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Perspective on Translating Biomaterials Into Glioma Therapy: Lessons From *in Vitro* Models

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Glioblastoma (GBM) is the most common and malignant form of brain cancer. Even with aggressive standard of care, GBM almost always recurs because its diffuse, infiltrative nature makes these tumors difficult to treat. The use of biomaterials is one strategy that has been, and is being, employed to study and overcome recurrence. Biomaterials have been used in GBM in two ways: *in vitro* as mediums in which to model the tumor microenvironment, and *in vivo* to sustain release of cytotoxic therapeutics. *In vitro* systems are a useful platform for studying the effects of drugs and tissue-level effectors on tumor cells in a physiologically relevant context. These systems have aided examination of how glioma cells respond to a variety of natural, synthetic, and semi-synthetic biomaterials with varying substrate properties, biochemical factor presentations, and non-malignant parenchymal cell compositions in both 2D and 3D environments. The current *in vivo* paradigm is completely different, however. Polymeric implants are simply used to line the post-surgical resection cavities and deliver secondary therapies, offering moderate impacts on survival. Instead, perhaps we can use the data generated from *in vitro* systems to design novel biomaterial-based treatments for GBM akin to a tissue engineering approach. Here we offer our perspective on the topic, summarizing how biomaterials have been used to identify facets of glioma biology *in vitro* and discussing the elements that show promise for translating these systems *in vivo* as new therapies for GBM.

Keywords: glioblastoma, biomaterial, hydrogel, regenerative medicine, tissue engineering, brain, tumor microenvironment

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INTRODUCTION

Glioblastoma (GBM) is a high-grade brain cancer that almost always recurs (Cuddapah et al., 2014). Many *in vitro* and *in vivo* models of GBM have been developed in an effort to uncover new therapeutic strategies. Biomaterials are often primary components of *in vitro* models to chemically, mechanically, and/or topographically recreate the physiological tumor environment, as recently reviewed by Xiao et al. (2017), Gu and Mooney (2015), Pradhan et al. (2016), Cha and Kim (2017), and Heffernan and Sirianni (2018).

While GBM models are useful for studying glioma biology, the field is far from accurately predicting clinical success of a new therapy. It was recently suggested that all models, including gold-standard mouse xenografts, inherently cannot preserve the genetic landscape of patient-derived tumor cells (Ben-David et al., 2017). Where does this study (and others

like it) leave the field? Glioma is a tissue, with complex heterogeneity in tissue geometry, composition, biophysical properties, etc. Even if researchers can create sophisticated models of the tumor, these models cannot logistically account for every element of the *in vivo* environment. Therefore, a tissue-level approach may enhance our ability to treat this deadly disease.

In vitro biomaterial research has revealed crucial information about material-GBM cell interactions. And yet, implementation of biomaterials *in vivo* for treating GBM has been limited to anti-tumor drug delivery, such as BCNU-releasing Gliadel wafers (Wait et al., 2015). These wafers, made of a poly(lactic-co-glycolic) polymer backbone, have been used to line resection cavities in patients receiving surgical removal of primary tumors and offer a modest, yet significant, increase in survival. However, these systems are simply a conduit for therapy and thus in no way leverage glioma-biomaterial interactions as part of the therapy.

Many diseases are now being viewed from a regenerative medicine lens, using factors within the patient's own body to promote healing. Cancer is often described as a wound that does not heal and may similarly benefit from this approach. The fluid-filled cavity remaining after resection is a prime space in which to examine biomaterial-based therapies, analogous to experimental treatments for stroke or traumatic brain injury. In current literature, treating the post-resection cavity has primarily involved hydrogel biomaterials as passive vehicles for drug therapy (Bagó et al., 2016; Bastiancich et al., 2017). It is possible that translating collective knowledge from myriad *in vitro* models could instead transition biomaterials to an active avenue for cancer remediation. Below, we summarize current understanding of how glioma outcomes can be altered *in vitro* and offer perspectives for using this data to design biomaterials for promoting anti-tumor responses, tumor targeting, and treatment against glioblastoma.

TUNING THE EXTRACELLULAR MATRIX

Matrix Composition

While earlier experiments with glioma cells used 2D plastic, it is now understood that the underlying matrix plays an important role in glioma phenotype (Eke and Cordes, 2011; Florczyk et al., 2013; Heffernan et al., 2015). The composition of the brain matrix is different from most tissues, primarily comprising the polysaccharide hyaluronic acid (HA) and HA-binding proteoglycans, but few fibrillar proteins. Many engineered *in vitro* systems for GBM therefore employ HA-based matrices. These models have elucidated that HA increases stem cell maintenance, glioma cell adhesion and migration, and markers of malignancy (Pedron et al., 2013; Kim and Kumar, 2014; Tilghman et al., 2014; Cha et al., 2016). Other brain components, such as certain chondroitin sulfate proteoglycans (CSPGs), have also been shown to increase glioma invasion (Logun et al., 2016). However, CSPGs have also been suggested to inhibit glioma cell invasion (Silver et al., 2013), therefore the specific response may depend on CSPG sulfation pattern (Silver and Silver, 2014).

Several *in vitro* models have been developed with components not ubiquitous in the brain, like collagen I and laminin-rich basement membrane extract (Matrigel). While mixing these components with HA can recreate the invasive phenotypes observed in pure HA hydrogels (Munson et al., 2013; Gritsenko et al., 2017), collagen and Matrigel hydrogels alone comparatively limit glioma cell invasion. Some non-native components nonetheless increase invasion: The extracellular matrix (ECM) secreted by glioma cells is itself dissimilar to the native brain and is rich in aberrant proteoglycans, tenascin-c, and an overabundance of HA (Cuddapah et al., 2014; Xia et al., 2016). For example, glioma cells secrete a truncated form of the proteoglycan brevican which binds to fibronectin and promotes invasion (Hu et al., 2008). Incorporation of RGDS, the adhesive ligand found in fibronectin, similarly induced cell dissemination in poly(ethylene) glycol hydrogels (Beck et al., 2013). Further, glioma cells adhere more strongly in HA matrices that contain RGDS, potentially due to augmented integrin-mediated mechanotransduction in HA (Chopra et al., 2014; Kim and Kumar, 2014).

Topographical Cues

Topographical cues present within the tissue can also enhance migration. While the brain is relatively non-fibrous and amorphous, basement membrane-rich blood vessels are a prime substrate on which glioma cells migrate within perivascular spaces (Cuddapah et al., 2014). Herrera-Perez et al. (2015) showed that pseudovessels of Matrigel-coated collagen-oligomer fibrils increased the speed of glioma cell migration across a 3D collagen-HA matrix. White matter tracts in the brain are also a frequent route of migration. Using core-shell electrospun nanofibers to mimic white matter tracts, Rao et al. (2013) found that glioma cell morphology, migration speed, and focal adhesion kinase expression were all sensitive to fiber mechanics and composition. Altering the design parameters of fibrous biomaterials can therefore offer precise control over glioma migration.

Mechanical Forces

A major driving force for using biomaterials in cell culture platforms is the ability to control biomechanical forces, often independently from the chemical composition. The mechanical properties of a scaffold influence a wide range of cellular behaviors, including proliferation, migration, and stem cell fate (Engler et al., 2006; Ulrich et al., 2009; Seidlits et al., 2010). It is well described that many tumors outside the brain are stiffer than the surrounding tissue. In glioma, tissue mechanics appear to be extremely heterogeneous, but the tumor is likely stiffer than normal brain, which has a Young's modulus around 1.4 kPa (Miroshnikova et al., 2016). While the exact physiological properties are controversial, stiffer matrices promote glioma dissemination. Increasing the stiffness of PEG hydrogels decreased proliferation of U87 cells and increased the number of cell protrusions (Wang et al., 2014). Similar results were found using fibronectin-based matrices on which tumor cell spread and speed of migration increased with modulus while proliferation rate decreased compared to softer substrates (Ulrich et al., 2009).

Fluid flow and shear stress are also felt by glioma cells in the tumor microenvironment (Munson and Shieh, 2014). These forces have been recreated *in vitro* using HA matrices (Polacheck et al., 2011; Qazi et al., 2011; Munson et al., 2013). Interstitial flow on the order of 0.1–1 μm/s generally increased glioma cell invasion, although patient-derived glioma stem cells showed variable responses (Kingsmore et al., 2016). Manipulation of the matrix to reduce glycocalyx assembly (Qazi et al., 2013) or CD44-binding (Kingsmore et al., 2016) attenuated these effects, indicating a link between flow and the surrounding 3D matrix.

Implications for Therapeutic Translation

Biomaterials often promote cell recruitment into an implantation site after neural injury (Ghuman et al., 2016; Nih et al., 2017). A similar approach may be beneficial for promoting glioma migration into an implanted material following resection. The properties of the implanted matrix should overcome the malignancy-enhancing properties of HA in the brain, either by disrupting binding or providing effective competition. Using a low molecular weight version of HA instead of high molecular weight may promote local anti-tumor inflammation and disrupt growth factor signaling (Fuchs et al., 2013; Rayahin et al., 2015). Incorporation of components such as fibronectin or RGDS could also preferentially promote stronger binding between invaded cells and the material vs. the parenchyma (Kim and Kumar, 2014). Fibrous materials would likely increase glioma invasion into the cavity. In fact, inducing migration through topography has already proved feasible and beneficial for GBM therapy (Jain et al., 2014). Additionally, the implanted matrix should be relatively stiffer than the brain to promote durotaxis, or migration up a stiffness gradient, of glioma cells and stem-like cells but deter migration of neural cells, which prefer softer substrates (Flanagan et al., 2002; Hadden et al., 2017). The caveat is that mechanical mismatch can promote potentially detrimental astrogliosis (Prodanov and Delbeke, 2016). Matrices that are initially stiffer and gradually soften over time may have a defined niche, in this case.

CONTROLLING BIOCHEMICAL CUE PRESENTATION

Cytokine and Growth Factor Gradients

Cytokines and growth factors originating from both glioma and parenchymal cells are associated with the progression of glioma and response to therapy, as previously reviewed (Iwami et al., 2011; Zhu et al., 2012). *In vivo*, natural heterogeneity is formed as tumor and parenchymal cells secrete biological molecules, which then differentially bind to the surrounding matrix and form gradients, sources, and sinks within the tissue. Recreating gradients *in vitro* using combinations of microfluidics, biomaterials, and various cells has been a focus of models for the study of both cancer (Keenan and Folch, 2008; Pedron et al., 2015) and regenerative medicine (Khang, 2015). Microfluidic devices and tissue culture insert models have both been used to show that *in situ* gradients of CXCL12 within 3D hydrogels directly promote glioma migration up the chemokine gradient (Munson et al., 2013; Addington et al., 2015; Kingsmore et al., 2016).

Cytokines are also implicated in the maintenance of glioma stem cells, a potential driver of glioma recurrence. Glial cells and recruited endothelial cells secrete factors such as bFGF that promote stem cell maintenance (Fessler et al., 2015). Blocking the effect of these cytokines offers potential to slow or halt proliferation of glioma cells. Affinity binding peptides have been incorporated into biomaterials for controlling release of bFGF, but these materials could inversely act as effective cytokine sinks (Lin and Anseth, 2009). A similar approach using an RNA aptamer to block PDGFRβ was shown effective at slowing glioma growth (Camorani et al., 2014). Designing materials to promote cell differentiation, as is common in regenerative medicine, may be equally applicable to treating glioma (Benoit et al., 2008).

Oxygen

Aberrant vasculature and unchecked tumor growth produce hypoxic or low oxygen-containing regions within the tumor and invading tumor clusters (called pseudopalisades; Rong et al., 2006). Hypoxia is implicated in increasing angiogenesis, stem cell maintenance, immunosuppression, and cancer cell therapeutic resistance (Colwell et al., 2017). Thus, incorporation of oxygen gradients within *in vitro* systems has been used to study a major effector of glioma outcomes. Use of 3D systems or spheroid culture naturally introduce regions of hypoxia based on thickness and permeability of the materials used. Recently, an *in vitro* PEG-based system showed that immobilization of the O_2-consuming enzymes glucose oxidase and catalase effectively induced hypoxia and upregulated genes known to contribute to cancer metastasis (Dawes et al., 2017). The opposite would therefore be useful for glioma therapy: generating oxygen gradients and preventing hypoxia. Validating this approach, a paper-based PET mesh layering system showing that linear gradients of oxygen in culture functioned as a primary chemoattractant and increased invasion of lung adenocarcinoma cells (Mosadegh et al., 2015). Oxygen-creating biomaterials have been tested in regenerative medicine, showing sustain oxygen release for weeks and reducing hypoxia until angiogenesis can occur (Pedraza et al., 2012).

Implications for Therapeutic Translation

The ability to control spatiotemporal chemical gradients within the post-resection cavity has far-reaching implications for glioma therapy. An ideal biomaterial would trigger glioma cell egress from the brain parenchyma into the material through establishing chemical gradients of chemotactic factors such as oxygen or CXCL12. Alternatively, the material could eliminate or disrupt pro-malignant cytokine signaling through either release of receptor blockers or sequestration of factors that aid glioma stem cell proliferation and maintenance. Dual-release or multi-functional biomaterials would likely be optimal. Materials that enable temporally-regulated release and/or capture dynamics, similar to those used in regenerative medicine (Spiller et al., 2015), are particularly promising since they may simultaneously promote parenchyma egress, glioma stem cell differentiation, and loss of acquired drug resistance.

REMODELING THE CELLULAR MICROENVIRONMENT

Angiogenesis

One hallmark of cancer is the ability to induce aberrant angiogenesis (Hanahan and Weinberg, 2011). Multiple models of angiogenesis have been engineered and used *in vitro* (Kimlin et al., 2013), although few have been described for co-culture of glioma cells and endothelial cells (Nguyen et al., 2016). Glioma cells secrete high levels of pro-angiogenic vascular endothelial growth factor (VEGF)-A which promotes blood vessel sprouting (Folkins et al., 2009). The ECM can act to sequester or locally retain VEGF-A, thereby amplifying resultant uncontrolled angiogenesis (Belair et al., 2016). Additionally, glioma cells *in vivo* physically displace astrocytic endfeet from the surface of blood vessels, disrupting the blood-brain barrier (BBB) and astrocytic control of vascular tone (Cuddapah et al., 2014; Watkins et al., 2014). While anti-angiogenesis strategies were initially promising for limiting glioma progression, the VEGF-specific antibody bevacizumab (Avastin) completely ablated tumor blood vessels and actually enhanced tumor growth by upregulating hypoxia-inducible pathways (Conley et al., 2012). A more apt approach may be to control availability of pro-angiogenic factors to promote vascular normalization.

Immune Cell Modulation

Another hallmark of cancer is the promotion of pro-tumor inflammation (Hanahan and Weinberg, 2011). Monocyte-derived cells can account for nearly 60% of the tumor bulk (Yuan et al., 2014). Initial studies proposed that glioma-associated macrophages were conditioned toward alternative, M2 activation, but recent evidence suggests this characterization requires refinement (Mantovani et al., 2002; Szulzewsky et al., 2015; Gabrusiewicz et al., 2016). Early in tumor development, anti-inflammatory cytokines enable tumor cells to evade the host immune response (Zitvogel et al., 2006; Razavi et al., 2016). Later, immunotolerance can occur due to secretion of tolerogenic cytokines and ligands such as TGFβ, IL-10, and PD-L1 (Razavi et al., 2016). Glioma-derived ECM molecules also alter immune cell phenotype, with periostin acting to recruit and train monocytes toward pro-tumor phenotypes and tenascin-c protecting tumor cells from immune surveillance by arresting T-cell activation (Jachetti et al., 2015; Zhou et al., 2015).

While early biomaterials aimed to reduce the immune response (Bryers et al., 2012), more recent advances have resulted in development of immunomodulatory biomaterials (Hubbell et al., 2009) and immunotherapeutic biomaterials (Swartz et al., 2012). Biomaterial-based regulation of macrophage polarization was recently reviewed elsewhere (Sridharan et al., 2015). Although regenerative approaches typically focus on promoting anti-inflammatory immune cell phenotypes, the opposite is also conceivable. These approaches could easily be tailored toward anti-cancer immunotherapy, as well. T cell modulation is a rapidly growing and promising field, with several strategies currently being tested: checkpoint inhibitor targeting of programmed cell death protein (PD)-1, chimeric antigen receptor (CAR) T cell therapy, and dendritic cell therapy (Tumeh et al., 2014; Garg et al., 2017; O'Rourke et al., 2017).

Glial Cell Modulation

The glioma tumor microenvironment uniquely contains a brain-specific class of cells known collectively as glia, in part comprising astrocytes and microglia. Astrocytes provide trophic and functional support for neurons, and microglia are the resident immune cells of the central nervous system. Glioma-associated factors such as CCL21 and the proteoglycan versican promote a pro-tumor phenotype in microglia (Vinnakota et al., 2013; Hu et al., 2015). Glioma cells communicate with astrocytes via connexin-43 gap junctions to promotes glioma invasion, potentially through exchange of double stranded DNA, as was observed with metastatic breast cancer cells (Chen et al., 2016; Sin et al., 2016).

There is limited knowledge on the effects of combining glial cells in 3D culture with glioma cells. Recent histological evidence revealed that the balance between reactive astrocytes and microglia correlated with GBM patient prognosis; therefore, it will be important to investigate the combination of these cell types in the future (Yuan et al., 2016). It also remains unclear if tumor-associated astrocytes are functionally different than other reactive astrocytes, particularly after the mechanical stress of surgical resection. Nonetheless, material interventions for tissue regeneration often target astrocytic "glial scarring." A mixture of collagen, hyaluronic acid, and Matrigel maintained astrocytes in a quiescent state *in vitro* (Placone et al., 2015). Additionally, a laminin-inspired self-assembling peptide hydrogel attenuated glial scarring following a stab injury (Maclean et al., 2017).

Implications for Therapeutic Translation

Biomaterials are routinely used to target the cellular microenvironment to promote healing. A similar approach may prove useful for limiting glioma recurrence. Implanting a material with immobilized pro-angiogenic factors may help constructively direct angiogenesis within the resection cavity to promote BBB formation and oxygen normalization while restricting vessel development in the parenchyma (Li et al., 2017). A matrix that irreversibly sequesters VEGF-A from the surrounding tumor microenvironment may have similar effects. The adaptive immune system can be redirected using biomaterial-based vaccines to elicit potent, antigen-specific T cell responses, including in glioma (Ali et al., 2011; Purwada et al., 2014; Cheung et al., 2018). Reversing pro-tumor polarization in innate immune cells and glia will likely require a nuanced balance between pro- and anti-inflammatory phenotypes. In this case, it would be useful to temporally control release and/or presentation of different factors (Spiller et al., 2015). Enzyme-releasing materials could assist in mitigating the effects of glioma-derived ECM molecules (Qu et al., 2013). Additionally, astrocytes may be specifically targeted using therapeutic connectosomes to override cell-cell communication with glioma (Gadok et al., 2016). The foremost objective must remain eliminating the cancer cells, therefore fibrous materials may again be preferred given it proves desirable to promote pro-healing phenotypes in the long run (Sridharan et al., 2015).

CONCLUSIONS

Although we use the *in vivo* environment to educate development of defined *in vitro* models, we rarely do the inverse in cancer. The complexity of glioblastoma has thus far proven difficult to capture *in vitro*, and unfortunately no current model can accurately predict the translational success of a therapy. Here, we proposed synthesizing the collective knowledge from *in vitro* models to inform tissue-level interventions through rational design of therapeutic biomaterials. Several strategies may be particularly relevant: Controlling angiogenesis by presentation of VEGF-A and FGF to enable better drug delivery to tumor remnants; induction of immunogenic response through growth factor and chemokine presentation to induce immune infiltration and anti-tumor differentiation; or increased stiffness coupled with topography and/or chemokines such as CXCL12 to encourage tumor cell migration away from healthy tissue. Regardless, using biomaterials as a tissue engineering approach to treat glioblastoma is an unexplored possibility. Because a plethora of *in vitro* models have used a host of different biomaterials and approaches, there may already be a strategy hidden within these studies that could assist in the fight against this deadly disease.

AUTHOR CONTRIBUTIONS

All authors developed the presented conceptual ideas, conducted literature review, drafted the manuscript, and approved it for publication.

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