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Shooting for the moon: using tissue-mimetic hydrogels to gain new insight on cancer biology and screen therapeutics

Samantha E. Holt, Department of Biomedical Engineering, Texas A&M University, College Station, Texas, USA
E. Sally Ward, Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, Texas, USA
Raimund J. Ober, Department of Biomedical Engineering, Texas A&M University, College Station, Texas, USA; Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, Texas, USA
Daniel L. Alge, Department of Biomedical Engineering, Texas A&M University, College Station, Texas, USA; Department of Materials Science and Engineering, Texas A&M University, College Station, Texas, USA

Address all correspondence to D. L. Alge at **dalge@tamu.edu**

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Abstract

Tissue engineering holds great promise for advancing cancer research and achieving the goals of the Cancer Moonshot by providing better models for basic research and testing novel therapeutics. This paper focuses on the use of hydrogel biomaterials due to their unique ability to entrap cells in three-dimensional (3D) matrix that mimics tissues and can be programmed with physical and chemical cues to recreate key aspects of tumor microenvironments. The chemistry of some commonly used hydrogel platforms is discussed, and important examples of their use in tissue engineering 3D cancer models are highlighted. Challenges and opportunities for future research are also discussed.

Introduction

The National Cancer Moonshot, announced in January 2016, has been compared with the more literal "moonshot" of the 1960s to send a man to the moon and guarantee his safe return within a decade. One important similarity between these two initiatives is that, much like models and simulations were critical to prepare the Apollo astronauts for different aspects of their mission, well-designed models will play an important role in the success of researchers in the Cancer Moonshot. Importantly, our scientific understanding of cancer has expanded in the past 35 years largely because of advancements in modeling cancer, and we now know that cancer is a complex disease encompassing a remarkable level of case-by-case and intratumor heterogeneity.[1] However, as our understanding of the tumor microenvironment (i.e., the mechanical, chemical, structural, and biological factors that surround the tumor and influence its progression and metastasis) has increased, it is clear that more sophisticated and reproducible tumor models are needed to interrogate cancer biology and improve the efficiency of the drug development pipeline. But how does one improve upon existing tumor models?

Traditionally, cancer biologists have utilized in vitro approaches in which cancer cells are grown on plastic dishes and in vivo methods in which tumors are grown in animals, mostly mice. However, these methods have important limitations, and tissue engineering has recently emerged as a powerful alternative strategy. While tissue engineering is generally thought of as a method for repairing the body, its more immediate promise is arguably in creating in vivo-like disease models for in vitro experimentation and drug screening. The concept of tissue engineering cancer models in particular has gained traction in recent years, and in 2014 the National Cancer Institute (NCI) convened a strategic workshop titled "Biomimetic Tissue-Engineered Systems for Advancing Cancer Research".[2] There is currently an NIH request for applications by the same name (PAR-17-171), which seeks to catalyze the development of innovative and well-characterized in vitro and ex vivo systems available for cancer research through collaborative projects that engage the fields of regenerative medicine, tissue engineering, biomaterials, and bioengineering with cancer biology.

Tissue-engineered technologies will undoubtedly play an important role in the Cancer Moonshot. Thus, the objective of this prospective is to discuss the state-of-the-art, challenges, and emerging trends in the development of tissue-engineered models for cancer research. Special emphasis will be given to approaches that are based on hydrogels. Importantly, hydrogels are the most tissue-like class of materials and are widely used for fundamental studies of cell biology in a biomimetic three-dimensional (3D) environment. We will briefly review the chemistry of the most widely used hydrogel platforms and then highlight some important examples of their application in creating tumor models for studying cancer biology and screening therapeutics, specifically for melanoma, glioblastoma, and breast cancer. We will also discuss some of the key challenges faced in the use of these platforms, particularly

with regards to molecular biology characterization. Finally, we will provide an outlook on the future of the field by discussing emerging trends and opportunities for further development and impact.

Critical need for tissue-engineered models

To fully appreciate how tissue-engineered cancer models can enable success in the Cancer Moonshot, we must first consider the limitations of conventional tumor models. The development of cell culture platforms and immortalized cell lines in the 1950s set the stage for the study of the genetic and proteomic features of cancer cells. Since the creation of the first immortalized cancer cell lines, several genetic features of cancer and therapeutic strategies have been tested and validated in cell lines. The immortalization process also enabled the discovery of key genetic mutations that enable the endless replicative potential of cancer cells, including the role of telomerase in the growth of cancer cells.[3] However, traditional two-dimensional (2D) tissue culture presents several limitations as a model for tumors. First, cancer cells are grown in total isolation, without systemic cues or cues from the stroma that are present in whole organisms. Second, the physical environment of the cell culture on tissue-culture-treated polystyrene dishes is dramatically different from a tumor in vivo. The elastic modulus of polystyrene dishes is approximately 1 GPa, which is orders of magnitude higher than most tissues in the body. It is well known that substrate modulus influences cell phenotype via mechanotransduction. For example, Leight et al. showed that TGF-β (transforming growth factor beta) induces EMT (epithelial-to-mesenchymal transition), a key step in metastasis, in cells cultured on top of rigid but not soft hydrogel substrates.[4] Cells in 2D culture are also artificially polarized in that one side of the cell body is attached to the dish, while the other side of the cell interacts with the culture media and receives soluble cues. For these reasons, many cancer researchers have adopted alternative in vitro models such as spheroids and Matrigel culture.

Animal models are at the opposite end of the spectrum and are widely used for in vivo cancer research. Tumor xenograft models, in which human cancer cells are implanted into immunocompromised mice, provide greater physiologic relevance than culturing cells on a plastic dish. However, they fail to recreate tumor–immune cell interactions and the initial phases of tumor growth, and results can vary based on implantation site. Genetically engineered, immunosufficient mouse models with induced tumor-initiating mutations are an alternative in vivo platform for studying human cancer, but they are limited to certain cancer types. Moreover, specific mutations and tumor development can be slow and inconsistent and fail to recapitulate tumor heterogeneity, which has necessitated the development of conditionally activated and chimeric genetically engineered models.[5] Methods and challenges for development of xenograft and genetically engineered mouse models of cancer have been reviewed comprehensively by Sharpless and DePinho.[6] It is critical to recognize that even a well-designed animal model can fail to accurately recapitulate human physiology. Fundamental species-specific differences in cancer susceptibility and intracellular processes such as genome repair and cell cycle arrest have motivated the search for better models to recapitulate specifically human biology.[7] Difficultly in comparing parameters for success in mouse models to success in human subjects is one of the contributing factors in the high failure rate of drug candidates in clinical trials.[8] Animal models are also expensive to create and maintain, and because of their complexity it is more difficult to elucidate the molecular mechanisms of cancer. Specific cell–cell interactions of interest are difficult to image in vivo, and the protein-level interactions that enable them are practically impossible to visualize. Thus, most animal studies are limited to reporting larger scale effects, such as tumor volume, number of tumors or metastases, and, of course, survival.

Tissue-engineered cancer models are a middle ground that emerged from research using 3D culture models in which cells were embedded in an extracellular matrix (ECM) material. Importantly, tissue-engineered 3D cultures recapitulate aspects of the in vivo environment better than conventional 2D culture methods. Moreover, they are simpler, cheaper, and provide more control over experimental conditions than animal models, which can enable greater insight into the disease mechanisms (Fig. 1). One of the most popular 3D culture materials used by biologists is Matrigel, which is a basement membrane gel made from the secreted ECM of Engelbreth–Holm–Swarm sarcoma cells. Notably, Matrigel has been used as a 2D and 3D substrate for the culture of various cancer cells. Seminal works by the Bissell laboratory and the Brugge laboratory have shown that non-cancerous breast epithelial cells form hollow spheres that mimic the physiology of breast acini in 3D culture in Matrigel,[9,10] whereas breast epithelial cancer cell lines and primary tumor cells form disorganized structures.[9] Matrigel is also commonly used in invasion assays to assess the invasiveness of tumor cells.[11] Despite the benefits and progress that has been achieved with Matrigel, this material is ill defined and is well known to display batch-to-batch variability.[12] Moreover, the composition of Matrigel is not necessarily reflective of the ECM composition of all tissues. Collectively, these limitations have led to interest in using tissue-mimetic hydrogels that provide greater control and reproducibility over the tumor microenvironment. In the following sections, we will review tissue-mimetic hydrogel platforms and highlight examples of their use in cancer research.

Overview of tissue-mimetic hydrogels

A comprehensive review of the polymers and crosslinking chemistries that can be used to create tissue-mimetic hydrogels is beyond the scope of this review. Instead, we will focus on the most widely used materials and those which are discussed in our selected examples of tissue-engineered 3D cancer models (Fig. 2).

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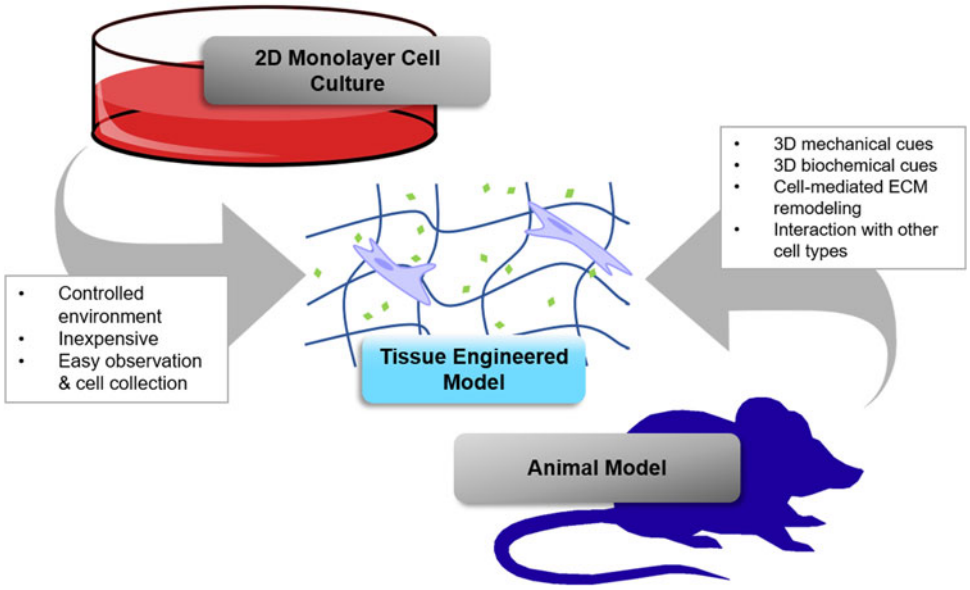

Figure 1. The advantages of a tissue-engineered model of cancer. The tissue-engineered 3D culture model shares advantages with 2D conventional monolayer cell culture models and animal models. While this is not a comprehensive list of advantages of tissue-engineered models, it highlights some of the common considerations and highlights what each model has in common with tissue-engineered models.

Hydrogels consist of hydrophilic polymers that are crosslinked, either chemically or physically, to form a 3D network that imbibes large amounts of water.[13] They can generally be classified as natural or synthetic, depending on the polymer used to make them, and both types have been used as tissue-mimetic platforms for cancer research. Natural hydrogels can be further sub-divided into ECM-derived and non-ECM derived. Moreover, a diverse toolkit for chemical crosslinking of both natural and synthetic hydrogels exists.[14–17] The most commonly used crosslinking reactions, and consequently the ones used most frequently to engineer 3D cancer models, are: (1) (meth)acrylate polymerization, (2) Michael-type thiol-ene click reactions, and (3) thiol-ene photoclick reactions. (Meth) acrylate polymerization occurs via a free radical-mediated chain-growth mechanism and is usually photopolymerized with cytocompatible initiators such as Irgacure 2959 or lithium acylphosphinate.[18] Michael-type thiol-ene click reactions are useful because they enable facile incorporation of cysteine-containing peptides to provide cell adhesion motifs (e.g., RGD, YIGSR, IKVAV) and enzyme-sensitive crosslinks for cell-mediated remodeling.[15,19] They proceed under mildly basic conditions and require an electron deficient alkene such as a (meth)acrylate, vinyl sulfone, or maleimide. Thiol-ene photoclick reactions offer the same advantages as the Michael-type thiol-ene reactions for peptide incorporation, but with the added advantage of spatiotemporal control via photopolymerization. The electron rich, ring-strained alkene norbornene is most commonly used as it undergoes step-growth rather than mixed-mode polymerization with thiols. Importantly, multiple studies have shown that thiol-ene photoclick crosslinking is more cytocompatible than (meth)acrylate photopolymerization.[20,21]

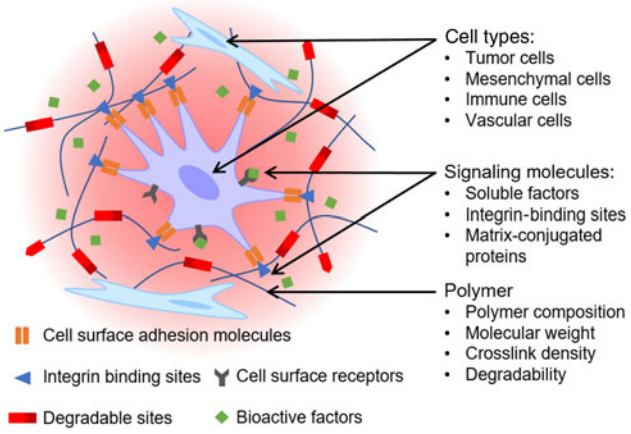

Figure 2. Design features of a tissue-engineered 3D culture tumor model. Tuning hydrogel crosslink density can change the mechanical stiffness and the ease with which cells can migrate. Biochemical cell signaling molecules, including different cell adhesion moieties, growth factors, and cytokines, can also be incorporated into the model at controlled concentrations to simulate microenvironmental signaling. A variety of cell types can be used to create co-culture models to study the interaction between different cell types in the tumor microenvironment.

Type I collagen (typically bovine or rat) is a popular commercially available ECM-derived natural hydrogel material that provides encapsulated cells with a nanoscale fibrillar network that they can attach to via integrin-binding motifs and

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remodel enzymatically via matrix metalloproteinases (MMPs). The mechanical properties of collagen hydrogels, which self-assemble in approximately 30 min at physiologic conditions, can be manipulated by the collagen concentration or through a variety of chemical and physical crosslinking strategies (e.g., with formaldehyde, genipin), as reviewed by Parenteau-Bareil et al.[22] However, these materials allow limited decoupling of biochemical and mechanical signals, and batch-to-batch variability is a concern. Consequently, many tissue engineers utilize gelatin in lieu of collagen. Gelatin, which is denatured collagen, also provides cell adhesion cites and can be remodeled by encapsulated cells. The most common implementation is gelatin-methacrylamide, or GelMA, which is prepared by functionalizing lysine amino groups in gelatin (e.g., with methacrylic anhydride).[23] GelMA hydrogels are generally photopolymerized, and their mechanical properties can be tuned by manipulating both the percent functionalization and the polymer concentration.

Other natural polymers that have been used to synthesize tissue-mimetic hydrogels for cancer models include hyaluronic acid and alginate. Hyaluronic acid is a polysaccharide found in the ECM of many tissues. Similar to gelatin, hyaluronic acid can be functionalized with polymerizable groups to varying degrees to control the crosslink density and properties.[24] For example, (meth)acrylates have been added to enable both free radical chain-growth polymerization and Michael-type thiol-ene crosslinking, the latter of which has been leveraged to incorporate integrin-binding peptides and MMP-cleavable crosslinkers. Alternatively, some platforms have been based on thiolated hyaluronic acid, which is then crosslinked by thiol-ene Michael-type addition reaction (e.g., with a diacrylate polymer). Alginate is a polysaccharide derived from seaweed that can be used to synthesize physically and chemically crosslinked hydrogels.[25] Because it is not an ECM-derived material, it lacks cell adhesive moieties, which provides an opportunity to introduce specific integrin-binding peptides. Alginate is most often physically crosslinked with divalent cations, namely calcium, in which case the calcium concentration controls crosslink density and mechanical properties. However, similar to hyaluronic acid, alginate can also be modified with polymerizable chemical groups such as (meth)acrylates.[26]

The most widely used synthetic polymer for engineering tissue-mimetic hydrogels is poly(ethylene glycol) (PEG). PEG is considered to be a "blank slate" biomaterial due to its resistance to protein adsorption.[14,27] Consequently, cells are unable to bind to and interact with PEG hydrogels unless they are modified with bioactive components, which makes them a powerful platform for investigating how specific cues in the cancer microenvironment influence signaling pathways and susceptibility to treatment. Myriad strategies for synthesizing PEG hydrogels via chemical crosslinking have been reported in the literature. The most common strategy has been to end-functionalize linear PEG with (meth)acrylates and then perform free radical chain-growth polymerization. However, the Michael-type thiol-ene and thiol-ene photoclick reactions have become extremely popular in recent years because bioactive peptide functionalization is simpler with these chemistries. These step-growth polymerizations, which utilize multi-arm PEG star polymers synthesized by living polymerization methods, are also believed to produce more uniform polymer networks. Typically, Michael-type thiol-ene crosslinking is performed using PEG macromers functionalized with vinyl sulfone[28] or maleimide[29] groups, since the acrylate–thiol reaction produces esters with neighboring sulfides that are readily hydrolyzed under physiologic conditions.[30] The thiol-ene photoclick reaction is performed using norbornene-functionalized PEG.[31] Regardless of the crosslinking chemistry, PEG hydrogel crosslink density and physical properties can be tuned by changing the PEG molecular weight or concentration.

Examples of tissue-engineered cancer models

The following sections will review selected examples of models of melanoma, glioblastoma, and breast cancer from the recent literature. While tissue-engineered models of other cancers have been developed (e.g., non-small cell lung cancer,[32,33] PDAC (pancreatic ductal adenocarcinoma),[34] hepatocellular carcinoma,[35–37] oral squamous cell carcinoma,[38] prostate cancer,[39,40] ovarian cancer[41–43]), these examples were selected to highlight the role of materials innovation in developing hydrogel-based 3D tissue-engineered models.

Melanoma

Melanoma is the deadliest form of skin cancer, and the 19th most common form of cancer worldwide.[44] It is a tumor of melanocytes, the pigment-producing cells of the skin. Incidence is highly linked to exposure to UVA and UVB radiation from the sun or tanning beds. Overall, the clinical burden of melanoma is increasing. In 2016, the lifetime risk of an American developing melanoma was 1 in 54,[45] and incidence rates of melanoma are increasing faster than any other solid tumor type.[44] However, it should be noted that the incidence of pre-invasive melanoma is rising at a higher rate than invasive melanoma, which may indicate more effective methods of detection and may decrease mortality rates long term.[45] Additionally, melanoma is unusually common compared with other forms of cancer in young people. Incidence rates have been increasing in particular in young Caucasian women, with one study showing an eightfold increase from the 1970s to the 2000s.[46] It is thought that this trend is attributable to behavioral risk factors, in particular the use of UV tanning beds.

Several studies on tissue-engineered models of melanoma have been reported by the Anseth research group.[47–49] These have been based on the PEG thiol-ene photoclick hydrogel platform that is enzymatically degradable and incorporates a cell-adhesive RGD-containing peptide. As noted earlier, this platform allows for control of matrix stiffness by modulating

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the PEG molecular weight as well as the concentrations of PEG and crosslinker. In a fundamental study of cell–material interactions, Singh et al. leveraged this control and observed an effect of matrix stiffness in 2D culture on the proliferation rate of WM239A cells, a metastatic melanoma cell line.[48] They also made a 3D culture model by encapsulating WM239A cells in the PEG hydrogel and then surrounding them with human dermal fibroblasts seeded in a collagen gel for indirect co-culture. The WM239A cells proliferated and formed clusters in 3D culture, though the cluster size was significantly reduced in the fibroblast co-culture. This may suggest a realistic tumor–stroma interaction, as healthy fibroblasts are thought to inhibit tumor formation.[50] However, after 4–5 days in co-culture, the WM239A cells appeared to protrude and invade into the surrounding hydrogel. Transformation of fibroblasts to a tumor-promoting phenotype is well reported in tumor biology research,[50] which may explain this switch in this model. Altogether, these findings suggest that matrix stiffness influences tumor cell proliferation and 3D culture can, to an extent, recapitulate a physiologically relevant phenotype in tumor cells that includes interactions with stromal fibroblasts.

With that precedent, Tokuda et al. used a similar tissue-engineered melanoma model based on PEG thiol-ene click hydrogels to study the effects of BRAF-kinase inhibition.[47] They found that a different metastatic melanoma cell line, A375, exhibited less sensitivity to the small-molecule BRAF inhibitor PLX4032 when cultured on soft but not stiff gels (Young's modulus ~0.6 kPa versus 1.6 and 13.1 kPa), while the early-stage radial growth melanoma line WM35 showed higher sensitivity to the same drug. Similar trends emerged when they advanced to 3D culture.[49] Briefly, PLX4032 treatment induced higher levels of caspase 3 activity, a marker of apoptosis and an indicator of drug efficacy, in WM35 cells grown in soft gels (Young's modulus ~0.4 kPa) compared with conventional 2D culture on tissue culture-treated polystyrene. In contrast, A375 cells were even less sensitive to the drug in 3D culture conditions than in 2D hydrogel culture. Moreover, 3D spheroids of WM35 cells encapsulated in the same PEG hydrogels appeared less sensitive to PLX4032, as the spheroids showed significantly higher relative DNA content after treatment compared with single-cell 3D encapsulation conditions, suggesting higher proliferation. These findings suggest that early stage radial growth melanoma cells and late stage metastatic melanoma cells respond to PLX4032 differently, and that the biophysical cues of the model microenvironment influence this response.

Leight et al. went a step further by incorporating a fluorogenic sensor for MMP activity into the PEG thiol-ene photoclick hydrogels and testing the effects of multiple BRAF inhibitors on several melanoma cell lines.[51] The fluorogenic sensor enabled quantitative analysis of MMP expression. Their sensor, which was covalently attached to the hydrogel network, consisted of an MMP-cleavable peptide (the same GPQGIWGQ sequence) flanked with a fluorophore (fluorescein) and a quencher (dabcyl). When the peptide was cleaved by cell-secreted MMPs, separation of the fluorophore and quencher resulted in a fluorescent signal that was used to quantify the activity of cell-secreted MMPs in the gel. Four melanoma cell lines were tested, representing different points in tumor progression: WM35 (a radial growth line mentioned earlier), WM239A and A375 (two metastatic lines mentioned earlier), and WM115 (a vertical growth line). They also tested four small molecule BRAF-inhibitors: PLX4032 (tested by Tokuda et al.), sorafenib, AZD6244, and CI-1040. Importantly, they found that in 3D culture the metastatic line WM239A was highly drug resistant, with no significant change in metabolic activity, and exhibited higher levels of MMP activity compared with the control for all four drugs tested.[51] In contrast, the A375 metastatic cell line exhibited lower metabolic activity after treatment with all four drugs but differences in levels of MMP activity. While A375 cells treated with sorafenib showed no significant increase in MMP activity compared with the control, PLX4032 cells showed significantly higher MMP activity (Fig. 3).[51] Morphologically, PLX4032-treated A375 cells were elongated, with F-actin-rich protrusions into the surrounding matrix.[51] In addition, PLX4032 treatment increased both the number and the speed of migrating A375 cells in the matrix (Fig. 3).[51] These results suggest that while PLX4032 may be effective in reducing tumor cell metabolism, it may do so at the cost of increasing the invasive and migratory characteristics of the cells, especially in metastatic melanoma. This result would be nearly impossible to observe in vivo, which shows the promise of using tissue-engineered models for drug screening. It may also explain why melanoma patients taking PLX4032 were observed to relapse with metastatic lesions in clinical trials.[52] However, it should be noted that this model omitted the tumor stroma, which is a limitation of the study since tumor-associated endothelial cells, fibroblasts, and macrophages can be important sources of MMPs and contributors to tumor dissemination. Thus, to get an accurate picture of how drug treatment influences matrix remodeling in the tumor microenvironment, future work should also incorporate stromal cells in the model.

Glioblastoma

Glioblastoma is the most aggressive form of brain cancer. Glioblastomas arise from the astrocytes of the brain and are highly metastatic, though they rarely metastasize outside the central nervous system. While it is one of the rarer forms of cancer, it is nonetheless deadly. The 5-year survival rate in 2010 was <5%.[53] Recurrence after the first round of treatment is common, with virtually all patients experiencing disease progression within 7–10 months post-treatment.[54] The median survival post-diagnosis is 15 months.[53] Some genetic and environmental risk factors for glioblastoma have been studied, but none have been identified that account for a large portion of cases.[53] Its aggressive and rapid metastasis and low sensitivity to available therapies make glioblastoma extremely difficult to treat and provide a strong impetus for tissue-engineering glioblastoma models.

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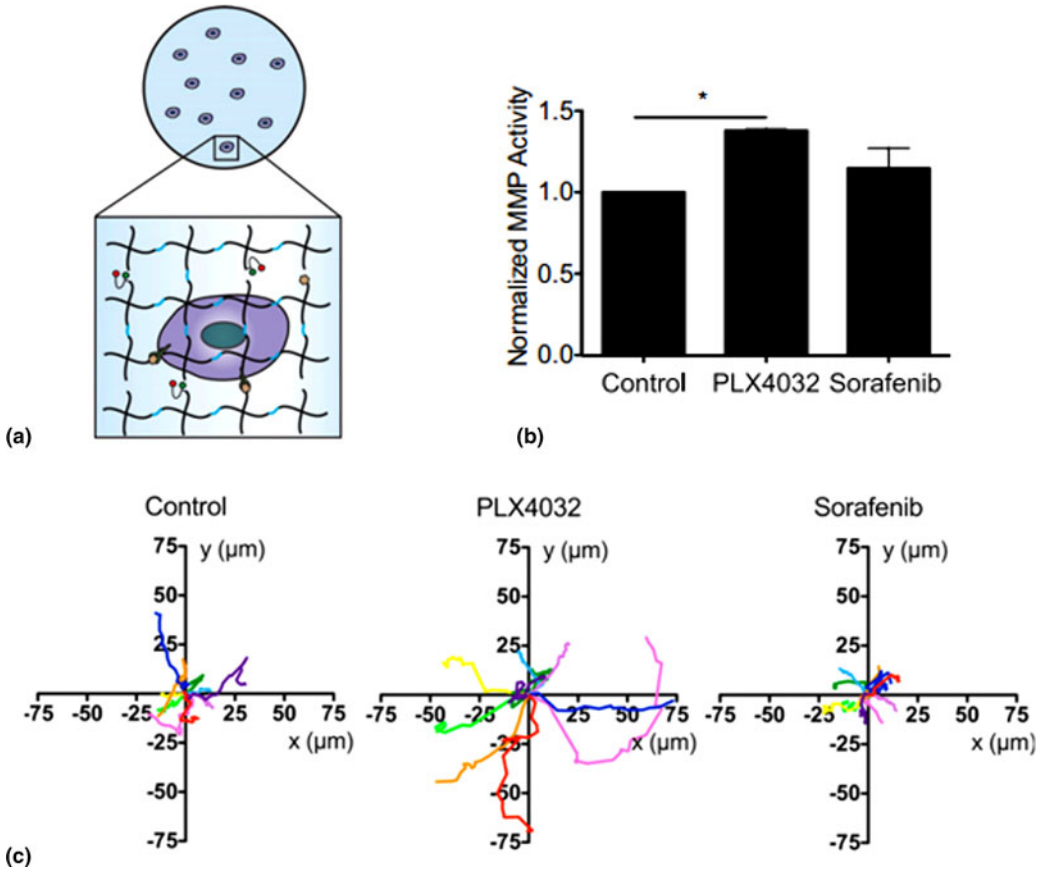

Figure 3. Engineered hydrogel scaffold enables real-time visualization and quantitation of MMP activity in tumor models. (a) Schematic representation of a cancer cell encapsulated in an MMP-degradable PEG-peptide hydrogel scaffold modified with a fluorogenic sensor of MMP activity. Melanoma cells treated with small-molecule BRAF inhibitor PLX4032 showed (b) increased MMP activity and (c) increased migration over melanoma cells treated with a different small-molecule BRAF inhibitor, sorafenib. Adapted from Leight et al.[51] with permission from the National Academy of Sciences, USA.

A significant focus of glioblastoma research using tissue-mimetic hydrogels has been elucidating the connection between matrix stiffness and glioblastoma cell migratory phenotype. Ulrich et al. sought to accomplish this using polyacrylamide gels surface functionalized with plasma fibronectin for 2D culture substrate.[55] Using hydrogels with Young's moduli between 0.08 and 119 kPa, they found that cells from multiple glioma lines (U373-MG, U87-MG, U251-MG, SNB19, C6) isolated from both rat and human tumors exhibited greater spreading, faster migration, and faster proliferation on stiffer gels. Moreover, they showed that these effects could be attenuated by inhibiting molecular signaling pathways involved in mechanotransduction and cytoskeletal remodeling, including non-muscle myosin II and ROCK.[55] Pathak and Kumar also used a similar polyacrylamide gel platform to study glioblastoma cell migration in microchannels made using photolithography and found that the previously observed effects of stiff substrates on migration speed were enhanced in the microchannels, with cells in thinner microchannels migrating faster.[56] The result corroborating that glioblastoma migration speed is increased on stiffer substrates is particularly interesting, since the opposite trends have been observed in 3D models.

A number of studies have used natural polymer-based hydrogels to create tissue-engineered 3D glioblastoma models to investigate tumor angiogenesis. Verbridge et al. used RGD-functionalized alginate hydrogels for 2D culture and 3D encapsulation under hypoxic conditions to study angiogenic signaling in U87 MG (also known as U87) glioblastoma cells as well as OSCC-3 oral squamous cell carcinoma cells.[57] They found that U87 cells exhibited elevated pro-angiogenic signaling via cytokine IL-8 in gels in both ambient and hypoxic conditions. Nguyen et al. investigated the use of a GelMA hydrogel "microwell" as a potential platform for high-throughput testing of tumor cultures and examined endothelial cell migration toward U87 glioblastoma cell spheroids.[58] Human umbilical vascular endothelial cells (HUVECs) were found to migrate through the gelatin matrix that held a U87 cell spheroid grown in a well at the center of the gel, suggesting angiogenesis.[58] Subsequent testing of the anti-angiogenic drug TNP-470 in this same platform showed fewer HUVECs

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in the vicinity of the glioblastoma spheroids, suggesting inhibition of angiogenic-like endothelial cell migration.[59] While additional molecular and cellular biological analyses are needed to verify that these models recapitulate the angiogenic signaling from tumor cells observed in vivo, microwell platforms like this one could accelerate the identification of novel angiogenesis inhibitors for clinical testing.

Tissue-engineered models are also being used to investigate the role of hyaluronic acid in the glioblastoma ECM, since this biomolecule is present at higher concentrations in the brain than most other tissues.[56] Ananthanarayanan et al. used hydrogels comprising hyaluronic acid methacrylate functionalized with cysteine-containing RGD peptides and crosslinked with dithiothreitol in a Michael-type addition reaction to investigate the effects of matrix stiffness on glioblastoma phenotype.[60] They found that spheroids of glioblastoma cells displayed a more invasive phenotype marked by more infiltration into the surrounding matrix from the spheroid boundary in 3D culture in soft (150 Pa) gels compared with stiff gels. Importantly, this result was in contrast to 2D culture experiments on the hydrogels, in which the glioblastoma cells displayed more spread morphology, faster migration, and more proliferation on stiff gels (1 and 5 kPa), similar to what was observed by Ulrich et al.[55] These conflicting responses to matrix stiffness in 2D and 3D models may indicate that the dimensionality of the culture has a significant impact on the cellular response. However, it could also be an indication of a limitation of the 3D platform. The higher crosslinking and tighter mesh of the stiffer gels may be more difficult for the cells to migrate through, which may also explain why 3D migration was faster in a softer gel while the reverse was shown in 2D culture.

Hydrogels containing mixtures of hyaluronic acid and other polymers have also been investigated. Rao et al. used composite hydrogels comprising mixtures of type I collagen and thiolated hyaluronic acid to examine the effects of ECM composition on primary human glioblastoma-derived cell motility.[61] They found that higher concentrations of hyaluronic acid correlated with a slower migration speed and more rounded cell morphology, as well as a higher elastic modulus of the gels.[61] The Harley group has utilized hydrogels comprising a combination GelMA, acrylated PEG, and methacrylated hyaluronic acid crosslinked via radical-initiated chain polymerization.[62–64] In one study, they found that increasing the relative amount of hyaluronic acid in the gel induced U87 glioblastoma cells to increase expression of genes associated with malignancy, including the angiogenic factor VEGF and the hypoxia marker HIF-1.[62] Interestingly, wild-type U87 cells showed decreasing levels of MMP-2 expression, a marker for invasiveness, in gels with higher concentrations of hyaluronic acid, while U87 cells modified to overexpress the epidermal growth factor receptor (EGFR) displayed the opposite trend. Pedron et al. suggested that this effect may be due to the "more malignant" phenotype of the EGFR+ cells, as EGFR overexpression is commonly seen in more metastatic forms of glioblastoma.[62] In a separate study, they demonstrated that a less degradable matrix, with a higher ratio of PEG tetra-acrylate to GelMA, also increases U87 expression of VEGF, HIF-1, and MMPs in 3D culture,[64] further demonstrating that microenvironmental composition is important in tissue-engineered glioblastoma models. Future studies may include comparisons of drug response in matrices with different concentrations of hyaluronic acid. Additionally, correlating features of the tumor cell phenotype and the composition of the surrounding matrix could be valuable in determining prognosis and treatment in a clinical setting.

PEG-based thiol-ene photoclick hydrogels have been used by the Yang research group to create 3D glioblastoma models.[64,65] Their hydrogels were functionalized with RGD adhesive peptide, crosslinked with a mixture of PEG-dithiol and MMP-degradable crosslinker (GPQGIWGQCK), and also contained sodium hyaluronate. In their initial work, the sodium hyaluronate was physically trapped in the gel after crosslinking, and they studied the effects of matrix stiffness by creating soft (1 kPa) and stiff (26 kPa) hydrogels. They found that U87 cells encapsulated in soft gels showed greater proliferation, whereas cells in stiff gels exhibited denser aggregation as well as cellular protrusions into the surrounding gel, suggesting a propensity to invade.[65] These results are similar to previously referenced results in 3D culture platforms, but conflict with the rates of proliferation seen in 2D culture studies. However, the changes in cellular morphology may indicate a more invasive phenotype, which was further investigated through gene expression data. Cells in stiff gels showed increased expression of HA synthase 1 and MMP-1 genes and downregulation of hyaluronic acid synthase 2 and MMP-9 genes, all associated with matrix remodeling, compared with cells in soft gels,[65] and increased expression of Hras, RhoA, and ROCK1, which are associated with mechanotransduction.[65] Overall, these data suggest that the substrate stiffness influences the mechanosensing and ECM remodeling response of the glioblastoma cells. In a separate study using the same material platform, but with thiolated hyaluronic acid that can be chemically conjugated to the matrix, the gel stiffness was maintained between 1.2 and 2.0 kPa but the degradability of the matrix was adjusted by varying the ratio of MMP-degradable crosslinker peptide to PEG-di-thiol.[66] They observed that U87 cells in degradable matrices (50% or 100% degradable crosslinker) exhibited a spread morphology and actin-dense structures extending into the surrounding gel, while cells in the non-degradable gels maintained a round morphology.[66] Interestingly, cells in all gels exhibited increased expression of MMP-1 and MMP-9 as well as significantly increased expression of hyaluronic acid synthase 2 compared with pre-encapsulation levels, but the differences in MMP and hyaluronic acid synthase expression between gels of different levels of degradability were not significant. In addition, gene expression levels in these gels, normalized to pre-encapsulated gene expression, were similar to the soft gels of the previous study. Taken together, these results suggest that matrix stiffness may influence the nature of ECM

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remodeling in glioblastoma more strongly than hyaluronic acid presentation (free versus matrix conjugated) or matrix degradability.

The aforementioned studies utilized homogenous hydrogels of varying composition, but there has also been interest in incorporating spatial gradients of microenvironmental cues in 3D glioblastoma models to recapitulate tumor heterogeneity. For example, Pedron et al. used a microfluidic gradient mixing device to create gradients in chain-growth photopolymerized GelMA hydrogels used for 3D cell culture.[63] The degree of methacrylation of the gelatin macromer, concentration of methacrylated hyaluronic acid added, and cell number were varied throughout bulk gels. They observed that cell proliferation of U87 cells increased in regions containing gelatin with a higher percent methacrylation, and consequently a higher stiffness. MMP expression levels as well as gene expression levels for fibronectin, HIF-1, and VEGF were also observed to vary across the gradient.[63] In gels with varying concentration of hyaluronic acid, U87 cells displayed a tendency to aggregate more and showed decreased cell proliferation in regions with high hyaluronic acid concentrations. Varying the concentration of cells in the gel also created a gradient of pro-invasive and pro-angiogenic gene expression, as expression of genes for hypoxia marker HIF-1, pro-angiogenic growth factor VEGF, and ECM protein fibronectin all increased with increasing cell concentration. Since ECM density, ECM composition, and cellular composition of tumors can vary spatially, it may be necessary to use spatiotemporal patterning approaches like this to properly account for tumor heterogeneity.

Ultimately, tissue-engineered glioblastoma models may provide a better testing platform for cancer therapeutics and lead to better treatment strategies for patients. Work by Jiglaire et al. supports this promise.[67] They used a commercially available hydrogel platform comprising a mixture of thiolated hyaluronic acid and thiolated gelatin crosslinked with PEG-diacrylate via a Michael-type addition for 2D and 3D culture of U87 glioblastoma cells, primary murine glioblastoma cells, and primary human patient-derived glioblastoma cells. They investigated the cell responses to radiation therapy as well as three common cancer chemotherapeutics: cisplatin, carmustin, and temozolomide. Notably, U87 cells showed more resistance to cisplatin, carmustin, and radiation in 3D culture than in 2D culture, but they were more sensitive to temozolomide in 3D culture than in 2D culture. While primary patient-derived cells showed variable responses to all treatments, cells from the same tumor often differed in response to treatment between 2D and 3D culture and generally displayed more sensitivity to therapy in 2D culture.[67] While further studies are needed before this effect of 3D culture can be generalized to all patient-derived glioblastomas, this study suggests that 3D tumor models may prove useful for drug screening by simulating resistance to therapy. It would be particularly interesting to see similar studies performed using the tissue-engineered glioblastoma models described earlier, as these could provide greater insight on how the tumor ECM and biophysical cues affect drug efficacy, similar to what has been done with the melanoma models.

Breast cancer

Breast cancer is the most common form of cancer in American women, and an estimated 249,260 new cases were diagnosed in 2016 in women and men in the USA.[68] Breast cancer is a clinical and research priority worldwide; 1.7 million women across the globe were diagnosed with breast cancer in 2012.[69] Overall, 5-year survival rates are good for breast cancer, with an average of 89.7% from 2006 to 2012, according to the National Cancer Institute's SEER data. Early detection methods have been critical in raising this survival rate from 75.2% in 1975. However, breast cancers detected post-metastasis remain deadly, with a 5-year survival rate of 26.3%. Early detection with mammography and screening does carry the risk of overdiagnosis, treatment when the disease would not have caused symptoms within the patient's lifetime. It is estimated that overdiagnosis has affected 1.3 million women in the USA in the past 30 years, since the implementation of mammography.[70] The advent of mammography has allowed for the detection of pre-cancerous lesions before the tumor breaches the basement membrane of the tissue, known as ductal carcinoma in situ (DCIS). DCIS presents a clinical challenge because there are no known markers of future metastasis.

The seminal work in 3D cancer models demonstrated that breast epithelial and epithelial carcinoma cells can form acini-like structures as they proliferate in Matrigel,[9,10] with highly ordered structures forming from non-cancer cells and markedly more disordered structures forming from malignant cells.[9] Understanding how microenvironmental cues lead to this behavior has been a major focus in tissue-engineered breast cancer models. As one example, Pradhan et al. utilized fibrinogen-conjugated PEG diacrylate crosslinked via a radical-initiated chain polymerization to encapsulate breast cancer cells.[71] They adjusted the stiffness of the gels from 3.2 ± 0.5 kPa to 5.4 ± 0.5 kPa to 9.0 ± 1.4 kPa (Young's modulus) by introducing increasing concentrations of PEG diacrylate that was not fibrinogen-conjugated to the gel and observed that two breast cancer cell lines, MCF-7 and SK-BR-3, formed cell clusters after 15 days of culture and had comparable morphologies across gels of differing stiffness. These cells formed orderly spherical cell clusters, similar to the acini-like structures observed in Matrigel.[9,72] However, MDA-MB-231 breast cancer cells, which are noted for their aggressive, invasive phenotype, did not cluster in the gels but rather showed a more disordered, spread morphology, with protrusions into the surrounding matrix.[71] Collectively, these results suggest that the engineered PEG-fibrinogen platform is a suitable surrogate for Matrigel in breast cancer studies. Building on this work, a subsequent study used the PEG-fibrinogen platform to produce MCF-7 breast cancer cell spheroids via microsphere encapsulation in the hydrogels.[73] They found that this approach resulted in greater uniformity and induced a more cancer-like cell morphology (i.e., less apico-basal polarity, more cellular and

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nuclear atypia, elevated nuclear to cytoplasmic ratio and nuclear density, and reduced cell–cell junction length) compared with self-aggregated spheroids produced by the hanging-droplet technique.[73] Thus, hydrogel microsphere encapsulation could be a superior method for drug screening studies with breast cancer cell spheroids.

Several studies have used "blank slate" PEG-based hydrogels to investigate the role of different integrin-binding peptide motifs in breast cancer. Weiss et al. used plasmin-degradable Michael-type addition crosslinked PEG hydrogels to compare the effects of RGD and the laminin-derived YIGSR adhesion motif.[74] They tested three cell types: MDA-MB-231 cells, a triple-negative breast cancer cell line, ErbB2.MCF10A cells, non-cancerous breast epithelial cells modified to overexpress the HER2 receptor also known as ErbB2, which is a common molecular feature of HER2+ breast cancer subtypes, and MCF10A cells, which were used as a control breast epithelial cell. The control MCF10A cells formed ordered spherical structures in the 3D PEG hydrogels, with some exhibiting a lumen reminiscent of acini. As with the PEG–fibrinogen hydrogel studies, these results were consistent with results previously observed in Matrigel experiments. Thus, engineered PEG-peptide hydrogels are also a good surrogate for Matrigel. Interestingly, the acini-like structures were formed more readily in RGD-functionalized gels than in YIGSR-functionalized gels or blank control gels, demonstrating the impact that differing integrin engagement has on cellular organization. However, the MDA-MB-231 cells and ErbB2.MCF10A cells exhibited less organization in 3D culture, and tended to form smaller and less developed structures in the YIGSR-functionalized gels. Overall, MDA-MB-231 cells were also more likely to form loose, non-spherical cellular aggregates than ErbB2.MCF10A cells in all matrices. The three cell types also showed distinctly different patterns of transcriptional factor activation in the different adhesive peptide-functionalized PEG gels. These results support previous results of abnormal breast cancer cell morphology observed in Matrigel culture, but they also indicate that matrix composition and breast cancer subtype significantly influence these morphological differences.

In another study, Taubenberger et al. used PEG hydrogels to investigate the effects of different cell-adhesive peptide motifs and co-culture with vascular cells on tumor cell phenotype of breast cancer cells.[75] They specifically used a Michael-type addition crosslinked hydrogel comprising a PEG-based macromer functionalized with cysteine-terminated peptides with either a MMP-degradable or a cell adhesive motif and maleimide-functionalized heparin.[75] They tested three cell adhesive peptides—RGD, the laminin-derived IKVAV motif, and the collagen type I-derived GFOGER motif—on two breast cancer cell lines, MCF-7 (ER+/PR+/HER2- subtype) and MDA-MB-231 (triple negative subtype). Interestingly, they observed that neither of the breast cancer cell lines exhibited significant differences in proliferation or invasiveness into the surrounding matrix with different adhesive peptides. However, the MDA-MB-231 breast cancer cells, unlike the MCF-7 breast cancer cell line, did not form spherical cell aggregates in the 3D culture. This result is similar to the results seen by Weiss et al. and may be a feature of the more invasive triple negative phenotype. Unfortunately, the MDA-MB-231 were excluded from subsequent experiments because of their non-spherical phenotype. However, tumor angiogenesis was studied for MCF-7 breast cancer cells, and they were compared with two prostate cancer cell lines (LnCaP and PC-3). Interestingly, regardless of the matrix composition, few endothelial-tumor cell interactions were observed when MCF-7 cells were in co-culture with HUVECs and mesenchymal stem cells (MSCs), which formed vessel-like structures in the hydrogel. This observation was in contrast to what was seen with PC-3 cells, which influenced angiogenesis significantly in hydrogels functionalized with IKVAV and GFOGER but not RGD. While additional studies are needed to elucidate the biologic mechanisms responsible for these differences, this study nonetheless demonstrates how the modularity of PEG-peptide hydrogels can be leveraged study the effects of microenvironmental cues on tumor cell phenotype.

One exciting area of investigation that has not been highlighted in the other example sections is the development of mechanically dynamic microenvironments. Outside of cancer research, softening via photodegradable crosslinkers has been used extensively to create mechanically dynamic hydrogels and probe the effects of matrix stiffness in cell biology.[76–78] However, matrix stiffening is more desirable for mimicking cancer progression, and platforms to achieve stiffening are not as well developed. Stowers et al. achieved this goal using an alginate-based platform.[79] The key to their approach was the incorporation of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine based liposomes loaded with $CaCl_2$ and gold nanorods.[79] Matrigel was also included to facilitate cell adhesion. Irradiation with an 808 nm laser induced surface plasmon resonance in the gold nanorods, resulting in localized heating that triggered the release of calcium ions to increase alginate crosslinking and stiffness. Their platform exhibited a high degree of stiffening over a physiologically relevant range, from an elastic modulus of 151 Pa pre-irradiation to 1074 Pa post-irradiation. Notably, non-malignant MCF10A breast epithelial cells encapsulated in gel initially formed acini-like structures, but after stiffening formed multicellular protrusions into the surrounding matrix with beta-catenin-staining cell-cell contacts (Fig. 4). Acini-like structures in stiffened gels also had larger cross-sectional area and higher levels of nuclear staining for the proliferation marker Ki67, and laminin staining indicated that the layer of basement membrane surrounding the outside of the structure became disorganized post-stiffening (Fig. 4). Collectively, these results indicate that stiffening induced a more tumor-like phenotype. However, the effects were dampened by treatment with small molecule inhibitors of Rac1 and ROCK, suggesting that dynamic material platforms like this one could provide new insight on the mechanisms of mechanotransduction that influence the progression of breast cancer.

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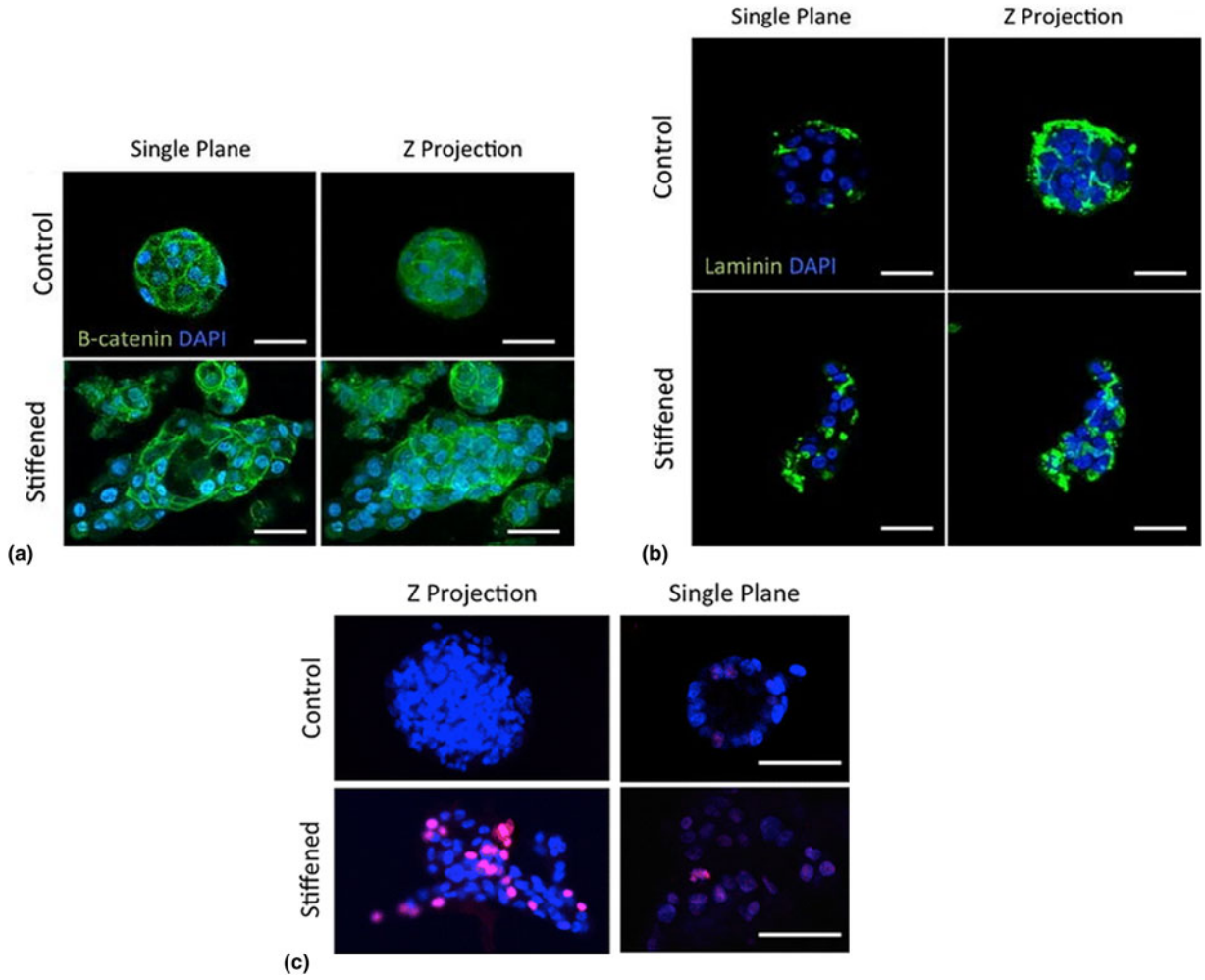

Figure 4. Progressive matrix stiffening induces tumor-like morphology in non-cancer human mammary epithelial cells. (a) MCF10A non-cancer epithelial cells form less spherical, acini-like structure after matrix stiffening and (b) exhibit less acini-like organization of laminin-rich basement membrane and around cell clusters. (c) MCF10A cells exhibit greater nuclear staining of Ki67 (pink), a proliferative marker, after gel stiffening. Adapted from Stowers et al.[79] with permission of Springer.

Challenges of studying cancer biology in 3D cultures

While 3D tissue-engineered models can provide a more physiologically relevant environment for in vitro culture, it is generally recognized that molecular and cell biological analyses become more difficult in these systems. Immunostaining and 3D fluorescence imaging techniques generally work well and can provide valuable insight on structure, organization, and cell–matrix interactions. However, implementing quantitative methods in 3D tissue-engineered culture systems to study intercellular and intracellular signaling pathways is not trivial since these methods were developed for simpler 2D culture systems. While methods for protein and nucleic acid detection and quantitation have been adapted to whole tissue samples, whole tissue samples have more cells and more ECM proteins than 3D tissue-engineered systems. This yields a stronger signal in protein and gene expression that is less vulnerable to significant degradation from common digestion methods used for quantitative protein or gene expression analysis, such as enzymatic digestion or phenol–chloroform extraction, compared with tissue-engineered models. Consequently, it is possible that signal loss from processing for molecular and cell biologic analyses could be limiting our understanding of cancer biology in 3D environments.

Perhaps the best evidence that the challenges of extending molecular biology tools to tissue-engineered systems could be limiting our understanding is a recent paper from Valdez et al.[80] They developed a novel PEG hydrogel crosslinked with a peptide susceptible to enzymatic cleavage by both cell-secreted MMPs and Sortase A.[80] Because Sortase A is a bacterial enzyme and is not produced by mammalian cells, this approach enabled on-demand exogenously controlled degradation of cell-laden hydrogels for protein expression analysis. Using a Sortase A variant engineered to have faster kinetics,

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they achieved complete degradation in approximately 5 min. In contrast to degradation with trypsin digestion and a commercially available collagenase digestion, which are common enzymatic digestion methods, they demonstrated that Sortase-mediated digestion was mild and preserved the integrity of cell-secreted cytokines in the matrix. Moreover, they detected significant differences between cytokines present in the matrix compared with the media. This finding suggests that only analyzing the media (an approach used from 2D studies) provides an incomplete picture of cellular signaling in 3D models, and studies that use this method may be missing important proteomic information. This work is a case study on how materials innovation can provide new insight on cancer biology.

Gene expression analysis is another staple in molecular biology analysis. Myriad kits for mRNA isolation from 2D cultures and bulk tissue samples are commercially available and are frequently used in conjunction with quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR) to measure gene expression. However, isolating sufficient amounts of high-quality mRNA from 3D hydrogel cultures can be challenging. Work from the Gasparian et al. suggests that, at least in PEG-based hydrogels, this may be due to RNase-mediated degradation.[81] They showed that while low quantities and poor quality mRNA were obtained using standard procedures, the yield was drastically improved by flash freezing in liquid nitrogen and then adding sacrificial tRNA to protect the desired mRNA. While their study is useful for method development, it also raises the question of whether mRNA signals have been missing in previous work and to what degree RNA isolation methods impact signal strength and quality, especially with the advent of more sensitive RNA detection methods such as RNA sequencing (RNA-Seq).

Cell migration, which is of course fundamental to metastasis but is also important for evaluating cellular interactions (e.g., between fibroblasts and cancer cells), also has challenges in 3D. Live-cell microscopy techniques are frequently employed and used to perform real-time tracking of cell migration in 3D matrices, as in the study by Leight et al.[51] Thus, methods and tools are not a limitation. However, a critical effector of cell migration in hydrogel cultures is mesh size, which is determined by the crosslink density and is inversely related to modulus. Typical pore sizes in tissue mimetic hydrogels being used for 3D culture are on the order of tens of nanometers. Thus, the polymer network constitutes a restrictive mesh to cells, which are microscale, and it must be degraded to permit cell spreading and migration. While this problem is generally circumvented in migration studies by choosing low-modulus matrices, it is potentially confounding since tumors are known to be stiffer than healthy tissues and it may be desirable to increase the matrix modulus to provide a more physiologically relevant microenvironment. Douglas et al. recently reported an intriguing solution to this problem when they demonstrated that ultrasoft microgels could be incorporated into stiff fibrin gels to form a connected network of tracks for cellular migration.[82] Because this approach decouples cell migration from matrix degradation, it could allow migration to be studied in stiffer matrices that better replicate certain tumor microenvironments.

Another challenge in developing in vitro tumor models is accounting for the complex cellular milieu of tumor tissue. Increasing evidence supports that the tumor stroma, which includes all the cells of the tumor that are not the neoplastic tumor cells (i.e., fibroblasts, endothelial cells, adipocytes, MSCs, macrophages, and other vascular, mesenchymal, and immune cells), is crucial for the formation and progression of a tumor. Tumor vascularization and angiogenesis in particular require tumor–stroma interaction. Thus, tumor cell expression of pro-angiogenic signaling molecules is often investigated in 3D hydrogel cultures.[38] However, more compelling and physiologically accurate models include vascular cells such as HUVECs and quantify the production of pro-angiogenic signaling molecules as well as endothelial cell migration.[57] More sophisticated models have been used to visualize the assembly of vessel-like structures in co-culture with tumor cells. For example, such co-culture models have included breast or prostate tumor cells, HUVECs, and MSCs[83] or lung tumor cells, HUVECs, and HVP (human vascular pericytes).[33] However, only a few of the known intercellular interactions crucial to tumor angiogenesis have been targeted to date, and it may be important for future investigations to also consider how other stromal cells contribute to tumor vascularization.

Additionally, while it is widely recognized in tumor biology that metastasis requires the tumor stroma, investigation of the intercellular interactions of metastasis is lacking in tissue-engineered models. Many studies of 3D tissue-engineered tumor models report cancer cell MMP production as a marker of metastatic potential, but MMP expression by stromal cells has not been considered. Huang et al. demonstrated in a mouse model that stromal expression of MMP-9 is more critical to metastatic progression than cancer cell expression of MMPs.[84] Within the stroma, tumor-associated macrophages in particular may be important to include in tissue-engineered models. In vivo studies have shown that macrophages are necessary for the formation of metastases. For example, Lin et al. prevented the recruitment of macrophages by tumors by knocking down the gene for macrophage colony-stimulating factor (CSF-1) in a MMTV-PyMT mouse model of breast cancer, which is known to exhibit lung metastases, and found that CSF-1 knockdown mice resulted in delayed histological progression and almost no metastases.[85] According to Pollard and Condeelis, tumor-associated macrophages are not only important in metastasis but necessary for metastasis to occur.[86] Incorporating stem cells like adipose-derived stem cells is also of interest for tissue-engineered models, as recently reviewed by Wittmann and Fischbach.[87]

Another challenge is that gradients of oxygen concentration and regions of hypoxia are common in tumors, especially larger tumors. Regions of hypoxia are thought to induce the development of cancer stem cells, cells with stem-like characteristics that are capable of regenerating full tumors in cases of recurrence and metastasis, and the emergence of these cells has

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been linked to the expression of hypoxia-induced factors, as reviewed in references.[88,89] Hypoxic gradients have been shown to induce cancer stem-cell-like phenotypes in glioblastoma cells[90] as well as in breast cancer cells in vitro and in mouse xenografts.[91] The most common way of modeling hypoxia in vitro is by use of hypoxia chambers, which have a limited volume and are expensive. Recently, creative methods of inducing hypoxia in vitro in tissue-engineered tumor models have been explored. For example, Rodenhizer et al. developed a TRACER (tumor roll for analysis of cellular environment and response), made from a cellulose scaffold in a strip, filled with collagen I and seeded with cells, then wound around a spool.[92] Importantly, the physical arrangement of the cells and scaffold around the spool creates a gradient in oxygen concentration, with hypoxia in the inner layers, and the scaffold can be unrolled for analysis. They observed several metabolic changes with increasing hypoxia, including increased levels of lactate, a marker of aerobic glycolysis. They also found that the innermost layers of the scaffold contained less doxorubicin after the drug was added to the media and were more resistant to radiation treatment. Incorporation of oxygen-consuming enzymes can also be used to induce hypoxia in tissue-engineered hydrogel models. For example, Park et al. developed a hypoxia-inducing 3D culture system using ferulic acid-functionalized gelatin.[93] Enzyme-mediated crosslinking of the ferulic acid molecules depleted the oxygen in the gel to create hypoxic conditions, which the authors reported lasted up to 50 h in gels with encapsulated cells due to cellular oxygen consumption. Lewis et al. subsequently used this platform to model hypoxia in soft tissue sarcoma.[94] Primary samples of tumors showed enhanced invasion into hypoxic gels over non-hypoxic controls, with greater speed and range of migration. In addition, individual sarcoma cells encapsulated in the gel were able to survive and maintain low oxygen levels for 7 days in culture. The encapsulated individual cells also showed enhanced migration along hypoxic gradients in the gels, as well as more evidence of matrix remodeling than cells in non-hypoxic gels. This evidence of more invasive behavior of tumor cells, expected under hypoxic conditions, suggests that this material may offer a useful platform for future investigation on the effects of tumor hypoxia and drug screening.

ECM nanoarchitecture and dynamics are also important challenges for future research. Most of the hydrogels discussed in this review are chemically crosslinked gels that do not recapitulate the nanofibrillar structure of the ECM. Platforms such as peptide-amphiphile or PEG-conjugated peptide self-assembling nanofiber hydrogel matricies[95,96] or electrospun methacrylated dextran[97] might be used in the future in tumor models to address this challenge. In addition, dynamic hydrogels that allow for spatiotemporal control over physicochemical properties could be used to mimic dynamic changes in the tumors. As discussed previously, Stowers et al. designed a controllably stiffened alginate platform to investigate the effects of dynamic matrix stiffness on breast cancer cells. However, other dynamically stiffening hydrogel platforms have been reported in the literature and could be useful for cancer research.[98,99] In addition, while photodegradation is perhaps less relevant to tumor progression, photocleavage reactions could be leveraged to dynamically release molecules of interest[76,100] in 4D experiments. This approach could provide a greater understanding of how various molecules of interest (e.g., hyaluronic acid in glioblastoma) influence tumor growth.

Conclusions and outlook

Tissue engineering holds great promise in creating cancer models. The diverse toolkit of tissue-mimetic hydrogels available now can be used to build models with more physiological relevance than current standard 2D culture models and greater control and reproducibility than current animal models. The potential of 3D tissue-engineered culture systems to recreate the physical, mechanical, biochemical, and cellular features of the tumor microenvironment ex vivo offers an exciting opportunity to fill the current gap in knowledge of how tumors create, maintain, and are sustained by their microenvironment. Several polymer strategies have been used to recreate the mechanically stiff matrix of the tumor, and material strategies for changing the stiffness spatially and temporally offer new insights into how mechanical features influence cancer promotion and progression. Additionally, modular scaffold designs have allowed for selective incorporation of cell-signaling molecules, cell-adhesive peptides, and degradable sites. This has enabled the investigation of microenvironmental biochemical features such as matrix degradability, specific cellular adhesion signaling motifs, and even the influence of differing concentrations of whole ECM proteins on tumor cells. Future models could potentially explore incorporating real-time sensing capabilities in the hydrogel platform, as has been explored with MMP activity.

In the future, it will likely be important to build additional microenvironmental features into cancer models to better account for tumor heterogeneity and complexity. To this end, emerging technologies in the field of tissue engineering will be enabling. Bioprinting, for example, has the potential to recreate the complex and heterogeneous spatial organization of cells and biochemical cues of the tumor microenvironment. Also, as more cell types are incorporated and sustained in 3D tissue-engineered culture models, better approximations of complex bi-directional cell-signaling networks will be able to be studied in vitro. However, moving forward, the balance between necessary and sufficient model complexity will have to be kept in mind. The value of increasing or reducing model complexity will have to be considered in the context of in vivo relevance, which will require continuing collaboration with cancer biologists. Ultimately, tissue-engineered tumor models will occupy a critical niche in studying how cancer works and be enabling tools for clinicians and scientists fighting the battle against cancer.

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References

1. D. Hanahan and R.A. Weinberg: Hallmarks of cancer: the next generation. *Cell* **144**, 646 (2011).
2. T.K. Schuessler, X.Y. Chan, H.J. Chen, K. Ji, K.M. Park, A. Roshan-Ghias, P. Sethi, A. Thakur, X. Tian, A. Villasante, I. K. Zervantonakis, N.M. Moore, L.A. Nagahara, and N.Z. Kuhn: Biomimetic tissue-engineered systems for advancing cancer research: NCI strategic workshop report. *Cancer Res.* **74**, 5359 (2014).
3. W.C. Hahn, S.A. Stewart, M.W. Brooks, S.G. York, E. Eaton, A. Kurachi, R.L. Beijersbergen, J.H.M. Knoll, M. Meyerson, and R.A. Weinberg: Inhibition of telomerase limits the growth of human cancer cells. *Nat. Med.* **5**, 1164 (1999).
4. J.L. Leight, M.A. Wozniak, S. Chen, M.L. Lynch, and C.S. Chen: Matrix rigidity regulates a switch between TGF-β1-induced apoptosis and epithelial-mesenchymal transition. *Mol. Biol. Cell* **23**, 781 (2012).
5. J. Heyer, L.N. Kwong, S.W. Lowe, and L. Chin: Non-germline genetically engineered mouse models for translational cancer research. *Nat. Rev. Cancer* **10**, 470 (2010).
6. N.E. Sharpless and R.A. DePinho: The mighty mouse : genetically engineered mouse models in cancer drug development. *Nat. Rev. Drug Discov.* **5**, 741 (2006).
7. A. Rangarajan and R.A. Weinberg: Comparative biology of mouse versus human cells: modelling human cancer in mice. *Nat. Rev. Cancer* **3**, 952 (2003).
8. I.W.Y. Mak, N. Evaniew, and M. Ghert: Lost in translation : animal models and clinical trials in cancer treatment. *Am. J. Transl. Res.* **6**, 114 (2014).
9. O.W. Petersen, L. Rønnov-Jessen, A.R. Howlett, and M.J. Bissell: Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 9064 (1992).
10. J. Debnath, S.K. Muthuswamy, and J.S. Brugge: Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods* **30**, 256 (2003).
11. A. Albini, I. Iwamoto, H.K. Kleinman, G.R. Martin, S.A. Aaronson, J. M. Kozlowski, and R.N. McEwan: A rapid in vitro assay for quantitating the invasive potential of tumor cells. *Cancer Res.* **47**, 3239 (1987).
12. C.S. Hughes, L.M. Postovit, and G.A. Lajoie: Matrigel: a complex protein mixture required for optimal growth of cell culture. *Proteomics* **10**, 1886 (2010).
13. A.S. Hoffman: Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **64**, 18 (2012).
14. C.M. Magin, D.L. Alge, and K.S. Anseth: Bio-inspired 3D microenvironments: a new dimension in tissue engineering. *Biomed. Mater.* **11**, 22001 (2016).
15. M.A. Azagarsamy and K.S. Anseth: Bioorthogonal click chemistry: an indispensable tool to create multifaceted cell culture scaffolds. *ACS Macro Lett.* **2**, 5 (2013).
16. K.T. Nguyen and J.L. West: Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials* **23**, 4307 (2002).
17. K.Y. Lee and D.J. Mooney: Hydrogels for tissue engineering. *Chem. Rev.* **101**, 1869 (2001).
18. B.D. Fairbanks, M.P. Schwartz, C.N. Bowman, and K.S. Anseth: Photoinitiated polymerization of PEG-diacrylate with lithium phenyl-2,4,6-trimethylbenzoylphosphinate: polymerization rate and cytocompatibility. *Biomaterials* **30**, 6702 (2009).
19. C.M. Nimmo and M.S. Shoichet: Regenerative biomaterials that "click": simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning. *Bioconjug. Chem.* **22**, 2199 (2011).
20. C.-C. Lin, A. Raza, and H. Shih: PEG hydrogels formed by thiol-ene photo-click chemistry and their effect on the formation and recovery of insulin-secreting cell spheroids. *Biomaterials* **32**, 9685 (2011).
21. A.D. Shubin, T.J. Felong, D. Graunke, C.E. Ovitt, and D.S.W. Benoit: Development of poly(ethylene glycol) hydrogels for salivary gland tissue engineering applications. *Tissue Eng. A* **21**, 1733 (2015).
22. R. Parenteau-Bareil, R. Gauvin, and F. Berthod: Collagen-based biomaterials for tissue engineering applications. *Materials* (*Basel*) **3**, 1863 (2010).
23. D. Loessner, C. Meinert, E. Kaemmerer, L.C. Martine, K. Yue, P.A. Levett, T.J. Klein, F.P.W. Melchels, A. Khademhosseini, and D.W. Hutmacher: Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms. *Nat. Protoc.* **11**, 727 (2016).
24. X. Xu, A.K. Jha, D.A. Harrington, M.C. Farach-Carson, and X. Jia: Hyaluronic acid-based hydrogels: from a natural polysaccharide to complex networks. *Soft Matter* **8**, 3280 (2012).
25. A.D. Augst, H.J. Kong, and D.J. Mooney: Alginate hydrogels as biomaterials. *Macromol. Biosci.* **6**, 623 (2006).
26. O. Jeon, K.H. Bouhadir, J.M. Mansour, and E. Alsberg: Photocrosslinked alginate hydrogels with tunable biodegradation rates and mechanical properties. *Biomaterials* **30**, 2724 (2009).
27. J. Zhu: Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials* **31**, 4639 (2010).
28. M.P. Lutolf, J.L. Lauer-Fields, H.G. Schmoekel, T. Metters, F.E. Weber, G.B. Fields, and J. Hubbell: Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 5413 (2003).
29. E.A. Phelps, N.O. Enemchukwu, V.F. Fiore, J.C. Sy, N. Murthy, T.A. Sulchek, T.H. Barker, and A.J. Garcia: Maleimide cross-linked bioactive PEG hydrogel exhibits improved reaction kinetics and cross-linking for cell encapsulation and in situ delivery. *Adv. Mater.* **24**, 64 (2012).
30. A.E. Rydholm, K.S. Anseth, and C.N. Bowman: Effects of neighboring sulfides and pH on ester hydrolysis in thiol-acrylate photopolymers. *Acta Biomater.* **3**, 449 (2007).
31. B.D. Fairbanks, M.P. Schwartz, A.E. Halevi, C.R. Nuttelman, C. N. Bowman, and K.S. Anseth: A versatile synthetic extracellular matrix mimic via Thiol-Norbornene photopolymerization. *Adv. Mater.* **21**, 5005 (2009).
32. B.J. Gill, D.L. Gibbons, L.C. Roudsari, J.E. Saik, Z.H. Rizvi, J.D. Roybal, J.M. Kurie, and J.L. West: A synthetic matrix with independently tunable biochemistry and mechanical properties to study epithelial morphogenesis and EMT in a lung adenocarcinoma model. *Cancer Res.* **72**, 6013 (2012).
33. L.C. Roudsari, S.E. Jeffs, A.S. Witt, B.J. Gill, and J.L. West: A 3D poly (ethylene glycol)-based tumor angiogenesis model to study the influence of vascular cells on lung tumor cell behavior. *Sci. Rep.* **6**, 32726 (2016).
34. C.S. Ki, T.-Y. Lin, M. Korc, and C.-C. Lin: Thiol-ene hydrogels as desmoplasia-mimetic matrices for modeling pancreatic cancer cell growth, invasion, and drug resistance. *Biomaterials* **35**, 9668 (2014).
35. Y. Liang, J. Jeong, R.J. DeVolder, C. Cha, F. Wang, Y.W. Tong, and H. Kong: A cell-instructive hydrogel to regulate malignancy of 3D tumor spheroids with matrix rigidity. *Biomaterials* **32**, 9308 (2011).
36. X. Xu, C. Liu, Y. Liu, N. Li, X. Guo, S. Wang, G. Sun, W. Wang, and X. Ma: Encapsulated human hepatocellular carcinoma cells by alginate gel beads as an in vitro metastasis model. *Exp. Cell Res.* **319**, 2135 (2013).
37. T.-Y. Lin, C.S. Ki, and C.-C. Lin: Manipulating hepatocellular carcinoma cell fate in orthogonally cross-linked hydrogels. *Biomaterials* **35**, 6898 (2014).
38. C. Fischbach, H.J. Kong, S.X. Hsiong, M.B. Evangelista, W. Yuen, and D. J. Mooney: Cancer cell angiogenic capability is regulated by 3D culture and integrin engagement. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 399 (2009).
39. S. Sieh, A.V. Taubenberger, S.C. Rizzi, M. Sadowski, M.L. Lehman, A. Rockstroh, J. An, J.A. Clements, C.C. Nelson, and D.W. Hutmacher:

Phenotypic characterization of prostate cancer LNCaP cells cultured within a bioengineered microenvironment. *PLoS ONE* **7**, e40217 (2012).
40. X. Xu, L.A. Gurski, C. Zhang, D.A. Harrington, M.C. Farach-Carson, and X. Jia: Recreating the tumor microenvironment in a bilayer, hyaluronic acid hydrogel construct for the growth of prostate cancer spheroids. *Biomaterials* **33**, 9049 (2012).
41. D. Loessner, K.S. Stok, M.P. Lutolf, D.W. Hutmacher, J.A. Clements, and S.C. Rizzi: Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells. *Biomaterials* **31**, 8494 (2010).
42. Z. Yang and X. Zhao: A 3D model of ovarian cancer cell lines on peptide nanofiber scaffold to explore the cell-scaffold interaction and chemotherapeutic resistance of anticancer drugs. *Int. J. Nanomed.* **5**, 303 (2011).
43. E. Kaemmerer, F.P.W. Melchels, B.M. Holzapfel, T. Meckel, D.W. Hutmacher, and D. Loessner: Gelatine methacrylamide-based hydrogels: an alternative three-dimensional cancer cell culture system. *Acta Biomater.* **10**, 2551 (2014).
44. D. Holmes: The cancer that rises with the Sun. *Nature* **515**, S110 (2014).
45. A.M. Glazer, R.R. Winkelmann, A.S. Farberg, and D.S. Rigel: Analysis of trends in US melanoma incidence and mortality. *JAMA Dermatol.* **153**, 225 (2017).
46. K.B. Reed, J.D. Brewer, C.M. Lohse, K.E. Bringe, C.N. Pruitt, and L.E. Gibson: Increasing incidence of melanoma among young adults: an epidemiological study in Olmsted County, Minnesota. *Mayo Clin. Proc.* **87**, 328 (2012).
47. E.Y. Tokuda, J.L. Leight, and K.S. Anseth: Modulation of matrix elasticity with PEG hydrogels to study melanoma drug responsiveness. *Biomaterials* **35**, 4310 (2014).
48. S.P. Singh, M.P. Schwartz, E.Y. Tokuda, Y. Luo, R.E. Rogers, M. Fujita, N.G. Ahn, and K.S. Anseth: A synthetic modular approach for modeling the role of the 3D microenvironment in tumor progression. *Sci. Rep.* **5**, 1 (2015).
49. E.Y. Tokuda, C.E. Jones, and K.S. Anseth: PEG-peptide hydrogels reveal differential effects of matrix microenvironmental cues on melanoma drug sensitivity. *Integr. Biol.* **9**, 76 (2017).
50. R. Kalluri and M. Zeisberg: Fibroblasts in cancer. *Nat. Rev. Cancer* **6**, 392 (2006).
51. J.L. Leight, E.Y. Tokuda, C.E. Jones, A.J. Lin, and K.S. Anseth: Multifunctional bioscaffolds for 3D culture of melanoma cells reveal increased MMP activity and migration with BRAF kinase inhibition. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 5366 (2015).
52. J.A. Sosman, K.B. Kim, L. Schuchter, R. Gonzalez, A.C. Pavlick, J.S. Weber, G.A. McArthur, T.E. Hutson, S.J. Moschos, K.T. Flaherty, P. Hersey, R. Kefford, D. Lawrence, I. Puzanov, K.D. Lewis, R.K. Amaravadi, B. Chmielowski, H.J. Lawrence, Y. Shyr, F. Ye, J. Li, K.B. Nolop, R.J. Lee, A.K. Joe, and A. Ribas: Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N. Engl. J. Med.* **366**, 707 (2012).
53. J.P. Thakkar, T.A. Dolecek, C. Horbinski, Q.T. Ostrom, D.D. Lightner, J. S. Barnholtz-Sloan, and J.L. Villano: Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiol. Biomark. Prev.* **23**, 1985 (2014).
54. A.M.P. Omuro, S. Faivre, and E. Raymond: Lessons learned in the development of targeted therapy for malignant gliomas. *Mol. Cancer Ther.* **6**, 1909 (2007).
55. T.A. Ulrich, E.M. de Juan Pardo, and S. Kumar: The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res.* **69**, 4167 (2009).
56. A. Pathak and S. Kumar: Independent regulation of tumor cell migration by matrix stiffness and confinement. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 10334 (2012).
57. S.S. Verbridge, N.W. Choi, Y. Zheng, D.J. Brooks, A.D. Stroock, and C. Fischbach: Oxygen-controlled three-dimensional cultures to analyze tumor angiogenesis. *Tissue Eng. A* **16**, 2133 (2010).
58. D.T. Nguyen, Y. Fan, Y.M. Akay, and M. Akay: Investigating glioblastoma angiogenesis using a 3D in vitro GelMA microwell platform. *IEEE Trans. Nanobiosci.* **15**, 289 (2016).
59. D.T. Nguyen, Y. Fan, Y.M. Akay, and M. Akay: TNP-470 reduces glioblastoma angiogenesis in three dimensional GelMA microwell platform. *IEEE Trans. Nanobiosci.* **15**, 683 (2016).
60. B. Ananthanarayanan, Y. Kim, and S. Kumar: Elucidating the mechanobiology of malignant brain tumors using a brain matrix-mimetic hyaluronic acid hydrogel platform. *Biomaterials* **32**, 7913 (2011).
61. S.S. Rao, J. DeJesus, A.R. Short, J.J. Otero, A. Sarkar, and J.O. Winter: Glioblastoma behaviors in three-dimensional collagen-hyaluronan composite hydrogels. *ACS Appl. Mater. Interfaces* **5**, 9276 (2013).
62. S. Pedron, E. Becka, and B.A.C. Harley: Regulation of glioma cell phenotype in 3D matrices by hyaluronic acid. *Biomaterials* **34**, 7408 (2013).
63. S. Pedron, E. Becka, and B.A. Harley: Spatially gradated hydrogel platform as a 3D engineered tumor microenvironment. *Adv. Mater.* **27**, 1567 (2015).
64. S. Pedron and B.A.C. Harley: Impact of the biophysical features of a 3D gelatin microenvironment on glioblastoma malignancy. *J. Biomater. Res. A* **101A**, 3404 (2013).
65. C. Wang, X. Tong, and F. Yang: Bioengineered 3D brain tumor model to elucidate the effects of matrix stiffness on glioblastoma cell behavior using PEG-based hydrogels. *Mol. Pharm.* **11**, 2115 (2014).
66. C. Wang, X. Tong, X. Jiang, and F. Yang: Effect of matrix metalloproteinase-mediated matrix degradation on glioblastoma cell behavior in 3D PEG-based hydrogels. *J. Biomed. Mater. Res. A* **105A**, 770 (2017).
67. C. Jiguet Jiglaire, N. Baeza-Kallee, E. Denicolaï, D. Barets, P. Metellus, L. Padovani, O. Chinot, D. Figarella-Branger, and C. Fernandez: Ex vivo cultures of glioblastoma in three-dimensional hydrogel maintain the original tumor growth behavior and are suitable for preclinical drug and radiation sensitivity screening. *Exp. Cell Res.* **321**, 99 (2014).
68. R.L. Siegel, K.D. Miller, and A. Jemal: Cancer statistics, 2016. *CA. Cancer J. Clin.* **66**, 7 (2016).
69. C. Woolston: Breast cancer. *Nature* **527**, S101 (2015).
70. A. Bleyer and H.G. Welch: Effect of three decades of screening mammography on breast-cancer incidence. *N. Engl. J. Med.* **367**, 1998 (2012).
71. S. Pradhan, I. Hassani, W.J. Seeto, and E.A. Lipke: PEG-fibrinogen hydrogels for three-dimensional breast cancer cell culture. *J. Biomed. Mater. Res. A* **105A**, 236 (2017).
72. P.A. Kenny, G.Y. Lee, C.A. Myers, R.M. Neve, J.R. Semeiks, P. T. Spellman, K. Lorenz, E.H. Lee, M.H. Barcellos-Hoff, O.W. Petersen, J.W. Gray, and M.J. Bissell: The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. *Mol. Oncol.* **1**, 84 (2007).
73. S. Pradhan, J.M. Clary, D. Seliktar, and E.A. Lipke: A three-dimensional spheroidal cancer model based on PEG- fibrinogen hydrogel microspheres. *Biomaterials* **115**, 141 (2017).
74. M.S. Weiss, B.P. Bernabé, A. Shikanov, D.A. Bluver, M.D. Mui, S. Shin, L.J. Broadbelt, and L.D. Shea: The impact of adhesion peptides within hydrogels on the phenotype and signaling of normal and cancerous mammary epithelial cells. *Biomaterials* **33**, 3548 (2012).
75. A.V. Taubenberger, L.J. Bray, B. Haller, A. Shaposhnykov, M. Binner, U. Freudenberg, J. Guck, and C. Werner: 3D extracellular matrix interactions modulate tumour cell growth, invasion and angiogenesis in engineered tumour microenvironments. *Acta Biomater.* **36**, 73 (2016).
76. A.M. Kloxin, A.M. Kasko, C.N. Salinas, and K.S. Anseth: Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* **324**, 59 (2009).
77. H. Wang, M.W. Tibbitt, S.J. Langer, L.A. Leinwand, and K.S. Anseth: Hydrogels preserve native phenotypes of valvular fibroblasts through an elasticity-regulated PI3K/AKT pathway. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 19336 (2013).
78. C. Yang, M.W. Tibbitt, L. Basta, and K.S. Anseth: Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* **13**, 645 (2014).
79. R.S. Stowers, S.C. Allen, K. Sanchez, C.L. Davis, N.D. Ebelt, C. van Den Berg, and L.J. Suggs: Extracellular matrix stiffening induces a malignant phenotypic transition in breast epithelial cells. *Cell. Mol. Bioeng.* **10**, 114 (2017).
80. J. Valdez, C.D. Cook, C. Chopko, A.J. Wang, A. Brown, M. Kumar, L. Stockdale, D. Rothenberg, K. Renggli, E. Gordon, D. Lauffenburger,

https://doi.org/10.1557/mrc.2017.86

F. White, and L. Griffith: On-demand dissolution of modular, synthetic extracellular matrix reveals local epithelial-stromal communication networks. *Biomaterials* **130**, 90 (2017).
81. A. Gasparian, L. Daneshian, H. Ji, E. Jabbari, and M. Shtutman: Purification of high-quality RNA from synthetic polyethylene glycol-based hydrogels. *Anal. Biochem.* **484**, 1 (2015).
82. A.M. Douglas, A.A. Fragkopoulos, M.K. Gaines, L.A. Lyon, A. Fernandez-Nieves, and T.H. Barker: Dynamic assembly of ultrasoft colloidal networks enables cell invasion within restrictive fibrillar polymers. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 885 (2017).
83. L.J. Bray, M. Binner, A. Holzheu, J. Friedrichs, U. Freudenberg, D. W. Hutmacher, and C. Werner: Multi-parametric hydrogels support 3D in vitro bioengineered microenvironment models of tumour angiogenesis. *Biomaterials* **53**, 609 (2015).
84. S. Huang, M. Van Arsdall, S. Tedjarati, M. McCarty, W. Wu, R. Langley, and I.J. Fidler: Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. *J. Natl. Cancer Inst.* **94**, 1134 (2002).
85. E.Y. Lin, A. V. Nguyen, R.G. Russell, and J.W. Pollard: Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J. Exp. Med.* **193**, 727 (2001).
86. J. Condeelis and J.W. Pollard: Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* **124**, 263 (2006).
87. K. Wittmann and C. Fischbach: Contextual control of adipose-derived stem cell function: implications for engineered tumor models. *ACS Biomater. Sci. Eng.* **3**, 1483 (2017).
88. J.M. Heddleston, Z. Li, J.D. Lathia, S. Bao, A.B. Hjelmeland, and J.N. Rich: Hypoxia inducible factors in cancer stem cells. *Br. J. Cancer* **102**, 789 (2010).
89. B. Keith and M.C. Simon: Hypoxia-inducible factors, stem cells, and cancer. *Cell* **129**, 465 (2007).
90. J.M. Heddleston, Z. Li, R.E. McLendon, A.B. Hjelmeland, and J.N. Rich: The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle* **8**, 3274 (2009).
91. S.J. Conley, E. Gheordunescu, P. Kakarala, B. Newman, H. Korkaya, A. N. Heath, S.G. Clouthier, and M.S. Wicha: Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 2784 (2012).
92. D. Rodenhizer, E. Gaude, D. Cojocari, R. Mahadevan, C. Frezza, B. G. Wouters, and A.P. McGuigan: A three-dimensional engineered tumour for spatial snapshot analysis of cell metabolism and phenotype in hypoxic gradients. *Nat. Mater.* **15**, 227 (2016).
93. K.M. Park and S. Gerecht: Hypoxia-inducible hydrogels. *Nat. Commun.* **5**, article number 4075 (2014).
94. D.M. Lewis, K.M. Park, V. Tang, Y. Xu, K. Pak, T.S.K. Eisinger-Mathason, M.C. Simon, and S. Gerecht: Intratumoral oxygen gradients mediate sarcoma cell invasion. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 9292 (2016).
95. J.D. Hartgerink, E. Beniash, and S.I. Stupp: Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 5133 (2002).
96. J.H. Collier and P.B. Messersmith: Self-assembling polymer-peptide conjugates: nanostructural tailoring. *Adv. Mater.* **16**, 907 (2004).
97. B.M. Baker, B. Trappmann, W.Y. Wang, M.S. Sakar, I.L. Kim, V. B. Shenoy, J.A. Burdick, and C.S. Chen: Cell-mediated fiber recruitment drives extracellular matrix mechanosensing in engineered fibrillar microenvironments. *Nat. Mater.* **14**, 1262 (2015).
98. M. Guvendiren and J.A. Burdick: Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nat. Commun.* **3**, 792 (2012).
99. J.L. Young and A.J. Engler: Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro. *Biomaterials* **32**, 1002 (2011).
100. A.M. Rosales, K.M. Mabry, E.M. Nehls, and K.S. Anseth: Photoresponsive elastic properties of azobenzene-containing poly (ethylene-glycol)-based hydrogels. *Biomacromoleules* **16**, 798 (2015).

https://doi.org/10.1557/mrc.2017.86