is the use of biomimetic materials that model specific *in vivo* characteristics, including chemical composition or stiffness.³⁵ Biomimetic materials have been developed for applications ranging from the regeneration of cartilage to wound healing.² Currently, these materials have also been used to create synthetic environments that can be used to study cell morphology as well as the underlying molecular causes of malignant cell morphology and invasion.

In addition to tumor cells alone, incorporating multiple cell types such as immune and stromal cell populations have allowed researchers to better model the *in vivo* microenvironment, cell–cell interactions, and cell–ECM interactions. This has led to an increased understanding of tumor cell behavior and improved tumor targeting therapeutic strategies. The immunological response is also an important factor when developing 3D microenvironments. Tumor-promoting inflammation is an additional hallmark of cancer, so understanding how immune cells interact with tumor cells in the microenvironment is imperative for determining the causes behind tumor progression and metastasis.^{36,44,123} In this review, we will outline the emerging materials utilized in designing biomimetic microenvironments for evaluating tumor and immune cell behavior.

THE EXTRACELLULAR MATRIX (ECM)

Defining the ECM

The ECM is a 3D network that consists of various proteins and macromolecules necessary for providing cellular, biochemical, and structural support. All tissues in the body contain ECM components but vary in their composition. Most mammalian ECM consists of collagen, fibronectin, and laminin, along with glycosaminoglycans that form proteoglycans.^{33,124} Table 1 lists the key components present in the ECM. In cancer studies, cell–ECM interactions are commonly studied (Fig. 2), which facilitate changes in cytoskeletal actin filaments, upregulation or downregulation of oncogenes, and expression of proteins associated with cancer cell invasion and proliferation.^{11,14}

ECM and the Tumor Microenvironment

The ECM is a prominent factor in cancer progression in the tumor microenvironment.⁷ There has been extensive research into how the chemical and physical properties of the ECM can affect the proliferation and migration of cancer and immune cells as the composition and structure of the ECM is integral to the regulation of cell growth, phenotype, and organization.¹³¹ Many recent studies have attempted to mimic particular ECM properties and structures to study cancer progression. For example, Narkhede *et al.* developed hyaluronic acid (HA)-based hydrogels since HA is a key component of the brain ECM and has similar viscoelastic properties to the ECM.⁹⁴ The HA hydrogel stiffness was varied from 0.2 to 4.5 kPa to understand how changes in mechanical cues can affect the behavior of brain metastatic breast cancer cells. According to their results, breast cancer cells proliferate and adhere to a greater extent in stiffer microenvironments, and this was mediated by the focal adhesion kinase (FAK)–phosphoinositide-3 kinase (PI3K) pathway.⁹⁴ Another example is the work by Kim *et al.* where they incorporated collagen I and Matrigel into a microfluidic device to study how the recruitment of immune cells alters the invasiveness of cancer cells. This work demonstrated monocyte and macrophage-induced remodeling of the ECM, which enhanced the invasiveness of cancer cells.⁶³ ECM components have also been utilized as bioinks in 3D printing technologies.^{46,71} For instance, Duarte Campos *et al.* used a type I collagen-based bioink to make a 3D mini-model of neuroblastoma. These models were able to mimic *in vivo* characteristics of neuroblastoma such as proliferative potential and the formation of Homer Wright-like rosettes.^{27,134}

Integrin Binding Patterns

One of the most important drivers of communication between cells and the ECM is the presence of integrins. Integrins have key functions in multiple processes that facilitate tumorigenesis, progression, and metastatic potential of cancer cells.⁵⁸ These cellular receptors are implicated in signaling molecules, cell migration machinery, the epithelial-to-mesenchymal transition (EMT), and mechanotransduction.^{42,133} For instance, integrins allow for the activation of epidermal growth factor receptor (EGFR) and the modulation of epithelial differentiation in 3D microenvironments. EGFR also promotes the formation of focal adhesions, structures that bind integrins to the cytoskeleton and organize the conformation, tension, and morphology of cells.⁵² Integrin $\alpha v\beta 6$ is the binding receptor in tumorigenic cells while $\alpha 5\beta 1$ is a constitutively expressed integrin that plays the same role in healthy cells.^{28,57} Many studies have identified the role of specific integrin subtypes in cancer progression mediated by tissue stiffness using diverse biomaterials. Using alginate–Matrigel interpenetrating networks, Chaudhuri *et al.* proposed a mechanism where junction $\alpha 6\beta 4$ integrins and laminin play an important role in the development of malignant phenotypes in breast cancer, which is dependent on ECM stiffness. If the stiffness of the ECM is in the normal range (30 Pa), the $\alpha 6\beta 4$ integrin–laminin units overlap and allow for hemidesmosome formation. However, high ECM stiffness (310 Pa) reduces hemidesmosome formation, and the free $\beta 4$ can lead to Rac1 signaling and PI3 K activation for phosphorylation by receptor tyrosine kinases.¹⁴ Moreover, it was shown using 2D tunable collagen I-coated polyacrylamide (PA) hydrogels that an increase in stiffness and availability of collagen binding sites leads to the upregulation of integrin $\beta 1$ in hepatocellular carcinoma

(HCC) that promotes cancer progression by the activation of transforming growth factor beta-1 (TGF β -1).⁹⁸

BIOMIMETIC TUMOR MICROENVIRONMENTS

To study various organs and diseases, biomimetic environments have typically been designed to provide structure as a scaffold for cell growth and adhesion. More recently, many emerging biomaterials have been used to evaluate EMT, cell–ECM interactions, and immune cell infiltration, including organoids, decellularized ECM, natural materials, and synthetic materials. These materials and applications are summarized in Table 2.

Organoid and Spheroid Models

Organoid and spheroid systems are useful models for biomimetic microenvironments. Organoids are organ-like structures and are derived from cells and tissues ranging from murine to human species (Fig. 3). Organoids recapitulate key structural and functional components of various sites of interest from the brain to retinal systems.^{17,31,110} Organoids have been used extensively in studying multiple cancer models.^{8,13,24,111} For example, CRISPR/Cas9 gene editing was performed to introduce KRAS, SMAD4, APC, and TP53 mutations into normal human intestinal organoids to form adenocarcinomas in mouse models during xenograft transplantation.²⁵ Organoid models have been developed to mimic drug resistant tumors and to evaluate therapeutic efficacy.^{4,17,26} Sun *et al.* reported the formation of organoids from reprogrammed hepatocytes to initiate HCC through c-Myc expression.¹²⁰ It was shown that these organoid models can form tumors *in vivo*, making them a useful system for generating mouse xenografts to study drug response. In addition to tumor systems, organoids have been used as normal tissue models to evaluate tissue development as well as to study how normal tissues interact with tumor and immune cells.^{39,54} For example, Hacker *et al.* derived mammary epithelial organoids from irradiated and normal murine mammary glands.³⁹ These organoids are being used to study the effects of normal tissue radiation damage on breast tumor-stromal and immune interactions, which may elucidate cancer recurrence mechanisms. Patient–derived organoids are also being explored to develop platforms for studying novel biomarkers and drug targets as well as tumor-immune and tumor-stromal interactions.^{95,104,113,127,129}

An additional approach to studying 3D tumor models is developing spheroids or micro-sized cell aggregates that function as *in vitro* models of the behavior of different tumor types.¹⁸ Limitations in the use of tumor spheroids include the lack of ECM interactions and heterogeneity in the size and shape of the aggregates. To address that variability, Pradhan *et al.* designed and modeled 3D tumor microspheres formed by encapsulating MCF7 breast cancer cells in a PEG-fibrinogen hydrogel. This new model presented more cancer hallmarks compared to classic spheroids, such as increased disorganization, loss in apicobasal polarity, elevated nuclear-cytoplasmic ratio, nuclear volume density, and the reduction of cell–cell junction length.¹⁰² Recently, alginate-based bioinks have been used to form 3D tissue spheroids of bone, cartilage, vascular tissue, and diverse tumors through bioprinting.^{5,22,37,71,92,132} Compared to previous spheroid formation techniques, this method has been shown to improve cell viability, function, and architecture. 3D printing can form a ready-to-

use tumor model that can mimic interactions with the ECM and with other cells in a co-culture system. Additionally, compared to self-assembled spheroids using Matrigel, this method does not require complex components such as binding sites, growth factors, diverse extracellular fibers to promote tumor formation.¹²² In their work, Swaminathan *et al.* not only studied the pre-spheroid formation of the MDA-MB-231 and MCF7 human breast cancer cells lines but also a non-tumorigenic MCF10A breast epithelial cell line. They performed a co-culture with vascular endothelial cells to examine drug response using Matrigel, gelatin–alginate, and collagen–alginate as bioinks. Laminin was found to be a key factor in the spheroid formation of breast epithelial cells, the spheroid structure was distinct when comparing tumorigenic and non-tumorigenic cell lines, and these morphologies were conserved post-bioprinting.¹²²

Decellularized ECM (dECM)

Decellularization is a process that removes cellular material from tissues while leaving the majority of the ECM structure intact.¹¹⁴ Since the 1990s, decellularized ECM (dECM) has been used for wound healing and regeneration but has been variable in its success.⁴⁷ dECM can be reconstituted into hydrogels and used for *in vitro* and *in vivo* experiments to study cancer progression (Fig. 4). These dECMs consist of structural and functional molecules that assist in the 3D organization of encapsulated cells. The organs from which these matrices are derived can produce distinct ECM components, and these differences alter cell–ECM interactions that serve to influence tumor cell proliferation and adhesion.⁶ dECM derived from tumors has been shown to promote angiogenesis, the EMT response, and MMP-9 production.⁴⁸ Many studies use ECM obtained from tumor tissue to establish the effect of the microenvironment on the behavior and progression of various types of cancer, including colorectal,^{48,50,100,101,109} breast,^{49,61,77,91} liver,⁸¹ brain,⁶⁶ and lung.⁷⁹ This approach allows for the evaluation of dECM obtained at different stages of tumorigenesis¹⁰⁹ and cancer cell interactions with immune cells.^{100,101} One of the most important advantages of dECM is recapitulating the ECM microenvironment of specific tissues. For example, murine mammary fat pads were decellularized and exposed to radiation *ex vivo*.³ Using these dECM hydrogels allowed for studying the effect of radiation on breast cancer proliferation and invasion in the context of recurrence after therapy. Wolf and coworkers also showed that the ECM derived from urinary bladder can support 3T3 fibroblast growth and proliferation.¹³⁶ Another notable characteristic of dECM is that it can promote a pro-regenerative immune phenotype and thus has been considered to combat tissue loss and promote wound healing following tumor resection.¹³⁷

Naturally-Derived Materials

Matrigel—Matrigel is a solubilized basement membrane matrix that is derived from murine Engelbreth-Holm-Swarm sarcomas, tumors rich with ECM proteins such as laminin, collagen IV, and other growth factors.⁶⁵ Matrigel has mostly been used as a 3D matrix for cell growth and adhesion in culture systems due to relevant cell adhesion sites. Matrigel has also been combined with other 3D systems such as alginate hydrogels as will be described below.^{11,12,14,73,135} Although abundantly examined, Matrigel can vary from batch to batch, and its components are undefined. Using Matrigel is nonetheless advantageous for studying

how biological, chemical, and mechanical characteristics of the ECM affect the development and progression of cancer.

Alginate—Alginic acid, or alginate, is an anionic polysaccharide derived from brown algae. It is mostly comprised of α -D-mannuronic acid and β -L-guluronic acid and can form crosslinked hydrogel networks of tunable mechanical properties through crosslinking using divalent ions such as calcium and manganese.⁶⁸ Alginate lacks cell adhesion binding sites and often requires modification with peptides or combination with collagen or Matrigel to form interpenetrating networks.¹⁴ Read *et al.* showed that human colorectal cancer cells were viable when encapsulated within unmodified alginate hydrogels to study metabolic effects after irradiation.¹⁰⁵ However, not having binding sites available for the cells reduces the accuracy of the biomimetic environment since the progression of cancer is connected to cell–ECM dynamics.¹³¹ In order to mitigate this, Cavo *et al.* developed an alginate hydrogel conjugated with RGD peptides that bind to integrins such as $\alpha_v\beta_3$ and $\alpha_5\beta_1$.¹¹ Other peptide sequences such as DGEA and YIGSR, which are involved in cell adhesion to the ECM, have been used in conjunction with alginate to further produce bio-mimetic environments. It has been shown that the expression of these peptides can control EMT.⁶⁸ Alginate has also been used with collagen for immunotherapy treatments in mouse studies by Wei and coworkers. They show that the alginate–collagen matrix along with the TLR7 agonist R837 and radiation decreases 4T1 primary tumor growth and reduces the volume of distal tumors.⁸⁷ Alginate-chitosan scaffolds have been designed to enhance cell adhesion. These scaffolds have been used to mimic stages of cancer progression and metastasis in prostate adenocarcinoma and to examine cancer stem-like enrichment in prostate, breast, liver, and brain cancer.^{32,138}

Since alginate can be easily tuned through the addition of divalent cations, it is widely used for evaluating biomimetic stiffnesses of the tumor microenvironment. Stiffness is an important factor when studying the tumor microenvironment as it has been shown that matrix rigidity can cause cells to undergo EMT that is characteristic of invasive phenotypes (Fig. 5a).^{10,21,108} It is also important to tune biomaterials to mimic the vast range of mechanical properties experienced by cells in various organ microenvironments, including breast tissue (0.5–1 kPa), the intestines (20–40 kPa), and bone (15,000–20,000 kPa) (Fig. 5b). Alginate–Matrigel hydrogels have been used for breast cancer dormancy exit studies in relation to microenvironmental stiffness. Chaudhuri *et al.* fabricated alginate–Matrigel interpenetrating networks to evaluate breast cancer cell behavior in environments ranging from 30 to 300 Pa to mimic progressive tumors. Using the MCF10A breast cancer cell line, they observed that increased stiffness can promote the transition from epithelial to mesenchymal phenotypes, which is observed when dormant tumor cells exit dormancy.¹⁴ This alginate–Matrigel model has also been used to study how mechanotransduction pathways such as YAP/TAZ signaling relate to breast cancer progression.⁷³ These models for integrin binding have also been used to study the invasiveness of tumor cells. Cavo *et al.* used alginate–Matrigel hydrogels to understand how stiffness can cause malignant progression as it has been shown that the biomechanical properties directly affect neoplastic disease.¹²¹ MCF-7 ER+ breast cancer cells were shown to exhibit a rounded morphology in

microenvironments of 5 kPa compared to MDA-MB-231 cells, an ER-cancer cell line with higher metastatic and invasive potential.^{11,12}

Synthetic Materials

Polyethylene Glycol (PEG)—PEG is a synthetic polymer that is used in a variety of biomedical applications.⁹⁰ PEG, when modified with methacrylate and dimethacrylate, is photocrosslinkable under UV light and can easily be conjugated with active protein sites for cell adhesion to better evaluate cell morphology and production of growth factors.^{55,117} This material has been used to fabricate biologically active microenvironments by conjugating PEG to different protein binding sites such as RGD, IKVAV, and GFOGER to mimic the cellular binding sites in the fibrous structure of the ECM.^{82,115,141} Gill and coworkers used PEG conjugated with RGDS (PEG-RGDS) for cell adhesion and GGGPQGIWGQ GK (PQ) for degradation by matrix metalloproteinases. In this model, lung adenocarcinoma cells were used to understand morphological and epithelial changes in response to microenvironmental stiffness, ligand adhesion concentration, and TGF β .³⁴ Stiffnesses were varied from 21 to 55 kPa in order to study the effect of stiffness on the morphology of lung adenocarcinoma cells. Higher stiffnesses promoted an increase in mesenchymal phenotypes. In addition, cells interacting with larger amounts of RGDS peptide presented an epithelial phenotype. Sawicki and coworkers varied the concentration of the peptides GFOGER, RGDS, and IKVAV that bind to collagen, fibronectin, and laminin, respectively.¹¹⁵ These concentrations were changed to mimic peptide concentrations associated with cancerous tissue, including increased collagen I binding sites.¹²⁸

Polyacrylamide (PA)—Acrylamide-based hydrogels are widely used to model the stiffness range present in biological matrices, typically from 0.1 to 119 kPa.¹⁵ PA can be functionalized and coated with ECM components such as fibronectin, vitronectin, and collagen to encourage cell adhesion.²⁰ A major challenge, however, in using this material is the cytotoxicity of acrylamide monomers, which skews studies toward 2D models.¹⁴⁵ Currently, PA hydrogels are being designed to understand how mechanical cues such as stiffness, topography, and geometry alter gene expression,⁸⁶ EMT, drug resistance,¹⁰⁸ migration,⁶⁹ and tumor progression.^{86,106,107} To evaluate mechanotransduction in gradient hydrogels, Hadden *et al.* fabricated PA-derived gels to create cell culture surfaces that have tunable stiffnesses.⁴⁰ Using human adipose-derived stem cells, it was reported that cells experienced durotaxis on fibronectin-coated PA gels with a steep 8.2 kPa/mm gradient while cells did not migrate in response to a more shallow gradient of 2.9 kPa/mm. This indicates that conditions separating the effects of durotaxis from stiffness response should be considered in studying tumor cell behavior. PA hydrogels have also been used to understand cancer stem cell (CSC) plasticity. Tian *et al.* used PA hydrogels to understand whether matrix rigidity has an effect on the phenotype of CSCs.¹²⁵ It was reported that HCCs had stem cell-like properties and poor spreading at 5.9 kPa corresponding to normal liver stiffness compared to stiffnesses in the range of cancerous tissues (48.1 kPa). PA surfaces have also been utilized to study the response of human primary thyroid cells (S747) and anaplastic thyroid carcinoma cells (S277) when sensing a range of mechanical environments.¹⁰⁷ It was found that normal cells adapt their viscoelastic properties in response to stiffness changes while the carcinoma cell viscoelasticity remained constant.

Other Synthetic Biomaterials—Other commonly studied synthetic biomimetic materials are polydimethylsiloxane (PDMS), polycaprolactone (PCL), and polyurethane (PUR) films.^{51,75,97} All of these materials allow for a higher degree of stiffness matching of tissues and organs compared to natural biomaterials, and they can also be functionalized or mixed with ECM components to form hybrid materials that can model the tumor microenvironment.⁹⁷ One notable example evaluates the development of porous and tunable alginate-PCL nanofiber scaffolds for investigating cancer stem-like cell enrichment, EMT, and cell distribution.⁵¹

IMMUNE CELL BEHAVIOR

Studying the Immunological Response in Cancer Microenvironments

There are several components of the microenvironment that influence tumor growth, including cancer-associated fibroblasts, tumor associated neutrophils, and tumor associated macrophages (TAMs).^{64,93} It has been shown that these cellular and immune populations, or lack thereof, can contribute to cancer progression and metastasis. For example, triple negative breast cancer tumor cell recruitment after radiation therapy was enhanced due to reduced lymphocytes and excess macrophages.¹⁰³ In addition, incorporating chemokines and cytokines into models are important as they enable pro-inflammatory responses that affect the growth of tumors and give signals to immune cells to encourage malignant behavior.^{64,140}

One overarching goal in designing 3D biomimetic systems is to understand how tumor cells evade the immune response.³⁶ TAMs are associated with poor prognosis in patients and promote tumor growth and migration.^{85,147} A biomimetic materials approach has been undertaken to evaluate immune cell interactions with stromal and tumor cells. Wolf *et al.* used urinary bladder matrix (UBM) scaffolds embedded with three different cancer cells, melanoma (B16-F10), colorectal carcinoma (CT26), and mammary carcinoma (4T1) to evaluate how the ECM microenvironment influenced tumor progression. UBM is a decellularized scaffold composed of basal lamina and lamina propria of the porcine urinary bladder that contains collagen I, ECM-associated factors, glycoproteins, and proteoglycans.¹¹² The UBM scaffold inhibited and delayed tumor formation while promoting a distinct immune signature that was dependent on CD4+ T cell and macrophage infiltration.¹³⁷ In addition, Zhu and co-authors have shown that cellular phenotypes and their responses can change within dECM hydrogels. dECM hydrogels were found to play a role in macrophage activation and polarization that was dependent on the organ from which the ECM was derived.¹⁴⁷ Keane *et al.* demonstrated that decellularized matrices can promote anti-inflammatory responses by reducing inflammatory macrophage infiltration.⁶² The mechanisms behind how dECM can activate these macrophages warrant further study in order to evaluate polarization that mimics the tumor microenvironment.

3D bioprinted tumor models have also been used to study the interactions between different cell types, including immune, cancer, endothelial, and fibroblast cells *in vitro*.^{46,71} Gelatin and gelatin methacrylate have been used for this application.⁸⁹ In their work, Heinrich *et al.* fabricated a mini-brain with encapsulated glioblastoma-associated macrophages (GAMs) and glioblastoma multiforme (GBM) tumor cells to study cell crosstalk *in vitro*. This work

demonstrated how the presence of GAMs promotes the invasion and proliferation of GBM as well as how GBM leads to GAM recruitment and proliferation.⁴⁶

An important application of biomimetic materials is to study the behavior of cancer cells treated with novel therapies.³ One approach is local cancer immunotherapy, a treatment intended to stimulate the immune system of cancer patients commonly achieved through T-cell infusion, vaccines, or antibodies to inhibit proteins produced by cancer cells using macroscale bio-materials.^{29,83} The principal advantage of local immunotherapy is specific immunomodulation at the tumor site, which prevents systemic toxicity. Injectable biomaterials can include cargo such as immunomodulators, immune cells, or cancer vaccines. Mesoporous silica microrods, PEG with polypeptide blocks, synthetic peptides with specific motifs, and DNA-scaffolded biomaterials are being developed for this purpose.^{53,56,74,142} Additionally, natural biopolymers such as alginate, chitosan, gelatin, and HA have been explored.^{74,139} Lee *et al.* used DNA polyaptamer hydrogels with Cas9/sgRNA to promote the controlled delivery of immune checkpoint inhibitors for cancer immunotherapy. The hydrogels are composed of two templates that contain PD-1 aptamers and prepared by rolling circle amplification containing complementary sequences of sgRNA to be cleaved by Cas9. The release of a programmed death receptor (PD-1) DNA aptamer promotes the activation of T-cells and reduces tumor growth.⁷² Furthermore, immune checkpoint inhibitors can be released with a hydrogel-based polypeptide vaccine to improve tumor immunotherapy. Song *et al.* developed an injectable PEG-b-poly(L-alanine) hydrogel with encapsulated granulocyte-macrophage colony-stimulating factor and anti-CTLA-4/PD-1 antibodies. The injectable hydrogel promoted the recruitment and activation of dendritic and T-cells. Moreover, the hydrogel vaccine led to the secretion of specific cytokines that reduced the growth of B16 melanoma tumors. These injectable biomaterials can be used as a sustained delivery platform with co-delivery of immunotherapeutics to promote immune cell activation and eliminate tumors.¹¹⁸

Cell Membrane-Mimicking Nanoparticles

Currently, one of the major limitations in the use of drug delivery nanoparticles in chemotherapy is low therapeutic efficacy and limited tumor penetration.⁵⁹ For that reason, developing biomimetic nanoparticles as drug delivery vehicles to promote an immunomodulatory response has been explored. These nanoparticles are composed of a cell membrane coating and can mimic specific cell types due to the surface functionalization of the originating cell's membrane proteins.^{16,23,60,67,119,126,143,146} An innovative system for targeting tumors is the design of paclitaxel-loaded polymeric nanoparticles coated with the cell membrane of a specific tumor cell. For example, Sun *et al.* fabricated cancer cell biomimetic nanoparticles from 4T1 murine triple negative breast cancer cell membrane-derived vesicles and paclitaxel-loaded polymeric nanoparticles composed of PCL. In contrast with other drug delivery systems, this class of biomimetic nanoparticle has a high specificity for targeting primary tumors.¹¹⁹ In conjunction with other materials, PEG has also been used to help create immunological responses in a drug delivery system. Lai and coworkers fabricated DSPE-PEG nanoparticles coated with macrophage plasma membranes as a functional macrophage mimic.⁷⁰ This approach showed minimal cytotoxicity and successfully allowed the nanoparticles to cross the blood brain barrier to selectively

accumulate at glioblastoma sites, making this an optimal delivery strategy for reducing tumor growth.¹⁴⁷

CONCLUSION

The development of biomimetic materials offers promising avenues of understanding tumor and immune cell behavior *in vitro* and *in vivo*. In this review, we have demonstrated how various materials and techniques are used to evaluate the phenotypic and functional changes in tumor and immune cells. Models composed of common biomaterials such as alginate and PEG, novel materials including DNA hydrogels and dECM, and new technologies like 3D bioprinting allow for recapitulating the complex tumor microenvironment and analyzing multiple variables that promote tumor progression.

Biomimetic materials and synthetic environments are being designed in order to replicate complex microenvironments of interest and answer relevant biological questions. Some biomimetic models, such as organoids, are being implanted *in vivo* to encourage a vascularized environment.⁸⁴ Other studies have also begun to incorporate hydrogels into bioreactors to better mimic physiological conditions.^{38,88,96} Despite the development of these biomimetic materials and new, innovative ways of using them, not all of the factors that influence tumor progression can be tested simultaneously. This necessitates the continued use of *in vivo* models to validate *in vitro* observations. The complexity of design and how closely these models will resemble living systems remains to be seen. Nonetheless, these biomimetic materials allow for the study of tumor cell crosstalk with immune cells, mechanotransduction, ligand density, and cell–ECM interactions, which can advance the development of more effective therapies leading to improved patient outcomes.

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FIGURE 1.

The tumor microenvironment and its components, including tumor cells, immune cells, stromal cells, ECM, and soluble factors.

FIGURE 2.
ECM-cell interactions.

FIGURE 3.

Normal tissue and tumor organoid model systems and uses. Tumor organoid model systems can be produced from tumors or by genetic modification of organoids generated from normal tissues. Organoids can be used to answer questions about cancer biology, cell–cell interaction studies, and therapy response mechanisms.¹³⁰

FIGURE 4.

The process of deriving decellularized ECM from various organs for hydrogel formation.
3,41,76,78,116

FIGURE 5.

Biomimetic materials have been developed to span the stiffness range observed in (a) the epithelial-to-mesenchymal transition (EMT) and (b) commonly studied tissues and organs.
9,19,45,108

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TABLE 1.

Components of the ECM.

Main components	Macromolecules	Function
Highly viscous proteoglycans ¹⁴⁴	Aggrecan	Interact with integrins
	Decorin	Hydration buffer
	Lumican	Mitigate shear stress
	Perlecan	
	Syndecan	
Insoluble collagen fibers ^{30,144}	Versican	
	Collagen I	Provide structure
	Collagen II	Cell adhesion
	Collagen III	
	Collagen IV	
Soluble multiadhesive proteins ^{143,144}	Collagen V	
	Laminin	Integrin binding
	Fibronectin	Cell growth
		Cell adhesion

TABLE 2.

Common biomimetic materials and their applications.

Biomaterial	Cancer	Application
Organoids		
Mammary epithelial cells	Breast	Normal tissue radiation effects on tumor-stromal and immune interactions ³⁹
Pancreatic cancer cells	Pancreatic	Tumor-stromal and tumor-immune interactions ¹²⁹
Decellularized ECM		
Bladder-derived	Skin, colon, breast	Tumor growth delay and immune signatures ^{137,147}
Mammary fat pad-derived	Breast	Radiation effects on tumor cell behavior, breast cancer proliferation, and invasion ³
Colorectal cancer-derived	Colon	Establishing the colorectal tumor microenvironment influence on macrophage-mediated cell invasion ^{100,101}
Breast cancer-derived	Breast	Evaluation of tumor development and progression ^{49,61,77,91}
Liver-derived	Liver	Determining how the mechanical properties of cirrhotic liver tissue alter tumor cell behavior ⁸¹
Human tumor-derived	Colon	Studying the mechanisms of chemotherapy resistance at different stages of malignancy ^{48,50}
Brain tissue-derived	Brain	Investigating glioblastoma cell invasion ⁶⁶
Colon tumor-derived	Colon	Evaluating tumor progression at different stages of tumorigenesis ¹⁰⁹
Xenograft-derived	Breast, liver, lung	Studying the behavior of cancer cells in crosslinked dECM ⁷⁹
Natural materials		
HA hydrogel	Breast	Stiffness effects on metastasis to the brain ⁹⁴
Collagen I, Matrigel	Breast	Recruitment of immune cells and their effect on the invasiveness of cancer cells ⁶³
Porous chitosan-alginate scaffolds	Prostate, breast, liver, brain	Evaluating cancer cell behavior on scaffolds mechanically mimicking cancer progression; investigating cancer stem cell enrichment ^{32,138}
Alginate-Matrigel	Breast	Effect of stiffness and ligand ratio on cell morphology and invasion ^{11,12}
Alginate-based	Lung, breast, pancreatic, neuroblastoma, pituitary	Co-culture model for a drug screening platform ^{22,27,46,71,92}
Synthetic materials		
PEG-b-poly(L-alanine) hydrogels	Breast	Linking chemical and mechanical ECM characteristics to tumor cell behavior ¹⁸
PA hydrogels	Thyroid, renal, breast	Stiffness and topography effects on normal and cancer cells ^{86,106,107}
Alginate-PCL nanofibers	Liver	Cancer stem cell enrichment ⁵¹